Effects of aspirin, carprofen, deracoxib, and meloxicam on platelet function and systemic prostaglandin concentrations in healthy dogs

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**Objective**—To determine effects of therapeutic dosages of aspirin, carprofen, deracoxib, and meloxicam on platelet function and systemic prostaglandin concentrations in healthy dogs.

**Animals**—10 hound-crossbred dogs.

**Procedures**—Aspirin (10 mg/kg, PO, q 12 h), carprofen (4.4 mg/kg, PO, q 24 h), deracoxib (2 mg/kg, PO, q 24 h), meloxicam (0.1 mg/kg, PO, q 24 h), and a placebo were administered for 7 days in a random order to each of 10 healthy dogs; there was a 21-day washout period between subsequent treatments. One-stage prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen concentration, and plasma concentrations of thromboxane (TXB)2 and 6-keto prostaglandin (PGF)1α were measured before and after treatment administration. Platelet function was assessed by use of a platelet-function analyzer and aggregation.

**Results**—Aspirin, carprofen, and meloxicam did not significantly affect platelet function. Deracoxib caused a mild decrease in platelet aggregation induced by 50 µM ADP. Platelet number, Hct, PT, aPTT, and plasma TXB2 and 6-keto PGF1α concentrations were unchanged after NSAID administration. Meloxicam administration resulted in a significant decrease in fibrinogen concentration, but results remained within the laboratory reference interval.

**Conclusions and Clinical Relevance**—Oral administration of commonly used NSAIDs at therapeutic dosages in healthy dogs did not alter plasma TXB2 and 6-keto PGF1α concentrations. Deracoxib administration resulted in a minor abnormality in platelet aggregation. Anti-inflammatory doses of aspirin did not affect platelet function as measured by use of optical aggregometry and a platelet-function analyzer. Further evaluation of the effects of aspirin and cyclooxygenase-2–selective inhibitors on hemostasis should be performed. (Am J Vet Res 2010;71:349–358)

Nonsteroidal anti-inflammatory drugs have been widely used to provide short- and long-term analgesia and anti-inflammatory benefits to human and veterinary patients. By inhibiting the key enzyme, COX, in the arachidonic acid pathway, NSAIDs induce both analgesic and toxic effects.1,2 At least 2 isoenzymes of COX (COX-1 and COX-2) have been identified in humans and other animals.1,3 Cyclooxygenase-1 is considered a constitutive form of the COX enzyme; it is found in basal conditions in many cells and is responsible for such functions as gastric cytoprotection, regulation of renal blood flow, and regulation of platelet activity.1 In contrast, COX-2 is considered an inducible form of the COX enzyme; it is found in extremely low amounts in physiologically normal tissues but is rapidly induced in inflammatory conditions.1,2 Toxic effects of NSAIDs are thought to result mainly from inhibition of COX-1.1,3 As such, COX-2–selective NSAIDs have been developed in an attempt to minimize the adverse effects associated with the COX-1 inhibition of traditional NSAIDs.1,3,6 Clinical trials7–11 in dogs have identified several NSAIDs that are COX-2–selective inhibitors, including carprofen, deracoxib, fi-
rocoxib, and meloxicam. Although these NSAIDs are selective for inhibition of COX-2, most have varying degrees of inhibitory activity against COX-1.

An increased incidence of myocardial infarction and other adverse cardiovascular events has been associated with the use of COX-2–selective NSAIDs in humans.12,13 To our knowledge, no such reports have been made for veterinary species. The increased incidence of adverse cardiovascular events associated with COX-2 inhibitors in humans may be attributed to the induction of an imbalance in 2 systemic prostaglandins, TXA2 and PGI1. Thromboxane A2 is a product of COX-1 in platelets and induces vascular constriction and platelet aggregation in the circulation, whereas PGI1, a COX product of endothelial cells, inhibits platelet aggregation and induces vasodilation.12,13 Cyclooxygenase-2 inhibitors may lead to decreased concentrations of PGI1, which would leave the actions of TXA2 unopposed and potentially contribute to a prothrombotic environment in the circulation.14,17

Aspirin (formerly known as acetylsalicylic acid) is a COX-1 inhibitor and is generally considered to have anti-thrombotic effects as a result of irreversible acetylation of COX-1 in platelets, which prevents TXA2 production and induces anti-thrombotic effects for the lifespan of the platelets.18 Studies19–23 have revealed platelet inhibition induced by aspirin administered at anti-thrombotic (0.5 mg/kg, PO, q 12 h) and anti-inflammatory (≤ 10 mg/kg, PO, q 12 to 24 h) dosages.

Because COX-2–selective inhibitors are expected to spare production of COX-1 in platelets, they are thought to have little effect on platelet function. However, despite the increasing use of COX-2–selective inhibitors in veterinary medicine, there is limited information available regarding the effects of these NSAIDs on hemostasis in dogs.

Studies on platelet function after administration of COX-2 inhibitors have provided variable results. Platelet inhibition has resulted from administration of carprofen or meloxicam in some studies19,21,22 but not in others.21 A study20 of dogs with osteoarthritis revealed an increase in coagulation measured by use of thromboelastography (which was suggestive of hyper-coagulability) after treatment with the COX-2–selective inhibitor deracoxib for 14 days. In that study,19 NSAID treatment did not result in an altered ratio of 6-keto PGF1α to TXB2 concentrations.

Controlled, blinded, randomized trials conducted to examine the effects of commonly used NSAIDs administered at therapeutic dosages on hemostasis in healthy dogs are lacking. The study reported here was conducted to examine the effects of 4 commonly used NSAIDs (aspirin, carprofen, deracoxib, and meloxicam) on platelet function and plasma 6-keto PGF1α and TXB2 concentrations in healthy dogs.

