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Kathleen Brannon Mullins

EFFECTS OF ASPIRIN, MELOXICAM, DERACOXIB, AND CARPROFEN ON PLATELET FUNCTION IN DOGS

By

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Little research has been published evaluating the effect of non-steroidal anti-inflammatory drugs (NSAIDs) on platelet function in dogs. The effects of aspirin and cyclooxygenase-2 (COX-2) selective NSAIDS on platelet function were evaluated. Eight dogs received aspirin 10 mg/kg PO every 12 hours for 10 days, and then were randomly assigned to 4 groups, with each group receiving one of the following PO for 7 days in a crossover study design: carprofen 2.2 mg/kg every 12 hours, carprofen 4.4 mg/kg every 24 hours, meloxicam 0.2 mg/kg every 24 hours first day then 0.1 mg/kg every 24 hours for 6 days, or deracoxib 2 mg/kg every 24 hours. PFA-100® platelet function analysis with collagen/ADP and collagen/EPI cartridges, viscoelastometry, and urine 11-dehydro-thromboxane B_2 were evaluated. All drugs significantly prolonged PFA-100® closure times with the collagen/EPI cartridge, but not with collagen/ADP. Urine 11-dehydro-thromboxane B2 levels were significantly lower after aspirin but no other drugs.

DEDICATION

I would like to dedicate this project to my parents, Dan and Vickie Mullins, who not only told me I could accomplish anything, but believed it and helped me achieve it. Their constant love and support have formed me into the person I have become.

I would also like to dedicate this to my husband, Jason Schultz, who has supported me all day every day throughout my residency.

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CHAPTER I

INTRODUCTION

PLATELET FUNCTION AND HEMOSTASIS

The platelet was first discovered in 1842 by Donné using microscopy, but it wasn't until 1885 that Lubnitzky discovered platelets' primary function in the formation of hemostatic plugs.[1] The platelet was once thought to function only in hemostasis, but it is now known to be a mediator in processes including inflammation and atherosclerosis.[2] Platelets contribute to primary hemostasis in five ways: adhesion, aggregation, secretion, providing a procoagulant surface, and clot retraction.[3]

The hemostatic function of platelets could not occur without the cyclooxygenase enzyme (COX). COX is a catalyst in the production of prostanoids, including prostaglandins, prostacyclins, and thromboxanes. In platelets, COX oxidizes arachidonic acid to form prostaglandins (PGG_2 and PGH_2), which are then isomerized by thromboxane synthase to form thromboxane A_2 (TXA_2), the major arachidonic acid metabolite in platelets. TXA_2 is synthesized and released by platelets in response to activation by conventional agonists, including thrombin and ADP, resulting in enhanced aggregation and platelet recruitment. In addition, TXA_2 in and of itself is also a platelet activator. When TXA_2 synthesis is inhibited, platelet function is altered.

Two isoforms of COX have been described, COX-1 and COX-2. A newer form, COX-3, is considered to be a variant of COX-1.[4,5] COX-1 is constitutively expressed and is responsible for prostaglandin regulation in basal physiologic functions, such as normal platelet activity, gastric cytoprotection, and renal blood flow. It is present in many cells under basal conditions, including platelets, endothelial cells, mucosal cells of the gastrointestinal (GI) tract, and renal medullary collecting ducts and interstitium.[6] It also plays a major role in homeostatic activities such as gastric protection, platelet aggregation, and electrolyte balance in the kidney. COX-2 is induced by various cytokines and growth factors and is responsible for the production of prostaglandins involved in the inflammatory process. COX-2 is also present in some cells under basal conditions, such as macrophages, monocytes, smooth muscle cells, fibroblasts, dendritic cells, and chondrocytes; as well as some tissues, including kidney and brain.[7]

The induction of COX-1 in platelets occurs during injury, when damage to blood vessels results in exposure of the vessel subendothelium. This allows platelets to come into contact with collagen, von Willebrand factor, and tissue factor, resulting in platelet adherence and aggregation. This platelet activation initiates the arachidonic acid pathway, leading to the production of TXA_2 by COX, and causing the activation of additional platelets. The release of ADP, TXA_2, and other factors from these activated platelets results in the aggregation and adhesion of more platelets leading to platelet plug formation.[1]

NSAIDS AND CYCLOOXYGENASE

Aspirin (acetylsalicylic acid) is a non-selective non-steroidal anti-inflammatory drug (NSAID) that inhibits both COX-1 and COX-2. Through this COX inhibition, aspirin has an anti-thrombotic effect by inhibiting TXA_2 formation by platelets.[6] Because COX-1 plays a role in both normal platelet function and prostaglandin-mediated cytoprotection of the gastrointestinal mucosa, inhibition of COX-1 can lead to gastrointestinal ulceration and bleeding. In the past decade, much research has been undertaken to develop NSAIDS that are specifically COX-2 selective in an effort to maintain the analgesic and anti-inflammatory properties of the drug while minimizing systemic side-effects related to inhibition of COX-1.[8,9,10]

Theoretically, COX-2 selective NSAIDS should have no effect on platelet function, as platelets have been thought to be the only cells in the dog that exclusively express COX-1.[11,12] However, recent research has shown that so-called COX-2 selective NSAIDS are not truly selective for only COX-2, and that selectivity may decrease as the dose increases.[8,13] The selectivity of an NSAID is now based on drug concentrations at which enzyme activity is inhibited by 50% (inhibitory concentration [IC_{50}]) that are calculated and described as a ratio of COX-1:COX-2,[11] since none appear to be completely COX-2 selective.[9] NSAIDS with a ratio of >1 are more COX-2 selective and generally cause fewer side effects when used clinically.[12,14,15,16] Therefore, platelet function may, in fact, be altered to varying degrees by all NSAIDS, even those thought to be COX-2 selective. This would help explain the continued incidence of complications (i.e., anorexia, vomiting, nausea, and gastrointestinal ulceration) seen clinically in dogs treated with more selective NSAIDS. In dogs, carprofen, deraxocib, meloxicam, and etodolac have

been shown to inhibit COX-1 less severely than previous non-selective NSAIDS, as determined by the continued synthesis of gastric PGE_1 and PGE_2 and the continued production of thromboxane B_2 (TXB_2 – a stable metabolite of TXA_2) by platelets.[9,10,17,18] However, another study reported a decrease in TXB_2 concentrations in pyloric mucosa after treatment with carprofen.[19]

EFFECTS OF NSAIDS ON PLATELET FUNCTION AND HEMOSTASIS

The effects and complications of NSAIDS are so concerning because they are very commonly used in both human and veterinary medicine for treatment of acute and chronic pain and inflammation. Reported systemic adverse drug reactions associated with NSAIDS comprise the largest database within the United States Food and Drug Administration's Center for Veterinary Medicine files, and most of these reactions were reported with the use of COX-2 selective NSAIDS.[20] Although reports conclude that there are fewer side effects associated with COX-2 selective NSAIDS, as compared to non-selective NSAIDS, severe systemic side effects can still occur.[13,21]

Scientists have investigated the effects of COX-2 selective NSAIDS on bleeding in dogs, with conflicting results. Carprofen is one of the most commonly used COX-2 selective NSAIDS in veterinary medicine, and conflicting reports of its effect on bleeding have been described.[21,22,23,24,25,26] In a recent study significant decreases in platelet aggregation and clot formation were reported in dogs treated with carporfen.[27] Another study, evaluating the effects of carprofen in healthy Labrador retrievers, demonstrated a decrease in platelet aggregation and delayed onset of aggregation, with no differences detected in buccal mucosal bleeding time (BMBT) or hematocrit.[22] A report comparing

the effects of several COX-2 selective NSAIDS in dogs found that bleeding time was increased after 1 month and 3 months of caroprofen administration.[21] However, in another report, dogs receiving carprofen after suffering a fracture had no changes or abnormalities in bleeding time, platelet aggregation, hematocrit, or platelet count.[26]

Other COX-2 selective NSAIDS used in dogs have also been evaluated. Grisneaux reported that bleeding time was prolonged in dogs administered ketoprofen as compared to carprofen or saline (control) prior to orthopedic surgery.[23] Another study demonstrated that flunixin, ketoprofen, and meloxicam all increased clotting times, and bleeding time was increased after 7 days of treatment.[21] However, the perioperative administration of meloxicam or ketoprofen was found to have no affect on BMBT.[28,29.30,31]

Veterinary medicine is not the only field investigating NSAIDS. The effects of NSAIDS on platelet function, and particularly the potential for intraoperative bleeding, is a concern in human medicine as well. NSAIDS are used with caution in people undergoing tonsillectomy due to the potential for intraoperative blood loss, postoperative bleeding, and reoperation because of bleeding.[32] The administration of a non-selective NSAID 2 weeks prior to total hip replacement in humans has resulted in significantly increased peri-operative blood loss.[33] A study of human patients on various COX inhibitors and undergoing total knee replacement surgery positively correlated PFA-100® EPI closure time with both postoperative drain output for the first 24 hours and the surgeon's rating of the difficulty of achieving hemostasis intraoperatively.[34] The PFA-100® is a commercial machine used to evaluate platelet function via exposure of platelets to agonists, mimicking platelet activation in response to contact with vessel subendothelium after trauma.

Potential complications of NSAID usage

Alterations in platelet function not only affect intraoperative bleeding, they also influence other side effects of NSAIDS, including changes in gastric mucosal protection, potentially resulting in bleeding gastrointestinal ulcers. Gastric ulcers have been documented after the administration of NSAIDS, and the incidence is increased if they are used in conjunction with steroids.[19,21,35,36] Just as with studies regarding NSAIDS effect on bleeding, results of their effects on the gastrointestinal system are conflicting. Aspirin has been found to decrease COX-1 and COX-2 concentrations in both pyloric and duodenal mucosa, which is to be expected as it is non-selective. However, in the same study, carprofen, a COX-2 selective NSAID, was also found to decrease total prostaglandin and TXB_2 concentrations in the pyloric mucosa as compared to deracoxib.[19] The administration of deracoxib or carprofen to healthy dogs for 5 days resulted in no significant gastroduodenal ulcers as evaluated endoscopically.[18] However, in another study, carprofen, etodolac, ketoprofen, meloxicam, and flunixin were administered to dogs for 90 days, and gastric lesions were recongnized endoscopically in all dogs .[21] In this study, carprofen administration was shown to cause the fewest gastrointestinal effects, followed by meloxicam, etodolac, flunixin, and ketoprofen.

The effects of long-term administration of COX-2 selective NSAIDS warrant continued investigation, as more drugs are being approved for chronic treatment of osteoarthritis. Previous studies have evaluated NSAIDS effects on the gastric mucosa, but also in the synovial fluid. Administration of firocoxib and meloxicam to osteoarthritic dogs has been shown to decrease concentrations of PGE_2 in synovial fluid in a similar

manner in which they decrease plasma PGE_2 concentrations,[17] confirming their benefit in treating joint inflammation and arthritis. A study of the effects of administering carprofen and deracoxib to dogs with chronic osteoarthritis reported simliar results, including decreased PGE_2 concentrations in blood and synovial fluid and no suppression of TXB_2 blood concentrations or gastric PGE_1 synthesis. These findings confirm the COX-2 selectivity of these drugs.[10]

Aspirin resistance

Aspirin is the most extensively used and researched antiplatelet drug in human medicine due to its benefit in cardiovascular disease. The antiplatelet function of aspirin is via irreversible acetylation of Ser-529 in COX-1 and Ser-516 in COX-2, resulting in blockage of TXA_2 synthesis. "Aspirin resistance" is a term used in human medicine to refer to patients who have thrombotic events despite aspirin treatment. The term is also applicable if a prolongation in bleeding time is detected or if the expected inhibition of platelet activation or TXA_2 synthesis is not achieved.[2,37] Research has confirmed that humans have a variable response to aspirin therapy.[38,39] This variable response is thought to result from differences in prostaglandin production whereby COX-2 restores the ability of platelets to produce TXA_2 in some patients,[38,40,41] although additional theories exist.

Aspirin resistance lacks uniformity, and previous studies estimate that 8-45% of the human population are aspirin resistant.[42,43,44,45] It was also determined that the optimal dosage of aspirin for complete inhibition of platelet aggregation by physiological agonists (i.e. arachidonic acid) is subject to great interindividual variability, the preferred tests for detecting aspirin resistance *in vitro* are subject to debate, and the mechanisms by which

some patients are resistant to aspirin remain to be determined. Clinical vs. biochemical aspirin resistance in humans was investigated by Kour, et al[46] and suspected mechanisms responsible for resistance include reduced accessibility of aspirin to receptor sites due to concomitant intake of other NSAIDS; genetic polymorphism of enzymes like COX-1, COX-2, or thromboxane A_2 synthase; increased reactivity of platelets towards other aggregating factors; increased rate of entry of new platelets into the circulation; alternate pathways of thromboxane synthesis; and poor patient compliance. Aspirin resistance has also been associated with reticulated platelets,[39] which are young platelets that contain mRNA. In addition, it has been discovered that variation in response to aspirin may be associated with time in humans, as Dalby, et al[47] found a diurnal increase in closure time of 30% from 8:30am to 5pm with both collagen/epinephrine (Epi) and collagen/ADP PFA-100 cartridges. However, a more recent study[48] found a less variable response to aspirin. In this study, repeated optical arachidonic acid-induced platelet aggregometry was used on 289 patients with stable congestive heart diseases and all the subjects demonstrated ability to respond normally to aspirin in low doses. The authors of this study concluded that pharmacodynamic aspirin resistance is rare.

Very little research in veterinary medicine has been performed regarding aspirin resistance. One published report on the effects of aspirin on platelet function in dogs demonstrated a variable response within and amongst breeds.[49] However, whether dogs, like humans, may be "responders", "semiresponders", or "nonresponders" to aspirin therapy remains in question.

CHAPTER II

EVALUATION OF THE EFFECTS OF COX-2 SELECTIVE NSAIDS ON PLATELET FUNCTION

INTRODUCTION

Nonsteroidal anti-inflammatory drugs are commonly administered to dogs for the control of pain and inflammation associated with osteoarthritis and other chronic and acute inflammatory conditions. In addition, nonsteroidal anti-inflammatory drugs (NSAIDs) are frequently used peri-operatively to reduce pain and inflammation and minimize post-operative opioid requirements, and thus speed recoveries and shorten hospital stays. The anti-inflammatory and analgesic effects of all NSAIDs result from the inhibition of COX, an enzyme that converts arachidonic acid to a number of different prostanoids including the prostaglandins, the prostacyclins, and thromboxane. There are two well described isoforms of this enzyme, COX-1 and COX-2, although more recently a third possible isoform or splice variant, COX-3, has been described. COX-1 is constitutively expressed and is responsible for prostaglandin regulation in basal physiologic functions, including normal platelet activity, gastric cytoprotection, and renal blood flow.[13,50] COX-2 is traditionally considered to be inducible and responsible for producing the prostaglandins that are involved in the inflammatory process. [13,50]

Aspirin (acetylsalicylic acid) is a non-selective NSAID that irreversibly binds to and affects the function of both COX-1 and COX-2, and impairs platelet function by inhibiting thromboxane A_2 (TXA_2) formation by platelets.[6] TXA_2 is synthesized and released by activated platelets resulting in vasoconstriction, further platelet activation, and enhanced platelet aggregation. Inhibition of COX-1 mediated TXA_2 synthesis leads to decreased platelet function. Inhibition of COX-1 also reduces prostaglandin-mediated cytoprotection of the gastrointestinal mucosa, potentially leading to gastrointestinal ulceration and bleeding. In the past decade, much research has been devoted to the development of COX-2 selective NSAIDs that maintain analgesic and anti-inflammatory effects while minimizing unwanted side-effects thought to result primarily from COX-1 inhibition such as gastrointestinal ulceration, renal damage and susceptibility to bleeding.[8,9,10]

Platelets, both in the dog and in man, have historically been described as cells that exclusively express COX-1. Therefore, it was assumed that the newer COX-2 selective NSAIDs should have no direct effect on platelet function.[11,12] Recent research, however, has shown that many 'COX-2 selective NSAIDs' are not purely selective for COX-2, and that selectivity may decrease as drug dose increases.[8,13] All NSAIDs, even those marketed as COX-2 selective, may therefore have the potential to inhibit COX-1 and thereby decrease platelet function.

Administration of NSAIDs before surgery has the beneficial effects of reducing inflammation at the surgical site, minimizing resultant peripheral and central amplification of nociceptive input, and decreasing pain after surgery.[29] Undesirable NSAID-associated decreases in platelet function, however, have the potential to

contribute to excessive peri-operative hemorrhage, leading to longer surgical and anesthetic times and an increased risk of intra- and post-operative complications. In theory, compared to non-selective NSAIDs, the more COX-2 selective NSAIDs would be expected to have far less effect on platelet function and thus should not lead to unwanted peri-operative hemorrhage. The effects of the newer, more selective NSAIDs on canine primary hemostasis, however, have not as yet been comprehensively evaluated using appropriate methodologies.

The goal of our study was to characterize changes in platelet function associated with the administration of standard anti-inflammatory doses of aspirin and other veterinary-approved NSAIDs of varying COX-2 selectivity in dogs. Our hypotheses were that aspirin at standard anti-inflammatory doses would predictably interfere with platelet function, and that the other more COX-2 selective NSAIDs, while less potent in their effects on platelets, would still cause detectable alterations in platelet function.

MATERIALS AND METHODS

Eight female intact purpose-bred adult Walker Hound dogs were used for the study. Normal health status was established based on physical examination and baseline testing including a complete blood count, serum chemistry, urinalysis, BMBT, prothrombin time, partial thromboplastin time, von Willebrand factor levels, heartworm testing and titers for rickettsial diseases and *Babesia* species. No vaccines or medications were administered to the dogs for at least one month prior to initiation of the project.