Materials and Methods

Animals—Ten healthy mature hound-crossbred dogs (5 sexually intact males and 5 spayed females) that were part of a research colony were used in the study. Dogs weighed between 23.8 and 32.3 kg (mean, 27.3 kg) and ranged from 15 to 24 months of age (mean, 19 months of age). The dogs were healthy as determined on the basis that results of a physical examination were within expected limits and that results for a CBC, serum biochemical analysis, and urinalysis conducted 1 week before onset of the study were all within respective laboratory reference intervals. Additionally, measures of primary and secondary hemostasis, including results of platelet aggregometry, closure time determined by use of a platelet-function analyzer,24 and results of a coagulation profile (PT, aPTT, and fibrinogen concentration), were within expected limits for healthy dogs before onset of the study. Laboratory reference intervals for PT, aPTT, and fibrinogen concentrations were established from a general canine population and were not breed-specific values. Dogs had not received any medication in the 8 weeks preceding onset of the study. All dogs were housed in indoor kennels, fed a standard commercial dog food twice daily, provided water ad libitum, and allowed walks on a leash outside in a restricted area. This study was designed in accordance with the standards of the Canadian Council on Animal Care and the Ontario Animals for Research Act and was approved by the University of Guelph Animal Care Committee.

Study design—Preliminary studies were performed to determine the concentrations of each agonist used for platelet aggregometry. Two platelet aggregation agonists, ADP and PAF, were used for aggregometry. The lowest concentration of ADP and PAF that consistently induced maximal platelet aggregation was determined to be 100µM ADP and 1µM PAF.

Ten dogs were orally administered 4 NSAIDs and 1 placebo in a crossover design; doses of NSAIDs were consistent with current anti-inflammatory recommendations.25 The dogs were administered aspirin4 (10 mg/kg, PO, q 12 h), carprofen5 (4.4 mg/kg, PO, q 24 h), deracoxib6 (2 mg/kg, PO, q 12 h), and meloxicam7 (0.1 mg/kg, PO, q 24 h). The placebo consisted of an empty gelatin capsule. Each of the 4 drugs and the placebo were administered for 7 consecutive days, with a 21-day washout period (minimum of 14 half-lives for each drug) between subsequent treatments. A Latin square randomization procedure was used to determine the order in which each drug was received and to control for period and carryover effects. The drugs were administered with food, except for the NSAIDs administered on day 7 that were administered alone to avoid lipemia, which can interfere with platelet aggregometry.

Blood collection—All dogs were acclimated to blood collection procedures before onset of the study, which allowed rapid and efficient collection of samples during the study. Blood collection for platelet function testing and measurement of TXB2 and PGI1 concentrations was performed on day 0 before initiating each treatment and on day 7 of each treatment. Blood was collected 2 to 3 hours after NSAID administration on day 7. Food was withheld for a minimum of 12 hours prior to collection of blood samples used for hemostatic testing.

Venous blood samples were collected from a jugular vein by use of a 20-gauge needle. The first 2 mL of blood collected was immediately transferred to an EDTA-containing plastic tube. The remaining 35 mL of
blood was collected into a syringe containing 3.2% sodium citrate (1 part sodium citrate:9 parts blood).

**Hematologic variables**—Approximately 2 mL of EDTA-anticoagulated whole blood was used to quantify platelet number, Hct, and leukocyte number by use of an automated hemato logic analyzer. Platelet-poor plasma was used to measure PT, aPTT, and fibrinogen concentration by use of an automated analyzer.

**Platelet function**—Assessment of platelet function was performed by use of a bench top point-of-care platelet function analyzer. A sample reservoir cartridge containing a membrane coated with collagen plus ADP was warmed to 22°C. The cartridge was placed into the analyzer, and an 800-µL aliquot of citrate-anticoagulated whole blood was transferred by use of an adjustable piston micropipette into a sample reservoir. The cartridge and sample were further warmed to 37°C within the analyzer prior to assessing platelet function. The sample was aspirated from the reservoir through a capillary and a microscopic aperture, and the time required to occlude the aperture with a platelet plug was reported as closure time. Analysis was performed within 1 hour after sample collection. The maximum closure time measured by the platelet function analyzer was 300 seconds; longer closure times were reported as > 300 seconds. An instrument self-test was performed daily prior to sample analysis to verify proper functioning of the analyzer. Use of the platelet function analyzer has been described in clinically normal dogs as well as dogs with primary hemostatic defects (eg, von Willebrand disease) and dogs treated with aspirin. In a previous study, clinically normal dogs had an ADP closure time of 52 to 86 seconds and an epinephrine closure time of 97 to 225 seconds. Sensitivity and specificity of the platelet function analyzer for detection of dogs with primary hemostatic deficits by use of ADP cartridges is 95.7% and 100%, respectively, and 95.7% and 82.8%, respectively, with epinephrine cartridges. Platelet hyperfunction secondary to endotoxia results in substantially shortened closure times detected by use of both the ADP and epinephrine cartridges.