Baseline samples were obtained for platelet function analysis using a point-of-care platelet function analyzer[a] (Siemens PFA-100®), viscoelastometry[b] (Sienco

SonoClot®), and measurement of urine 11-dTXB$_2$[c] (Luminex® 11-dehydro Thromboxane B2 Kit). Venous blood was collected from the jugular veins via a 20-gauge vacutainer needle directly into glass tubes containing either 3.8% sodium citrate or ethylenediaminetetraacetic acid (EDTA). Venipuncture was performed by alternating between both jugular veins. An automated hematologic analyzer[d] (Abbott Cell-Dyn® 3700) was used to determine an accurate platelet count and packed cell volume (PCV) on each sample. Urine was collected via cystocentesis using a 22 gauge needle. All urine samples were batched and stored at -80°C until analysis.

Platelet Function Analysis (PFA-100®)

The PFA-100® device was used according to the manufacturer's instructions, and has previously been described and evaluated in dogs.[51,52] The PFA-100® is a bench-top analyzer that measures the time needed to form a platelet plug in an environment designed to mimic the naturally-occurring situation in blood vessels. The PFA-100® moves whole blood at high shear forces across a membrane aperture during exposure to platelet agonists, thereby mimicking damaged subendothelium in a blood vessel. The PFA-100® evaluates primary hemostasis as a whole by recording closure time (CT), the time needed to close the aperture by forming a platelet plug after platelet activation. Sodium citrate anticoagulated blood at a 9:1 blood-to-citrate ratio was used. Samples were analyzed within 30 minutes of collection, and kept at room temperature until analysis. For each sample, 800 µl of citrated blood was deposited in a test cartridge, which contained a biologically active membrane coated with either collagen and adenosine diphosphate (collagen/ADP cartridge)[e] or collagen and epinephrine

(collagen/EPI cartridge)[f]. After a 3-minute incubation period at 37°C, the blood sample was aspirated through an aperture in contact with the coated membrane, and the time until occlusion of the aperture (closure time) was recorded.

Viscoelastometry (SonoClot®)

The SonoClot® device was used according to the manufacturer's instructions. The SonoClot® uses a viscoelastometric system to detect mechanical changes that occur within a whole blood sample as it clots, and provides information on many aspects of the hemostatic process, including coagulation, fibrin gel formation, and clot retraction. The SonoClot® contains a tubular probe that oscillates up and down within a blood sample, with detection circuitry that senses the resistance to motion that the probe encounters as the sample clots and generates an analog electronic signal which is processed by a microcomputer and reported as a clot signal or, graphically, as a SonoClot® signature. Clotting was triggered using the SonACT® Kit[g], which is a celite-activated whole blood clotting time test designed for use with the SonoClot®. Results calculated by the SonoClot® and the Signature Viewer Program[h] from the resultant clot signal included the activated clotting time (ACT; or the time until onset of the first detectable clot) in seconds, the clot rate (CR; or the slope of the SonoClot® signature during initial fibrin gel formation) in clot signal units per minute, the peak amplitude of the SonoClot® signature (AMP) in clot signal units, and the time to peak amplitude (TP) in minutes. The SonoClot® Signature Viewer Program utilizes AMP and TP to calculate 'platelet function' (PF), an index which is claimed by the manufacturer to reflect the role of platelets in clot formation. Sodium citrate anticoagulated blood at a 9:1 blood-to-citrate

ratio was used. Samples were analyzed within 30 minutes of collection, and kept at room temperature until analysis. Each sample was tested in duplicate.

Urine 11-Dehydro-Thromboxane B_2

A commercial competitive enzyme immunoassay kit validated for analysis of canine urine for 11-dTXB$_2$ was used to measure thromboxane (Cayman Chemical Company). Samples were analyzed according to the manufacturer's instructions. Urine samples were serially diluted with the manufacturer's assay buffer based on the original specific gravity in order to have the specific gravity of the sample within the working range of the instrument, which was isosthenuric. All samples were analyzed in duplicate, and results were reported in pictograms per milliliter of urine (a correction factor was applied to allow for the effects of sample dilution). Briefly, a 96-well Luminex® plate was prepared by adding 100 µl of the diluted sample to the appropriate well, followed by the addition of 11-dTBX$_2$ Phycoerythrin Tracer and 11-dTBX$_2$ XMAPR® Beads. The plate was placed on an orbital shaker and incubated in the dark at room temperature for four hours prior to analysis. Urine 11-dTBX$_2$ concentration was normalized to the individual's urine creatinine[i] by determining the 11-dTBX$_2$ to creatinine ratio using the diluted urine.

Buccal Mucosal Bleeding Time

A commercially available device (Triplett®[j] or HemoHeel®[k]) was used to perform a BMBT on the everted lip of all dogs on the last day of drug administration for all drugs according to a previously described technique.[53] The two devices function similarly,

creating a standardized 1 mm deep incision; the incision length made with the Triplett® is 5-6 mm and with the HemoHeel® is 2.5 mm. Filter paper was used to blot 1-2 mm from the incision until cessation of bleeding.

Study Design

This study was designed in accordance with the Policy on the Humane Care and Use of Laboratory Animals and was approved by the Mississippi State University Institutional Animal Care and Use Committee. All dogs were administered aspirin[l] at 10 mg/kg PO every 12 hours for 10 days in order to establish the effects of a standard non-selective NSAID on hemostatic test results. The dogs were then randomly assigned to one of four groups (2 dogs/group) and treated for 7 days with one of the following regimens with each group rotating through all drug regimens in a crossover design: carprofen[m] 2.2 mg/kg PO every 12 hours, carprofen[m] 4.4 mg/kg PO every 24 hours, meloxicam[n] 0.2 mg/kg PO the first day then 0.1 mg/kg PO every 24 hours, or deracoxib[o] 2 mg/kg PO every 24 hours. Drug doses were based on manufacturer's recommendations. A 14 day washout period was established between each drug. During drug administration, blood was obtained for PFA-100® analysis using collagen/EPI cartridges on days 1, 2, 3, 5, and 7. On day 7, blood was collected for PCV, platelet count, viscoelastometry, and PFA-100® analysis using collagen/ADP cartridges. During the washout period, PFA-100® analysis using collagen/EPI cartridges was performed every other day until measurements within 10% of each individual dog's normal baseline were obtained on two consecutive occasions. On Day 14 of the washout period, blood samples were obtained for PCV, platelet count, PFA-100® analysis using collagen/ADP cartridges and viscoelastometry.

Urine was collected for 11-dTXB$_2$ analysis on Day 7 of administration of each drug. BMBT was evaluated at baseline and on the last day of drug administration for each drug, including aspirin.

Statistical Analysis

Results of PFA-100® analysis, urine 11-dTBX$_2$, and viscoelastometry in dogs treated by aspirin were individually assessed by analysis of variance (ANOVA) using the GLIMMIX procedure in SAS for Windows version 9.2^p followed by separation of means using least significant difference. Sample time was included in the model as a fixed effect. The identity of the dogs was used in RANDOM and RANDOM_RESIDUAL statements to account for the repeated measures of dogs over time. For the PFA-100® analysis using collagen/EPI cartridge outcomes, which were measured at five time points, a first order autoregressive covariance structure was used. An unstructured covariance structure was used for PFA-100® analysis using collagen/ADP cartridges, urine 11-dTXB$_2$, and viscoelastometry (ACT, CR, PF) outcomes, which each had only two sample times.

The effects of carprofen (both dosing regimens), deracoxib, and meloxicam on PFA-100®, viscoelastometry, platelet count, and urine 11-dTXB$_2$ results were assessed by analysis of variance using the GLIMMIX procedure in SAS for Windows version 9.2 followed by separation of means using least significant difference. Drug, rotation, sample time, and the drug*sample time interaction were included in the models as fixed effects. In addition, dummy variables were created to account for the drug a dog had been treated with in the prior rotation. The identity of dog within rotation was used in

RANDOM and RANDOM_RESIDUAL statements to account for the repeated measures of dogs over time. For the PFA-100® analysis using collagen/EPI cartridges outcomes, which were measured at six time points, a first order autoregressive covariance structure was used. An unstructured covariance structure was used for the other dependent variables, which each had only two sample times. All analyses tested differences in drug, sample, time, rotation, and drug*sample time interaction.

A P value of less than 0.05 was considered to be significant for all analyses.

RESULTS

Eight dogs (intact females ranging in age from 1-6 years [median, 5.21 years]) were included in the study. All dogs were Walker Coonhounds. Weights ranged from 20.5 to 24.2 kg (median, 22.3 kg). All dogs completed the study, with one dog receiving oral sucralfate[q] 1 g PO q8h for 5 days due to hematochezia observed during the washout period after receiving meloxicam. No other adverse effects were observed.

After accounting for the dogs' individual weights, the mean administered dosages of drugs were aspirin, 10.62 ± a standard deviation of 0.610 mg/kg, PO, q 12 h; carprofen, 2.18 ± 0.124 mg/kg, PO, q 12 h; carprofen, 4.36 ± 0.247 mg/kg, PO, q 24 h; meloxicam, 0.19 ± 0.013 mg/kg, PO, first dose, then 0.097 ± 0.006 mg/kg, PO q 24 h; and deracoxib, 2.18 ± 0.122 mg/kg, PO, q 24 h.