**Platelet aggregation**—Platelet aggregation was evaluated within 4 hours after sample collection and was measured optically by use of a dual-channel aggregometer. An aliquot of citrate-anticoagulated whole blood was centrifuged (80 × g at 15°C for 15 minutes) immediately after collection to obtain PRP. After removing the PRP, the remaining sample was centrifuged (3,000 × g at 15°C for 15 minutes) to obtain PPP. Platelet count of the PPP was determined by use of a hemacytometer and a standard manual platelet counting technique. Briefly, PRP was used to fill the 2 counting chambers of a hemacytometer. The hemacytometer was placed in a humidified chamber for 10 minutes. By use of light microscopy, the chambers were viewed at 500× magnification, and a platelet count was obtained from the 25 main squares of each of the 2 counting chambers. When the counts for the 2 chambers differed by > 5%, the counts were repeated. The mean value was determined for the 2 counted chambers, and the mean value then was multiplied by 1,000 to provide an estimate of the platelet count of the PRP. The platelet count of the PPP was assumed to be zero. An appropriate volume of autologous PPP was mixed with the PRP aliquot in test tubes to yield PRP with a final platelet count of 200 × 10^9 cells/L for platelet aggregometry. The final platelet count was confirmed by use of the aforementioned hemacytometer counting method. The diluted PRP was gently mixed by rotation of the test tubes; the tubes were then sealed with a plastic cap and allowed to sit undisturbed for 30 minutes.

A 25-µL aliquot of platelet agonist was added to a 225-µL aliquot of autologous PRP in 1 channel of the aggregometer, and 250 µL of PPP was placed in the other channel. The samples were allowed to warm to 37°C in the aggregometer and were continuously stirred at 900 revolutions/min to allow for adequate platelet distribution. Maximal transmission was determined by measuring transmission of light through the PPP, which was assigned a baseline transmission of 100%. Minimum transmission was determined by measuring light transmission through the stirred PRP and assigned a baseline transmission of 0%. Samples were recorded continuously until aggregation was complete, at which time light transmission reached a plateau. In cases of partial aggregation, recording was continued until disaggregation was complete and light transmission reached 0%.

The agonists were kept on ice and used within 2 hours after preparation. Each agonist was used at a high concentration (100µM ADP and 1µM PAF) that consistently induced maximal platelet aggregation in samples obtained from each dog (as determined on the basis of preliminary studies) and a low concentration (50µM ADP and 0.5µM PAF) that was half of the high concentration. Each sample was assayed in duplicate at each agonist concentration. Aggregation was evaluated by measuring maximal aggregation (total percentage increase of light transmission) and initial aggregation velocity (percentage increase in aggregation per minute). In samples in which there was disaggregation, the time until disaggregation was measured.

**Plasma PG concentrations**—Aliquots of PPP were frozen at −70°C immediately after preparation and stored frozen for up to 30 days. Immediately before use, the PPP aliquots were thawed in a 20°C water bath; samples were assayed by use of commercially available competitive enzyme immunoassay kits to determine concentrations of TXB, and 6-keto PGF_1α, respectively. Thromboxane B_2 is a stable metabolite of TXA_2, and 6-keto PGF_1α is a PGF_1α metabolite. Assays were performed in 96-well enzyme immunoassay plates. The sample wells in the plate were coated with monoclonal mouse anti-rabbit antiserum binding sites. Plasma samples were assayed in duplicate by adding 50 µL of PPP to each well. The concentration of prostaglandin was determined spectrophotometrically at 412 nm by use of a computerized assay plate reader. Samples with a coefficient of variation > 10% between duplicates were assayed again in duplicate.

Measurement of plasma TXB and 6-keto PGF_1α concentrations in samples of dogs has been described elsewhere. The detection limit for the TXB assay was 11 pg/mL, and the detection limit for the 6-keto PGF_1α assay was 6 pg/mL. The manufacturer reports
Table 1—Mean (range) results of hematologic analysis of samples obtained from 10 healthy dogs before and after treatment for 7 days with each of 4 NSAIDs and a placebo.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hct (L/L) Before</th>
<th>Hct (L/L) After</th>
<th>WBC count (x10^9 cells/L) Before</th>
<th>WBC count (x10^9 cells/L) After</th>
<th>No. of platelets (x10^9 cells/L) Before</th>
<th>No. of platelets (x10^9 cells/L) After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>0.49 (0.44–0.53)</td>
<td>0.49 (0.45–0.52)</td>
<td>7.9 (5.4–11.9)</td>
<td>8.5 (5.4–12.0)</td>
<td>241 (160–353)</td>
<td>243 (190–319)</td>
</tr>
<tr>
<td>Carprofen</td>
<td>0.50 (0.44–0.54)</td>
<td>0.50 (0.45–0.54)</td>
<td>7.2 (4.5–10.5)</td>
<td>7.0 (4.3–9.6)</td>
<td>252 (150–346)</td>
<td>248 (182–301)</td>
</tr>
<tr>
<td>Deracoxib</td>
<td>0.50 (0.45–0.54)</td>
<td>0.50 (0.45–0.56)</td>
<td>7.5 (5.4–10.2)</td>
<td>7.4 (5.2–9.8)</td>
<td>255 (156–363)</td>
<td>222 (151–304)</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>0.50 (0.45–0.54)</td>
<td>0.49 (0.45–0.52)</td>
<td>7.8 (4.8–9.4)</td>
<td>7.6 (5.5–9.1)</td>
<td>244 (190–358)</td>
<td>244 (190–330)</td>
</tr>
<tr>
<td>Placebo</td>
<td>0.51 (0.45–0.56)</td>
<td>0.50 (0.44–0.56)</td>
<td>7.2 (4.0–8.6)</td>
<td>7.3 (4.3–11.0)</td>
<td>237 (170–351)</td>
<td>242 (179–340)</td>
</tr>
</tbody>
</table>

Laboratory reference range for Hct was 0.39 to 0.54 L/L, for WBC count was 4.9 x10^9 cells/L to 15.4 x10^9 cells/L, and for platelet number was 117 x10^9 cells/L to 418 x10^9 cells/L.