Platelet Function Analysis (PFA-100®)

Pretreatment (baseline) closure times using collagen/EPI cartridges were not significantly different among groups for any drug. Collagen/EPI cartridge CTs were significantly

longer (P = <0.0001) than baseline values at all time points during treatment with aspirin, but there was no significant difference in closure time among any time points after baseline (Figure 1). Collagen/EPI cartridge CTs were significantly longer than baseline values (P = <0.0001) at all time points during treatment with carprofen 2.2 mg/kg twice daily (Figure 2), carprofen 4.4 mg/kg once daily (Figure 3), meloxicam (Figure 4), and deracoxib (Figure 5), but there was no significant difference in closure time among any time points after baseline, and there was no significant difference in closure time between the four drug regimens. When individual dog responses to various NSAIDs are evaluated, however, only aspirin caused consistently prolonged CTs in all dogs at all time points during drug administration (Figure 6). In contrast, 6 of 8 dogs returned to baseline CTs (defined as within 10% of their baseline value) at least once (range 1-3 times) while receiving carprofen 2.2mg/kg twice daily (Figure 7); 5 of 8 returned to baseline CTs twice while receiving carprofen 4.4mg/kg once daily (Figure 8); 3 of 8 returned to baseline CTs at least once (range 1-3 times) while receiving meloxicam (Figure 9); and 6 of 8 returned to baseline CTs at least once (range 1-4) times while receiving deracoxib (Figure 10). During the washout period, the average number of days until return to normal CTs using the collagen/EPI cartridges was 5.6 days for aspirin, 11.6 days for carprofen at 2.2mg/kg twice daily, 10.6 days for carprofen at 4.4mg/kg once daily, 11 days for meloxicam, and 10.6 days for deracoxib.

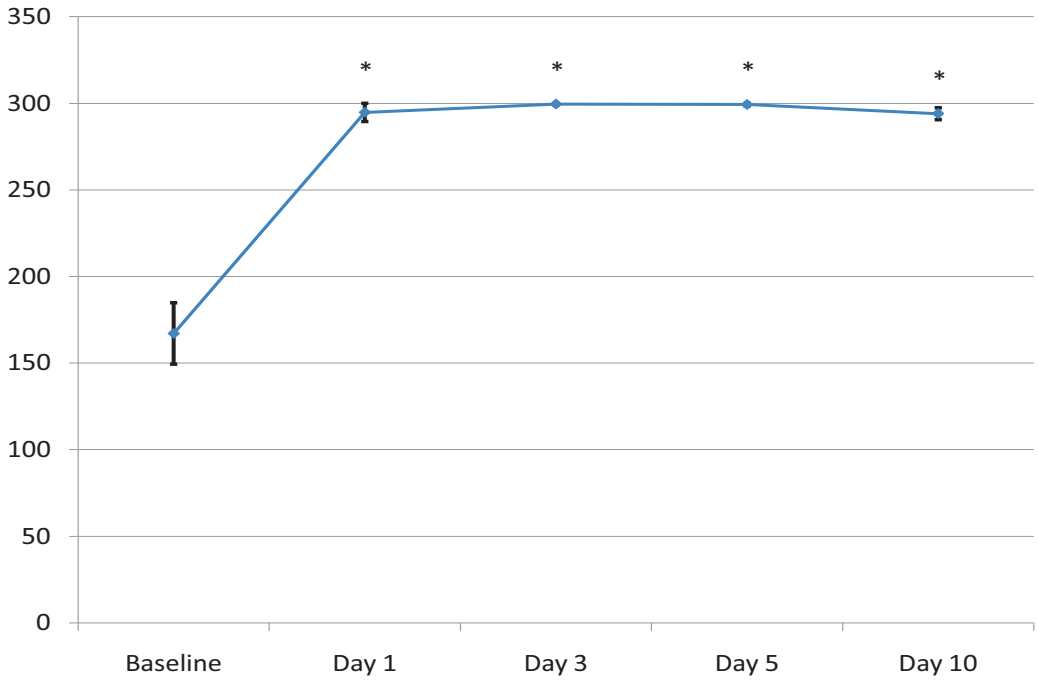

Figure 1 – Mean PFA-100 closure time with collagen/epinephrine cartridge over time for 8 dogs after treatment with aspirin

Mean PFA-100 closure time with collagen/epinephrine cartridge (+/- S. E) over time for 8 dogs after treatment with aspirin (10 mg/kg PO every 12 hours for 10 days).
* significantly increased over baseline value ($p>0.05$).

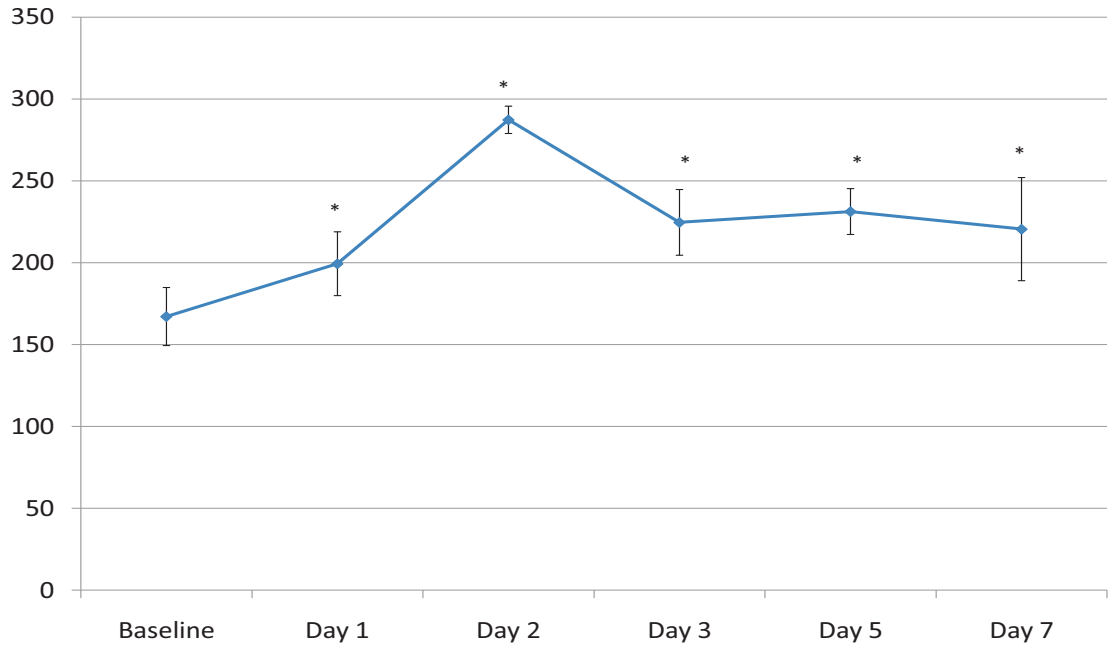

Figure 2 – Mean PFA-100 closure time with collagen/epinephrine cartridge over time of 8 healthy dogs after treatment with caprofen 2.2 mg/kg twice daily

Mean PFA-100 closure time with collagen/epinephrine cartridge (+/- S. E) over time of 8 healthy dogs after treatment with carprofen (2.2 mg/kg PO every 12 hours for 7 days).
* significantly increased over baseline value ($p>0.05$).

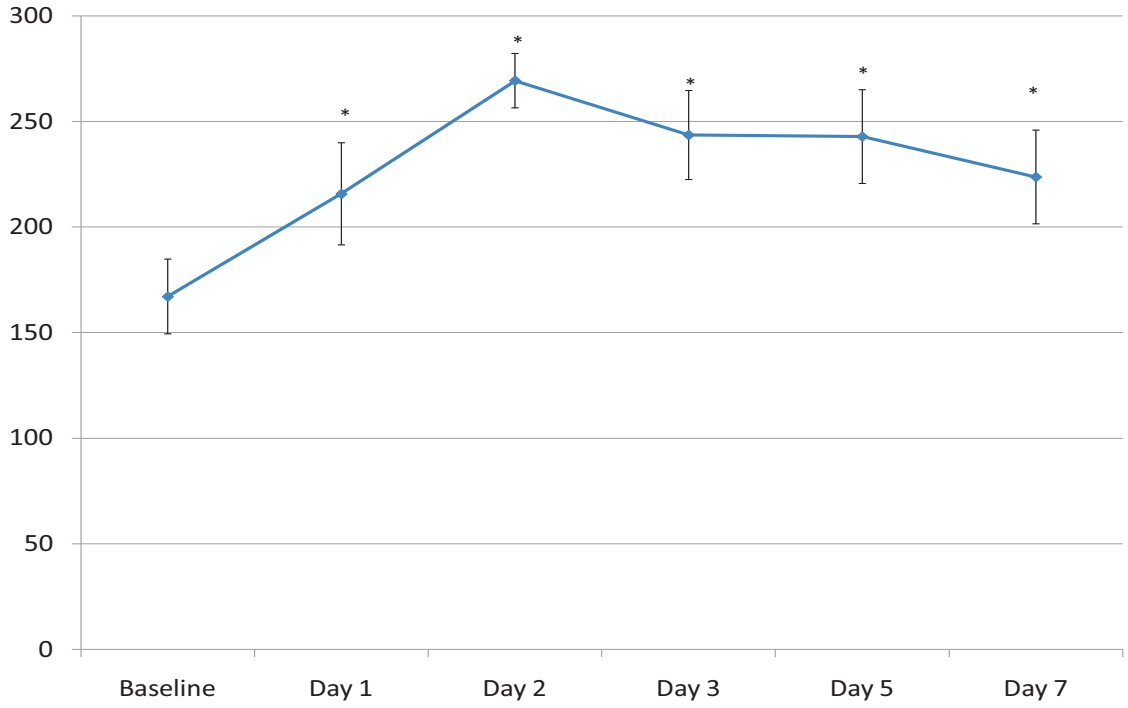

Figure 3 – Mean PFA-100 closure time with collagen/epinephrine cartridge over time of 8 healthy dogs after treatment with caprofen 4.4 mg/kg once daily

Mean PFA-100 closure time with collagen/epinephrine cartridge (+/- S. E) over time of 8 healthy dogs after treatment with carprofen (4.4 mg/kg PO every 24 hours for 7 days).
* significantly increased over baseline value ($p>0.05$)

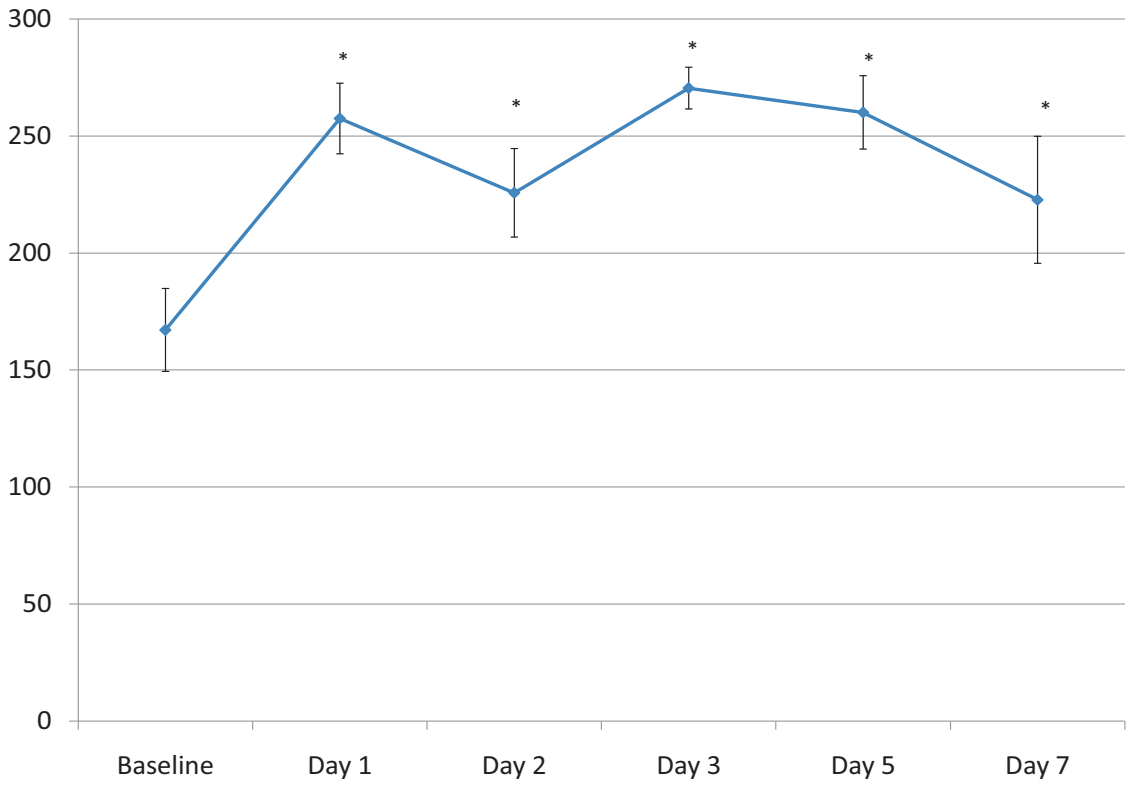

Figure 4 – Mean PFA-100 closure time with collagen/epinephrine cartridge over time of 8 healthy dogs after treatment with meloxicam

Mean PFA-100 closure time with collagen/epinephrine cartridge (+/- S. E) over time of 8 healthy dogs after treatment with meloxicam (0.2 mg/kg first day then 0.1 mg/kg PO every 24 hours for 6 days).
* significantly increased over baseline value ($p>0.05$).

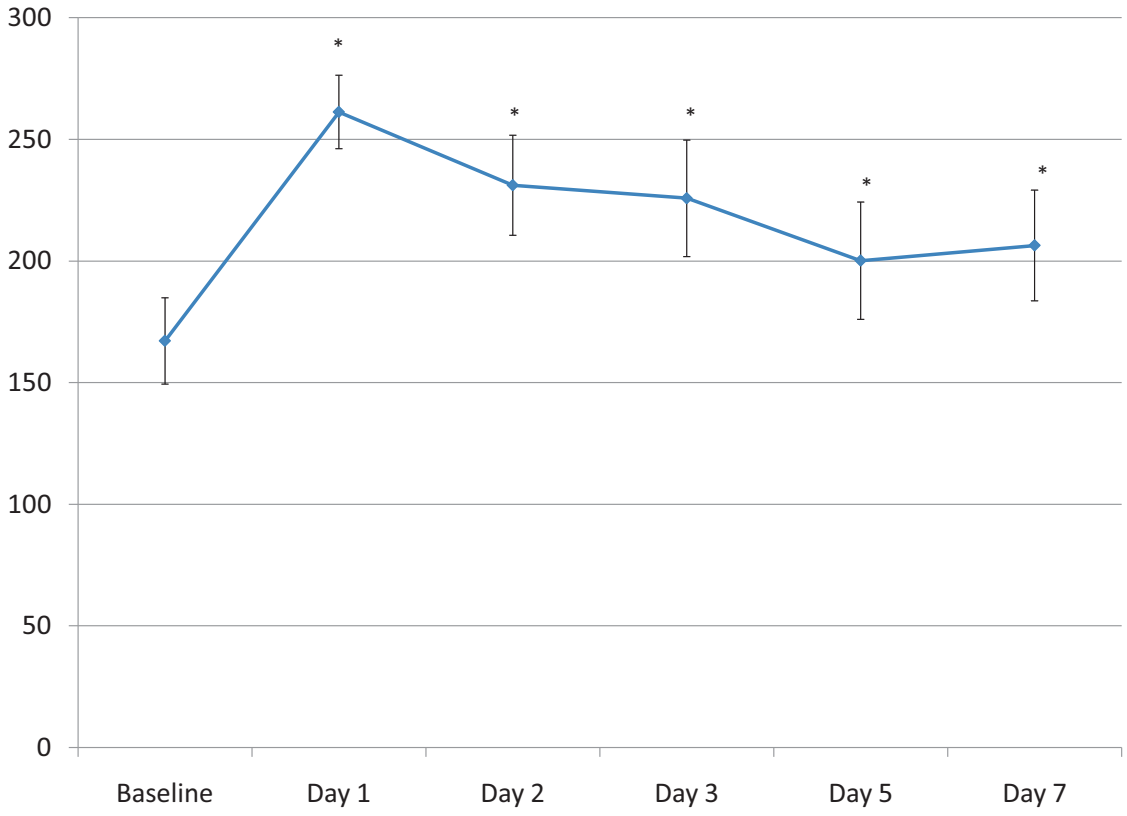

Figure 5 – Mean PFA-100 closure time with collagen/epinephrine cartridge over time of 8 healthy dogs after treatment with deracoxib

Mean PFA-100 closure time with collagen/epinephrine cartridge (+/- S. E) over time of 8 healthy dogs after treatment with deracoxib (2 mg/kg PO every 24 hours).
* significantly increased over baseline value ($p>0.05$).