Table 2—Mean (range) results of coagulation tests and fibrinogen concentration measured in samples obtained from 10 healthy dogs before and after treatment for 7 days with each of 4 NSAIDs and a placebo.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PT (s) Before</th>
<th>PT (s) After</th>
<th>aPTT (s) Before</th>
<th>aPTT (s) After</th>
<th>Fibrinogen (g/L) Before</th>
<th>Fibrinogen (g/L) After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>12.8 (11.4–16.6)</td>
<td>11.8 (8.7–15.3)</td>
<td>18.9 (15.9–23.2)</td>
<td>19.4 (17.7–21.7)</td>
<td>1.5 (1.2–2.1)</td>
<td>1.4 (1.2–1.3)</td>
</tr>
<tr>
<td>Carprofen</td>
<td>11.4 (9.6–13.8)</td>
<td>12.9 (9.0–18.7)</td>
<td>19.3 (19.0–23.0)</td>
<td>19.6 (17.2–20.5)</td>
<td>1.4 (1.2–1.7)</td>
<td>1.4 (1.2–1.7)</td>
</tr>
<tr>
<td>Deracoxib</td>
<td>13.0 (11.2–16.1)</td>
<td>12.2 (9.1–19.2)</td>
<td>20.1 (17.4–24.4)</td>
<td>19.6 (18.6–24.1)</td>
<td>1.5 (1.2–1.9)</td>
<td>1.4 (1.0–1.8)</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>11.5 (8.7–13.8)</td>
<td>11.6 (10.4–12.4)</td>
<td>19.8 (17.6–23.6)</td>
<td>20.1 (18.1–23.1)</td>
<td>1.5 (1.2–1.8)</td>
<td>1.3 (1.0–1.5)*</td>
</tr>
<tr>
<td>Placebo</td>
<td>12.2 (6.4–16.7)</td>
<td>12.9 (8.2–18.1)</td>
<td>19.5 (16.9–21.8)</td>
<td>19.6 (17.2–22.3)</td>
<td>1.5 (0.9–2.1)</td>
<td>1.1 (1.1–3.6)</td>
</tr>
</tbody>
</table>

Laboratory reference range for PT was 9 to 15 seconds, for aPTT was 15.0 to 23.5 seconds, and for fibrinogen concentration was 0.8 to 2.3 g/L.

*Value differs significantly (P = 0.03) from the value before treatment.

A 2-way ANOVA, which in the model, was used on the differences (before treatment vs after treatment) for the variables of interest. When the overall treatment effect was significant, a post hoc Tukey test was used to determine whether there was a greater change for that variable between treatments. A multivariate t adjustment was made to compare results with the baseline value within a specific treatment. Significance was set at values of P ≤ 0.05. All statistical analyses were performed by use of statistical software.*

Results

Hematologic analysis—All 10 dogs completed the study for each treatment, and no adverse effects were observed. Administration of NSAIDs did not result in a significant difference in platelet count, Hct, or WBC count before or after treatments (Table 1). Administration of NSAIDs did not have a significant effect on PT or aPTT (Table 2). Fibrinogen concentration was significantly (P = 0.03) decreased after meloxicam treatment but remained within the reference interval.

Platelet function—Platelet function measured by use of the platelet function analyzer did not differ significantly before and after administration of aspirin, carprofen, deracoxib, or meloxicam (Table 3). Prolonged ADP closure times were measured in 2 dogs (1 dog had a prolonged closure time [100 seconds] after deracoxib administration, and the other dog had a prolonged closure time [119 seconds] after meloxicam administration). None of the dogs had a closure time that was less than the reference interval.

Maximal platelet aggregation induced by 1µM or 0.5µM PAF was not significantly affected by NSAID treatment (Table 4). Maximal platelet aggregation induced by 50µM ADP was decreased significantly (P = 0.03) from 65.2% to 52.7% after deracoxib administration but was not significantly affected by deracoxib administration when platelet aggregation was induced by 100µM ADP. Aspirin, carprofen, and meloxicam administration did not significantly affect maximal platelet aggregation induced by either concentration of ADP. Rate of aggregation measured by the percentage change in aggregation during a 1-minute period was not significantly affected by NSAID administration.

Statistical analysis—A 2-way ANOVA, which included treatment carryover and period effect as factors in the model, was used on the differences (before treatment [baseline] vs after treatment) for the variables of interest. When the overall treatment effect was significant, a post hoc Tukey test was used to determine whether there was a greater change for that variable between treatments. A multivariate t adjustment was made to compare results with the baseline value within a specific treatment. Significance was set at values of P ≤ 0.05. All statistical analyses were performed by use of statistical software.*

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Interassay and intra-assay coefficients of variation for each assay to be < 10% and cross-reactivity of each assay with other eicosanoids to be < 1%.

Table 3—Mean ± SE platelet closure times measured* in samples obtained from 10 healthy dogs before and after treatment for 7 days with each of 4 NSAIDs and a placebo.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Closure time (s) Before</th>
<th>Closure time (s) After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>69.5 ± 3.77</td>
<td>71.0 ± 3.61</td>
</tr>
<tr>
<td>Carprofen</td>
<td>69.9 ± 2.58</td>
<td>74.2 ± 4.10</td>
</tr>
<tr>
<td>Deracoxib</td>
<td>71.0 ± 3.11</td>
<td>72.9 ± 3.63</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>65.0 ± 1.93</td>
<td>77.4 ± 5.53</td>
</tr>
<tr>
<td>Placebo</td>
<td>68.2 ± 3.26</td>
<td>73.7 ± 3.91</td>
</tr>
</tbody>
</table>

Laboratory reference range for closure time was 52 to 90 seconds.

*Measured by use of a platelet function analyzer and an ADP-collagen cartridge.
administration after aggregation was induced by PAF or ADP (Table 5).

Plasma PG concentrations—No significant differences in TXB2 or PG12 concentrations were detected after NSAID treatment (Table 6). Thromboxane B2 concentrations were significantly (P = 0.05) higher after deracoxib treatment, compared with TXB concentrations after aspirin treatment. The ratio of TXB2 to 6-keto PGF1α concentrations was decreased after aspirin administration, although this difference was not significant (P = 0.06). The ratio of TXB2 to 6-keto PGF1α concentrations was not affected by administration of any other NSAID.

Discussion

Nonsteroidal anti-inflammatory drugs are commonly used in veterinary medicine to provide analgesic and anti-inflammatory benefits to patients. Analysis of reports12–14 has suggested an increase in the incidence of myocardial infarction and other adverse cardiovascular events associated with the use of COX-2–selective NSAIDs in humans, possibly as a result of a relative increase in TXA2 concentrations, compared with PG12 concentrations. In contrast to the condition in humans, thromboembolic events leading to cardiovascular disease are infrequently identified in dogs.5 To our knowledge, there have been no reports associating the use of COX-2–selective drugs (eg, meloxicam, deracoxib, or carprofen) with thromboembolic disease in dogs. The objective of the study reported here was to investigate the effects of COX inhibition on hemostasis and concentrations of TXB2 and 6-keto PGF1α in healthy dogs.

Results of a CBC (ie, platelet count, Hct, and WBC count) and measures of secondary hemostasis (ie, PT and aPTT) were not significantly affected by NSAID administration in this study. Fibrinogen concentrations decreased significantly (P = 0.03) after meloxicam administration but remained within the laboratory reference range, and the clinical relevance of this change is considered to be low. Inhibition of COX by NSAIDs is not expected to alter CBC variables or measures of secondary hemostasis in veterinary patients or humans.

Analysis of primary hemostasis by use of a platelet-function analyzer and optical aggregometry in the study reported here revealed minimal effects of NSAID administration on platelet function. The ADP closure time measured by use of the platelet-function analyzer

Table 4—Mean ± SE maximal platelet aggregation measured in samples obtained from 10 healthy dogs before and after treatment for 7 days with each of 4 NSAIDs and a placebo.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0.5µM PAF Before</th>
<th>After</th>
<th>1µM PAF Before</th>
<th>After</th>
<th>50µM ADP Before</th>
<th>After</th>
<th>100µM ADP Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>66.25 ± 1.99</td>
<td>70.56 ± 2.16</td>
<td>69.44 ± 2.55</td>
<td>71.68 ± 1.74</td>
<td>58.25 ± 2.51</td>
<td>57.31 ± 3.67</td>
<td>50.75 ± 3.81</td>
<td>61.66 ± 4.25</td>
</tr>
<tr>
<td>Carprofen</td>
<td>65.39 ± 3.33</td>
<td>68.62 ± 2.46</td>
<td>65.56 ± 2.22</td>
<td>63.19 ± 3.08</td>
<td>50.88 ± 2.93</td>
<td>54.25 ± 4.35</td>
<td>57.70 ± 6.23</td>
<td>52.19 ± 3.55</td>
</tr>
<tr>
<td>Deracoxib</td>
<td>65.95 ± 2.10</td>
<td>69.25 ± 2.97</td>
<td>66.88 ± 1.74</td>
<td>61.19 ± 2.46</td>
<td>65.19 ± 2.77</td>
<td>52.69 ± 2.67</td>
<td>45.94 ± 6.23</td>
<td>61.38 ± 4.05</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>61.94 ± 4.88</td>
<td>68.44 ± 1.83</td>
<td>69.07 ± 1.73</td>
<td>65.69 ± 1.58</td>
<td>61.88 ± 3.70</td>
<td>51.06 ± 3.62</td>
<td>46.75 ± 5.83</td>
<td>59.19 ± 2.58</td>
</tr>
<tr>
<td>Placebo</td>
<td>66.63 ± 2.28</td>
<td>66.63 ± 2.28</td>
<td>62.45 ± 3.56</td>
<td>66.19 ± 2.30</td>
<td>64.13 ± 3.26</td>
<td>54.29 ± 4.83</td>
<td>53.94 ± 5.64</td>
<td>52.19 ± 5.51</td>
</tr>
</tbody>
</table>

*Values with different superscript letters differ significantly (P = 0.03).

Table 5—Mean ± SE rate of platelet aggregation measured in samples obtained from 10 healthy dogs before and after treatment for 7 days with each of 4 NSAIDs and a placebo.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0.5µM PAF Before</th>
<th>After</th>
<th>1µM PAF Before</th>
<th>After</th>
<th>50µM ADP Before</th>
<th>After</th>
<th>100µM ADP Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>14.35 ± 0.76</td>
<td>15.67 ± 1.02</td>
<td>15.48 ± 0.93</td>
<td>16.32 ± 0.50</td>
<td>11.59 ± 0.75</td>
<td>12.60 ± 0.85</td>
<td>12.62 ± 0.95</td>
<td>13.08 ± 0.58</td>
</tr>
<tr>
<td>Carprofen</td>
<td>15.01 ± 0.95</td>
<td>14.85 ± 0.83</td>
<td>14.14 ± 0.73</td>
<td>14.42 ± 0.70</td>
<td>12.45 ± 0.77</td>
<td>11.52 ± 0.68</td>
<td>13.16 ± 0.67</td>
<td>11.45 ± 0.75</td>
</tr>
<tr>
<td>Deracoxib</td>
<td>15.24 ± 0.70</td>
<td>15.67 ± 0.75</td>
<td>15.07 ± 0.91</td>
<td>14.40 ± 0.70</td>
<td>12.67 ± 0.87</td>
<td>11.44 ± 0.65</td>
<td>12.40 ± 0.71</td>
<td>11.98 ± 0.53</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>14.66 ± 0.94</td>
<td>15.09 ± 0.72</td>
<td>15.39 ± 0.58</td>
<td>14.28 ± 0.59</td>
<td>12.09 ± 0.59</td>
<td>11.65 ± 0.68</td>
<td>12.27 ± 0.43</td>
<td>11.50 ± 0.79</td>
</tr>
<tr>
<td>Placebo</td>
<td>15.77 ± 1.10</td>
<td>14.69 ± 0.85</td>
<td>14.51 ± 1.05</td>
<td>14.42 ± 0.98</td>
<td>12.27 ± 0.92</td>
<td>11.52 ± 0.73</td>
<td>11.77 ± 0.68</td>
<td>11.45 ± 0.79</td>
</tr>
</tbody>
</table>