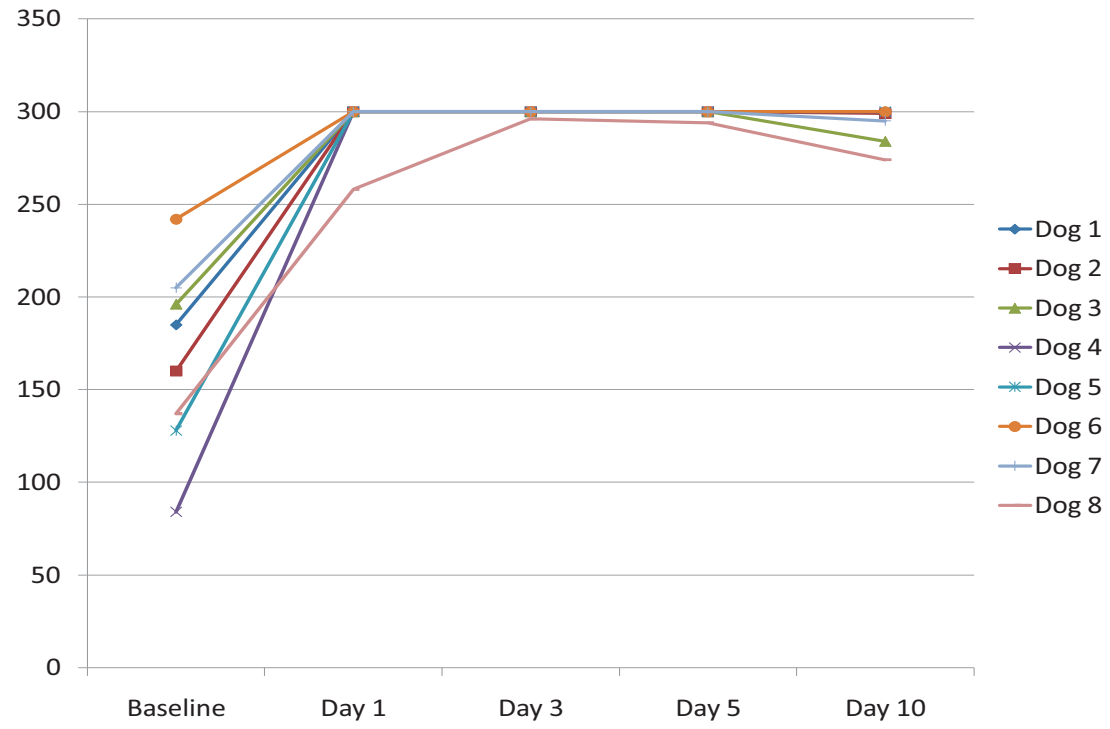

Figure 6 – Results of analysis with PFA-100 with collagen/epinephrine cartridge of 8 healthy dogs after treatment with aspirin.

Results of analysis with PFA-100 with collagen/epinephrine cartridge of 8 healthy dogs after treatment with aspirin (10 mg/kg PO every 12 hours).

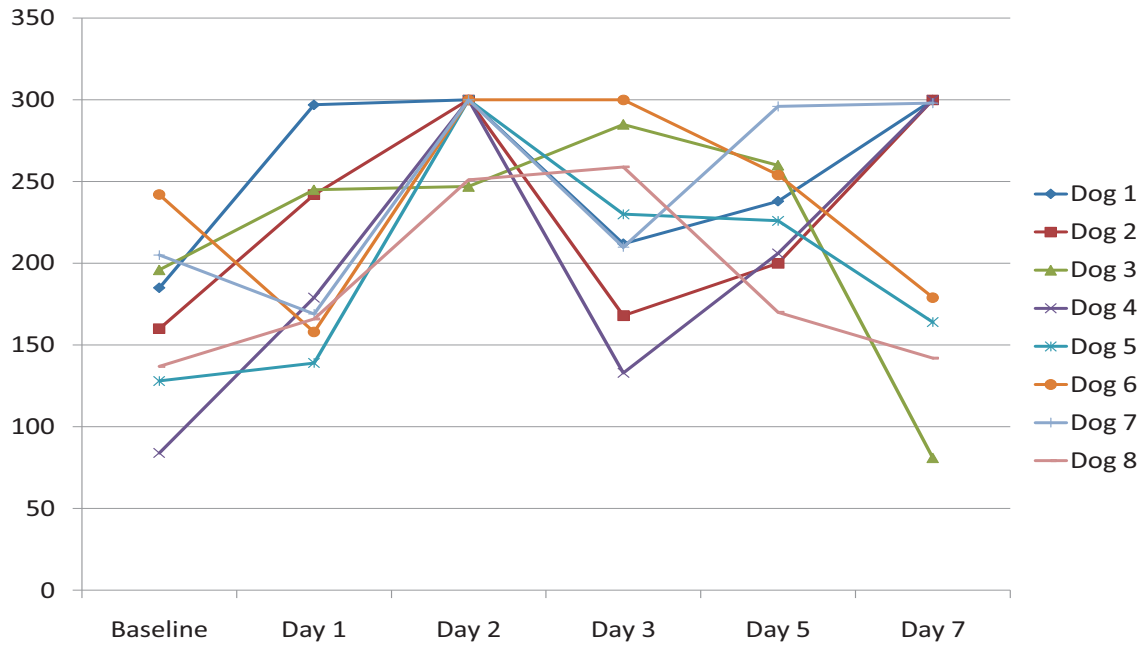

Figure 7 – Results of analysis with PFA-100 with collagen/epinephrine cartridge of 8 healthy dogs after treatment with carprofen 2.2 mg/kg twice daily.

Results of analysis with PFA-100 with collagen/epinephrine cartridge of 8 healthy dogs after treatment with carprofen (2.2 mg/kg PO every 12 hours for 7 days).

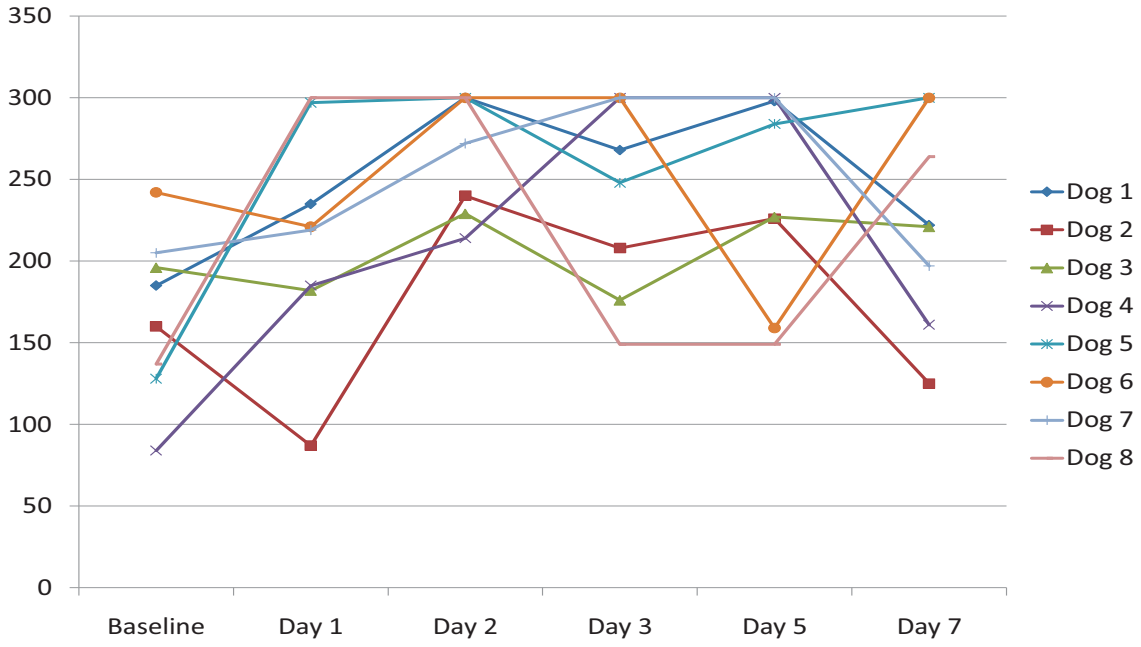

Figure 8 – Results of analysis with PFA-100 with collagen/epinephrine cartridge of 8 healthy dogs after treatment with carprofen 4.4 mg/kg once daily.

Results of analysis with PFA-100 with collagen/epinephrine cartridge of 8 healthy dogs after treatment with carprofen (4.4 mg/kg PO every 24 hours for 7 days).

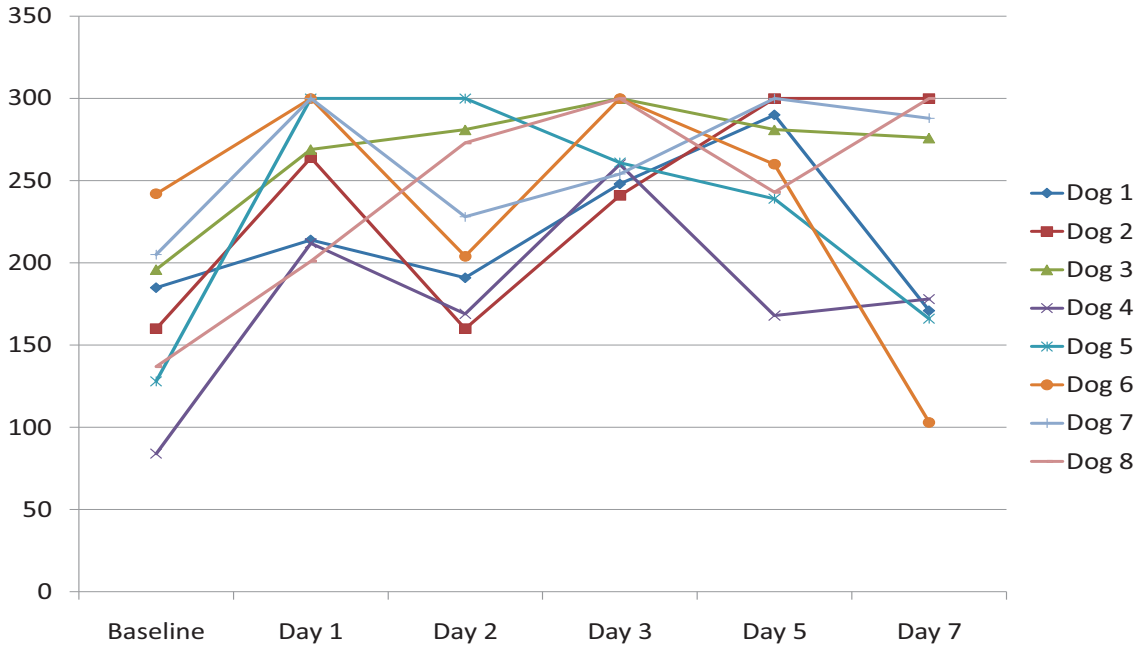

Figure 9 – Results of analysis with PFA-100 with collagen/epinephrine cartridge of 8 healthy dogs after treatment with meloxicam.

Results of analysis with PFA-100 with collagen/epinephrine cartridge of 8 healthy dogs after treatment with meloxicam (0.2 mg/kg first day then 0.1 mg/kg PO every 24 hours for 6 days).

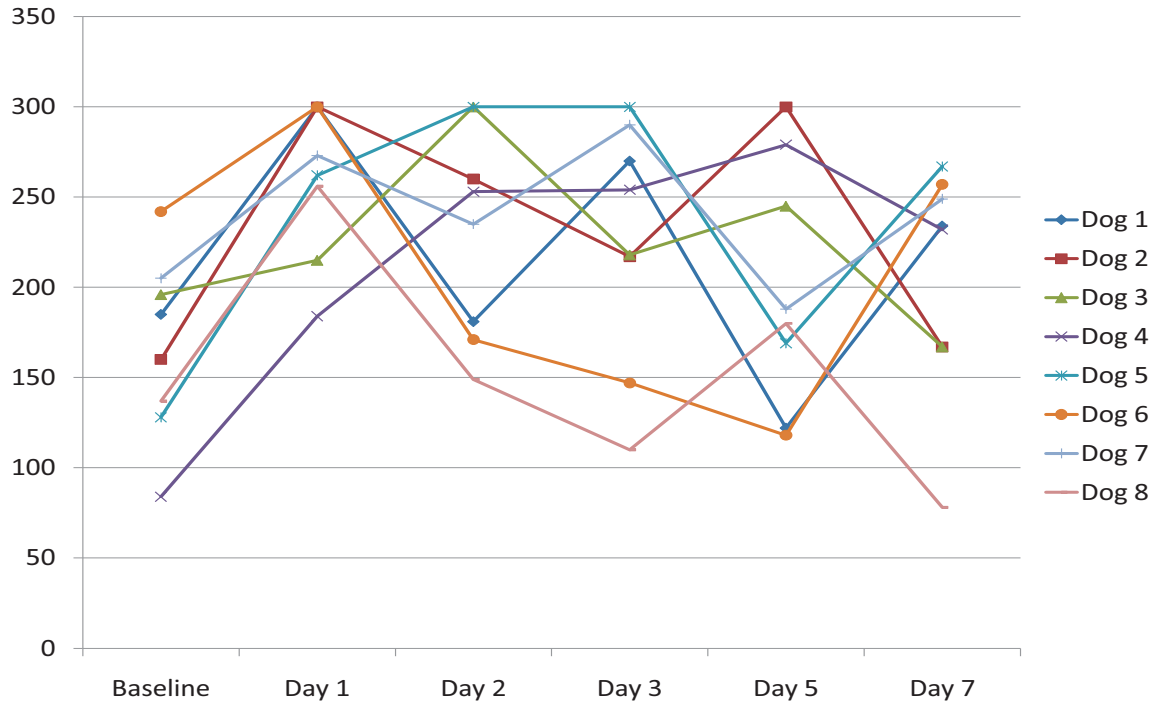

Figure 10 – Results of analysis with PFA-100 with collagen/epinephrine cartridge of 8 healthy dogs after treatment with deracoxib

Results of analysis with PFA-100 with collagen/epinephrine cartridge of 8 healthy dogs after treatment with deracoxib (2 mg/kg PO every 24 hours).

For collagen/ADP cartridge CTs, there was no significant difference seen with aspirin administration between baseline and Day 10 results, nor for the more COX-2 selective NSAIDs evaluated was there any significant difference noted between any drug or between baseline and Day 7 for any drug (Figure 11).

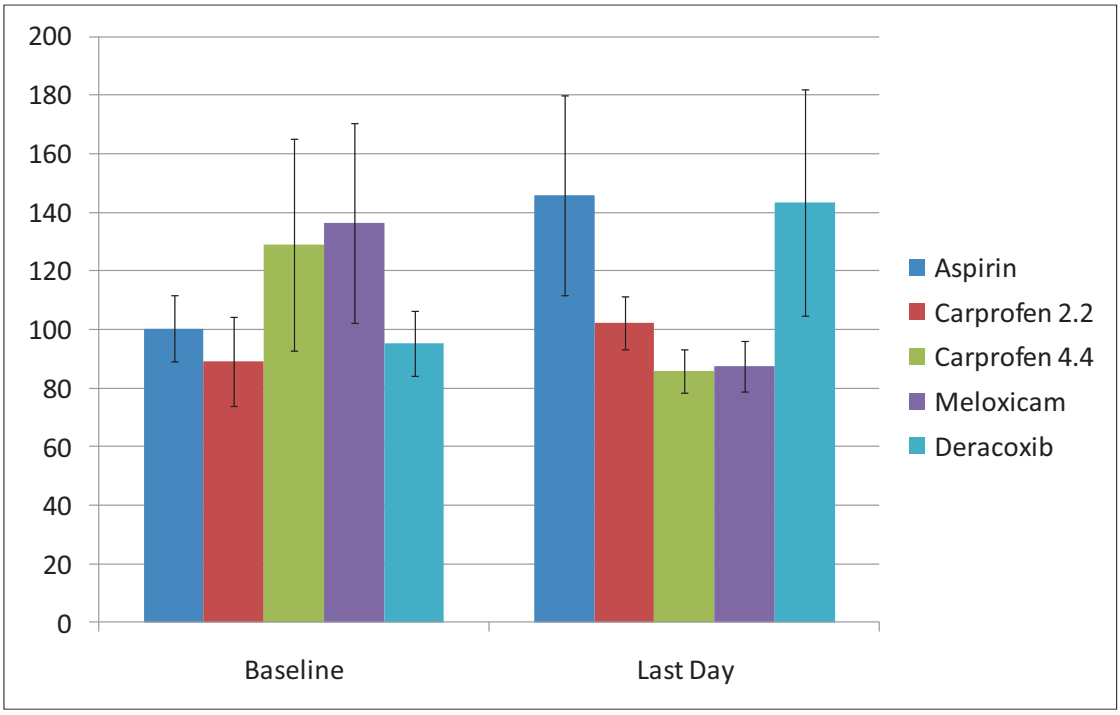

Figure 11 – Mean PFA-100 closure time with collagen/ADP cartridge over time of 8 healthy dogs after treatment with aspirin, carprofen, meloxicam, and deracoxib

Mean PFA-100 closure time with collagen/ADP cartridge (+/- S. E) over time of 8 healthy dogs after treatment with aspirin, carprofen, meloxicam, and deracoxib (aspirin 10 mg/kg PO every 12 hours for 10 days, carprofen 2.2 mg/kg PO every 12 hours for 7 days, carprofen 4.4 mg/kg PO every 24 hours for 7 days, meloxicam 0.2 mg/kg first day then 0.1 mg/kg PO every 24 hours for 6 days, and deracoxib 2 mg/kg PO every 24 hours for 7 days). No significant difference found.

Viscoelastometry (SonoClot®)

Viscoelastometry was performed at baseline and on the last day of drug administration for all drugs. A significant difference in ACT results was seen with drug but not time: carprofen 4.4mg/kg once daily had a longer ACT than carprofen 2.2mg/kg twice daily (P = 0.013), meloxicam (P = 0.046), and deracoxib (P = 0.019) (Figure 12). A significant difference in CR results was seen with drug but not time: carprofen

4.4mg/kg once (P = 0.027) and 2.2mg/kg twice daily (0.004) had a faster CR than did deracoxib (Figure 13). No significant differences in PF were seen between drugs or over time.