*Values with different superscript letters differ significantly (P = 0.05).

Table 6—Mean ± SE 6-keto PGF1α and TXB2 concentrations measured in samples obtained from 10 healthy dogs before and after treatment for 7 days with each of 4 NSAIDs and a placebo.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>6-keto PGF1α (pg/mL)</th>
<th>TXB2 (pg/mL)</th>
<th>6-keto PGF1α/TXB2 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>149.1 ± 13.6</td>
<td>171.8 ± 19.4</td>
<td>302.2 ± 66.0</td>
</tr>
<tr>
<td>Carprofen</td>
<td>162.5 ± 13.6</td>
<td>165.9 ± 14.8</td>
<td>206.4 ± 41.4</td>
</tr>
<tr>
<td>Deracoxib</td>
<td>178.2 ± 18.8</td>
<td>201.1 ± 20.2</td>
<td>265.2 ± 35.8</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>158.6 ± 15.8</td>
<td>191.3 ± 22.4</td>
<td>189.8 ± 33.2</td>
</tr>
<tr>
<td>Placebo</td>
<td>152.1 ± 13.3</td>
<td>165.9 ± 13.0</td>
<td>240.6 ± 49.9</td>
</tr>
</tbody>
</table>

*Values with different superscript letters differ significantly (P = 0.05).
was not significantly affected by administration of any of the NSAIDs. As a primarily COX-1 antagonist, aspirin was expected to decrease platelet function. There are conflicting results in the literature with respect to results of platelet function testing after administration of aspirin. Aspirin doses ranging from 0.5 to 3 mg/kg, PO, every 12 hours, decreased platelet aggregation in other studies in dogs. Additionally, 7 to 8 mg of aspirin/kg, PO, every 24 hours, administered to healthy dogs significantly decreased platelet aggregation responses detected with whole blood aggregometry induced by ADP and prolonged epinephrine closure times but did not significantly affect ADP closure times as measured by use of a platelet-function analyzer. However, in other studies, 3.5 mg of aspirin/kg, PO, every 12 hours, did not alter platelet aggregation in response to collagen, and aspirin at doses of 5 to 10 mg/kg, PO, every 24 hours, failed to decrease platelet aggregation in response to collagen and ADP. Similarly, the COX-1 inhibitor phenylbutazone failed to alter platelet aggregation in response to ADP but decreased aggregation induced by arachidonic acid.

Aspirin is used for its antithrombotic effects in veterinary patients at an ultralow dose (0.5 mg/kg, PO, every 24 hours), whereas anti-inflammatory doses for aspirin range from 10 to 35 mg/kg, PO, every 8 to 24 hours. It is unknown why aspirin at 10 mg/kg, PO, every 12 hours, failed to inhibit platelet function in the present study. Aspirin has a dose-related effect on primary hemostasis and platelet function. Aspirin administration at antithrombotic doses is reportedly sufficient to block platelet COX generation, which leads to decreased TXA₂ synthesis without affecting production of PGI₂. Doses exceeding 0.5 mg/kg administered during a period of several days may not result in platelet inhibition because of accumulation of the aspirin metabolite salicylate, which can reverse the antiaggregatory effects of aspirin. Additionally, the concurrent inhibition of endothelial PGI₂ production can also interfere with the antiplatelet activity of aspirin. Although a change in 6-keto PGF₁α concentrations was not detected in the study reported here, an accumulation of salicylate may have prevented aspirin from affecting platelet function tests. Both PAF and ADP induce irreversible aggregation in canine platelets. However, initial aggregation by ADP is not mediated by COX or TXA₂. Although aspirin blocks platelet COX generation, some dogs may still be able to produce sufficient amounts of other platelet agonists (such as PAF, ADP, collagen, and epinephrine) to induce platelet aggregation. Additionally, the concentrations of PAF and ADP used in our study may have been sufficient to overcome the inhibitory action of aspirin. Lower concentrations of ADP and PAF may have resulted in platelet dysfunction measured by use of aggregometry. Breed and interindividual variations in platelet function among dogs have been reported and may also account for the variability in the effects of aspirin on platelet function reported in our study and in that other study.

As expected, TXB₂ concentrations after aspirin administration were significantly lower, compared with those after deracoxib administration. However, free TXB₂ concentrations after aspirin administration were not significantly different from baseline free TXB₂ concentrations. This is consistent with previous findings that oral administration of anti-inflammatory doses of aspirin does not significantly impact circulating free TXB₂ concentrations. However, 5 mg of aspirin/kg, PO, every 12 hours, in dogs with osteoarthritis resulted in a marked decrease in platelet-produced TXB₂, which correlated with a mild and marked decrease in maximal platelet aggregation induced by ADP and collagen, respectively. The lack of platelet inhibition by administration of 10 mg of aspirin/kg every 12 hours in the study reported here is inconsistent with results of much of the previous research on the effects of aspirin on canine platelet function. Further investigation into the most appropriate platelet function tests to assess canine platelet function is warranted. Additionally, continued research into the effects of aspirin on canine platelet function and TXB₂ concentrations at various aspirin doses is needed to confirm these results.

Results of in vitro and in vivo studies indicate that carprofen, deracoxib, and meloxicam are preferential COX-2–selective inhibitors. The decrease in platelet aggregation induced by 50μM ADP after deracoxib administration was an unexpected finding in our study. This finding is contradictory to that in another study in which investigators found that deracoxib administration to dogs with osteoarthritis increased clot strength measured by use of thromboelastography (which is suggestive of hypercoagulability) but did not alter platelet aggregometry induced by ADP or collagen. Consistent with the results of that study, deracoxib did not alter TXB₂ or 6-keto PGF₁α concentrations in the present study. The lack of effect on platelet function after carprofen and meloxicam administration was an expected finding. Because they are COX-2–selective inhibitors, these NSAIDs are likely to have minimal impact on COX-1 in platelets. The effects of carprofen and meloxicam on platelet function have been variable in studies. Some studies have revealed that carprofen decreased platelet aggregation induced by ADP and collagen as well as decreased clot strength measured by use of thromboelastography and prolonged skin bleeding time. However, other studies have identified results within the reference range for platelet aggregation responses, closure times measured by use of a platelet-function analyzer, buccal mucosal bleeding times, or intraoperative bleeding score in other studies. However, a mild decrease in platelet aggregation induced by ADP was identified after meloxicam administration to dogs with osteoarthritis in 1 study.

Although deracoxib is considered the most selective COX-2 inhibitor used in the study reported here, it may still cause enough COX-1 inhibition to affect platelet function as indicated by the mild decrease in aggregation induced by 50μM ADP. Carprofen and meloxicam are considered to be less selective for COX-2 than is deracoxib, as determined on the basis of in vitro testing of COX inhibition. However, the in vitro testing of these medications may not accurately reflect the amount of COX-1 inhibitory activity that these agents have in vivo. Alternatively, deracoxib may de-
crease platelet function through a non–TXB<sub>2</sub>-mediated pathway in platelets. Deracoxib administration did not change free plasma TXB<sub>2</sub> concentrations in our study. However, platelet-produced TXB<sub>2</sub> concentrations were not measured; therefore, a subtle impairment of platelet COX-1 activity cannot be ruled out. No clinical signs of excessive bleeding (eg, hematoma formation after venipuncture) were detected after administration of any NSAID in any of the dogs of the study. The clinical importance of the mild reduction in platelet aggregation induced by 50μM ADP after deracoxib administration is likely low but warrants further investigation. The lack of effect of carprofen, deracoxib, and meloxicam on free TXB<sub>2</sub> and 6-keto PGF<sub>1α</sub> concentrations is not supportive of these COX-2–inhibitors inducing an im-

In the study reported here, we examined platelet function after 7 days of NSAID administration. The life span of canine platelets ranges from 5 to 7 days. As such, a 7-day treatment period may not have been long enough to alter function of a sufficient number of platelets to affect results of the platelet function tests. However, other studies revealed that oral administration of aspirin at doses ranging from 0.5 mg/kg, PO, every 12 hours, to 8 mg/kg, PO, every 24 hours, for 4 to 7 days resulted in detectable platelet dysfunction. Intravenous administration of 20 mg of aspirin/kg once resulted in a decrease in platelet function within 2 hours after injection. The 7-day treatment period was chosen to allow for at least 7 half-lives of the NSAIDs to elapse and each of the NSAIDs to reach a steady-state concentration. Future studies should include a longer duration of drug administration to evaluate the effect of long-term NSAID administration on platelets.

Epinephrine cartridges are used in platelet-function analyzers for samples obtained from humans to monitor response to aspirin administration because aspirin does not affect ADP-induced closure times. Another study revealed that IV administration of aspirin at doses ranging from 0.5 mg/kg, PO, every 12 hours, to 8 mg/kg, PO, every 24 hours, for 4 to 7 days resulted in detectable platelet dysfunction. Intravenous administration of 20 mg of aspirin/kg once resulted in a decrease in platelet function within 2 hours after injection. The 7-day treatment period was chosen to allow for at least 7 half-lives of the NSAIDs to elapse and each of the NSAIDs to reach a steady-state concentration. Future studies should include a longer duration of drug administration to evaluate the effect of long-term NSAID administration on platelets.

In that study, platelet aggregation induced by ADP was significantly decreased after treatment with aspirin, carprofen, and meloxicam. Aspirin also significantly decreased platelet aggregation induced by ADP (especially at high concentrations) may not be the most suitable agonist for evaluating aggregation in canine platelets to investigate the effects of COX inhibition on platelet function. Investigators of future studies should consider different aggregation agonists or alternative methods of evaluating platelet function.

Recently, the effects of NSAIDs on hemostasis in client-owned dogs with osteoarthritis were investigated. In that study, platelet aggregation induced by ADP was significantly decreased after treatment with aspirin, carprofen, and meloxicam. Aspirin also significantly decreased platelet aggregation induced by ADP (especially at high concentrations) may not be the most suitable agonist for evaluating aggregation in canine platelets to investigate the effects of COX inhibition on platelet function. Investigators of future studies should consider different aggregation agonists or alternative methods of evaluating platelet function.

Epinephrine cartridges are used in platelet-function analyzers for samples obtained from humans to monitor response to aspirin administration because aspirin does not affect ADP-induced closure times. Another study revealed that IV administration of aspirin at doses ranging from 0.5 mg/kg, PO, every 12 hours, to 8 mg/kg, PO, every 24 hours, for 4 to 7 days resulted in detectable platelet dysfunction. Intravenous administration of 20 mg of aspirin/kg once resulted in a decrease in platelet function within 2 hours after injection. The 7-day treatment period was chosen to allow for at least 7 half-lives of the NSAIDs to elapse and each of the NSAIDs to reach a steady-state concentration. Future studies should include a longer duration of drug administration to evaluate the effect of long-term NSAID administration on platelets.
embolic events in humans), the NSAIDs in that study are considered to have a much higher COX-2 selectivity than the COX-2–selective NSAIDs approved for use in dogs.\textsuperscript{7,8,10,13,33,35} In contrast to these data, selective COX-2 inhibition in another study\textsuperscript{37} in humans did not affect plasma TXB\textsubscript{2} or PGI\textsubscript{2} concentrations but did lead to an increase in urinary excretion of TXB\textsubscript{2}. Dogs are not as predisposed to development of myocardial infarctions and other thromboembolic complications as are humans,\textsuperscript{37} which suggests that there may be other factors in addition to selective COX inhibition involved in reports that suggest an increased rate of myocardial infarctions associated with COX-2–selective inhibitors in humans.

A limiting factor of our study was the small number of dogs used. Although aspirin administration was associated with lower plasma concentrations of TXB\textsubscript{2} compared with baseline concentrations, these concentrations did not differ significantly. Increasing the number of dogs in the study would have allowed for detection of modest effects of NSAIDs on platelet function and systemic PG concentrations.

The platelet-function analyzer that used ADP cartridges and optical platelet aggregometry that used ADP and PAF agonists may not have been sufficiently sensitive to detect platelet changes in this study. To ensure a more comprehensive evaluation of platelet function, future studies should incorporate the use of collagen plus epinephrine cartridges in evaluations conducted by use of a platelet function analyzer, as well as other platelet agonists such as collagen and different agonist concentrations when platelet aggregometry is used. Markers of platelet activity, such as P-selectin detected by use of flow cytometry, may aid in assessment of platelet function, and evaluation of global hemostasis by use of thromboelastography should also be considered for future studies. Measuring platelet-produced TXB\textsubscript{2} concentrations may be more accurate for determining the impact of NSAIDs on platelet COX function and should be performed along with measuring free TXB\textsubscript{2} and PGI\textsubscript{2} concentrations in future studies. Additionally, measurement of serum drug concentrations would be helpful for determining that each NSAID achieved therapeutic concentrations in the patients when platelet function and concentrations of TXB\textsubscript{2} and 6-keto PGF\textsubscript{1α} are evaluated.

Analysis of the results of the study reported here indicated the failure of aspirin given orally at 10 mg/kg every 12 hours to inhibit platelet function as measured via a platelet function analyzer that used ADP cartridges as well as via optical platelet aggregation that used up to 100$\mu$M ADP and up to 1$\mu$M PAF to induce aggregation. Further study of platelet function at various doses of aspirin is needed to determine whether this is a consistent finding. Another unexpected finding in this study was the decrease in platelet aggregation induced by 50$\mu$M ADP after deracoxib administration. Although deracoxib is considered a COX-2–selective inhibitor, it may retain enough COX-1 inhibition to decrease platelet function. The clinical importance of this finding is not known because deracoxib administration did not affect results of other platelet function tests. Further study of the effects of deracoxib and other COX-2–selective inhibitors is needed to determine their effects on primary hemostasis. The COX-2–specific inhibitors carprofen and meloxicam had no effect on platelet function in this study. Additionally, there was no evidence to suggest that the use of COX-2–selective inhibitors would result in a prothrombotic environment because an increase in platelet activity was not observed and an imbalance in free TXB\textsubscript{2} and 6-keto PGF\textsubscript{1α} concentrations was not detected. An understanding of the effects of NSAIDs on platelet function and concentrations of free and platelet-produced TXB\textsubscript{2} and 6-keto PGF\textsubscript{1α} in dogs with underlying disorders that predispose them to hypercoagulability is needed to fully evaluate the safety of these drugs. The small number of dogs in this study and the use of relatively high concentrations of aggregation agonists may have limited our ability to detect subtle platelet defects in this study.

\begin{itemize}
\item a. PFA-100, Dade-Behring, Mississauga, ON, Canada.
\item b. Equine muscle adenosine 5’-diphosphate, Sigma-Aldrich Co, St Louis, Mo.
\item c. Synthetic PAF-16, EMD Biosciences, San Diego, Calif.
\item d. Acrystalsaliclyl acid, Pharmscience Inc, Montreal, QC, Canada.
\item e. Rimadyl, Pfizer Animal Health Canada, Kirkland, QC, Canada.
\item f. Deramaxx, Novartis Animal Health Canada Inc, Mississauga, ON, Canada.
\item g. Metacam, Boehringer Ingelheim Canada Ltd, Burlington, ON, Canada.
\item h. Adiva 120, Bayer Inc, Toronto, ON, Canada.
\item i. Amelung KC 4 delta analyzer, Trinity Biotech, Ireland.
\item j. Research pipette, Eppendorf, Mississauga, ON, Canada.
\item k. Chrono-log 440VS, Chrono-log Corp, Haverton, Pa.
\item l. Thromboxane B2 ELIA kit, Cayman Chemical Co, Ann Arbor, Mich.
\item m. 6-keto-PGF\textsubscript{1α} ELIA kit, Cayman Chemical Co, Ann Arbor, Mich.
\item n. SpectraMax miniplate reader, Biotek Instruments, Winooski, Vt.
\item o. SAS, version 9.1.3, SAS Institute Inc, Cary, NC.
\end{itemize}

\textbf{References}