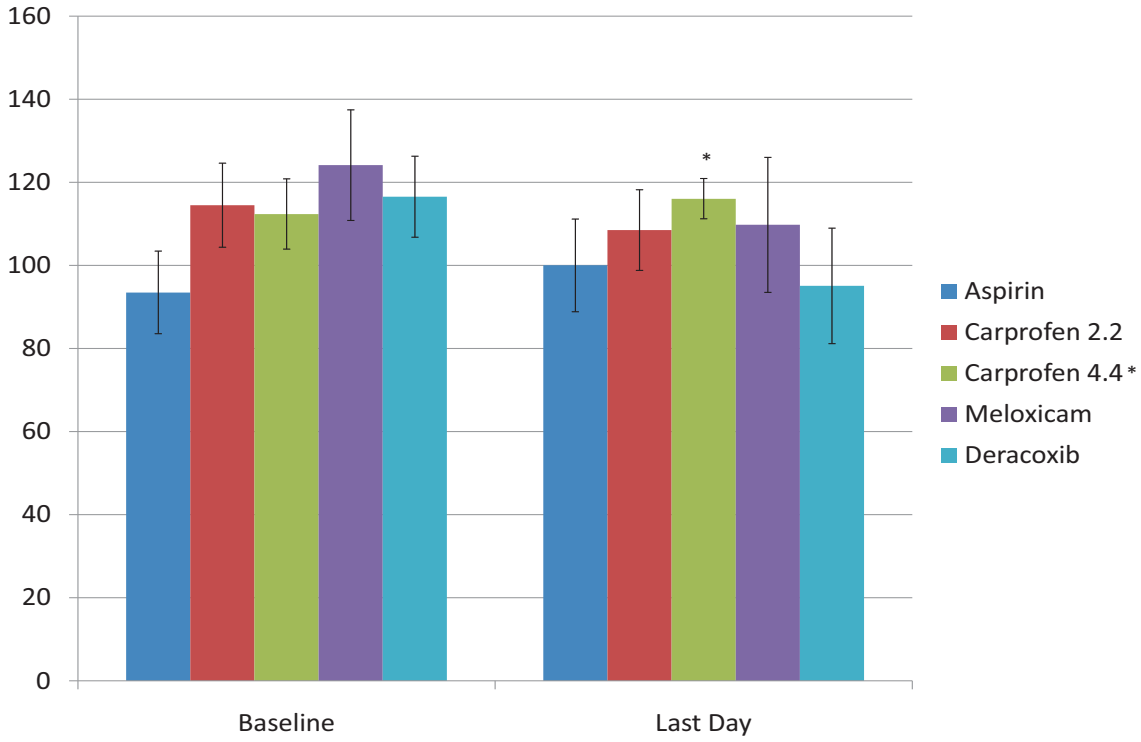

Figure 12 – Mean activated clotting time (ACT) with viscoelastometry over time of 8 healthy dogs after treatment with aspirin, carprofen, meloxicam, and deracoxib

Mean activated clotting time (ACT) with viscoelastometry (+/- S. E) over time of 8 healthy dogs after treatment with aspirin, carprofen, meloxicam, and deracoxib (aspirin 10 mg/kg PO every 12 hours for 10 days, carprofen 2.2 mg/kg PO every 12 hours for 7 days, carprofen 4.4 mg/kg PO every 24 hours for 7 days, meloxicam 0.2 mg/kg first day then 0.1 mg/kg PO every 24 hours for 6 days, and deracoxib 2 mg/kg PO every 24 hours for 7 days). * significantly increased over baseline value (p>0.05)

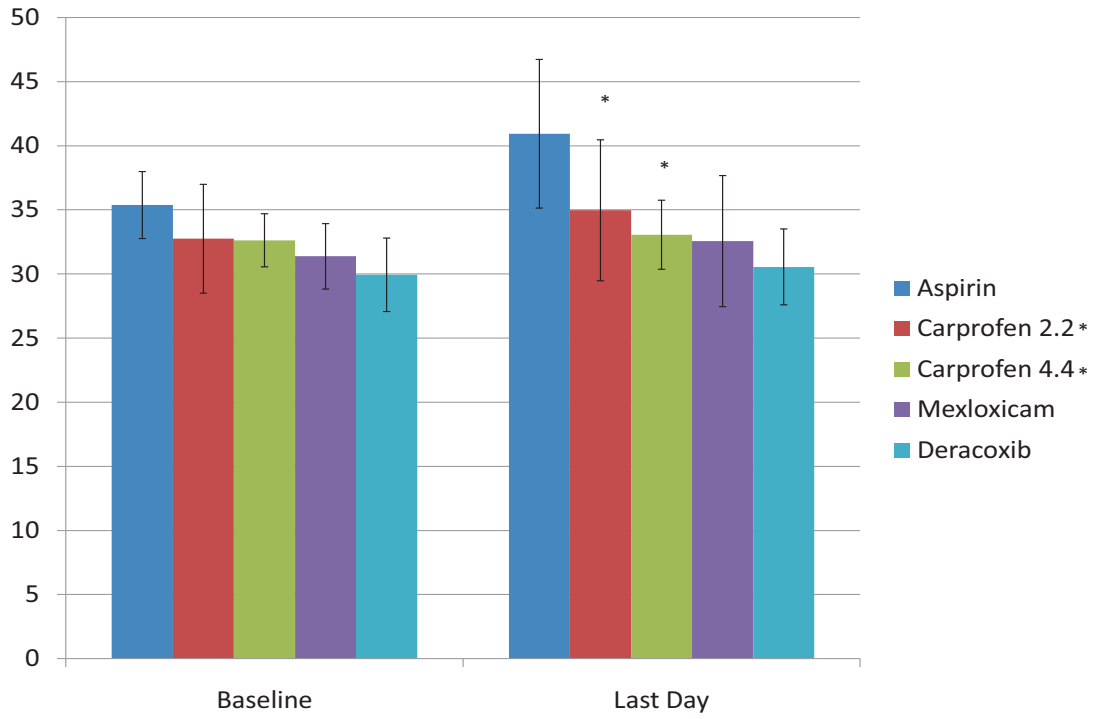

Figure 13 – Mean clot rate with viscoelastometry over time of 8 healthy dogs after treatment with aspirin, carprofen, meloxicam, and deracoxib

Mean clot rate with viscoelastometry (+/- S. E) over time of 8 healthy dogs after treatment with aspirin, carprofen, meloxicam, and deracoxib (aspirin 10 mg/kg PO every 12 hours for 10 days, carprofen 2.2 mg/kg PO every 12 h ours for 7 days, carprofen 4.4 mg/kg PO every 24 hours for 7 days, meloxicam 0.2 mg/kg first day then 0.1 mg/kg PO every 24 hours for 6 days, and deracoxib 2 mg/kg PO every 24 hours for 7 days).
* significantly decreased from baseline value ($p>0.05$)

Urine 11-Dehydro-Thromboxane B_2

Urine 11-dTBX$_2$ levels decreased significantly during aspirin administration from baseline to the last day of drug administration (day 10) ($P = 0.014$). There was no significant difference in urine 11-dTBX$_2$ levels among any other drugs or time points, although 11-dTBX$_2$ levels dropped below baseline levels for all drugs (Figure 14).

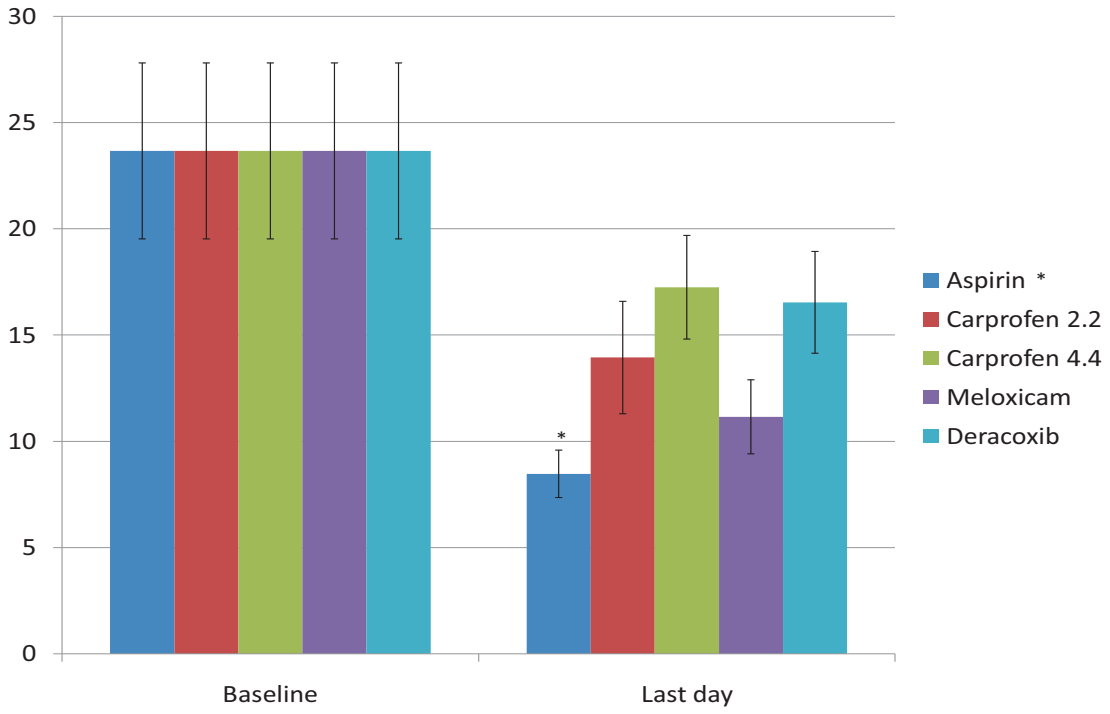

Figure 14 – Mean urine 11-dehydro-thromboxane B_2 (11-dTXB$_2$) levels after treatment with aspirin, carprofen, meloxicam, and deracoxib

Mean urine 11-dehydro-thromboxane B_2 (11-dTXB$_2$) levels after treatment with aspirin, carprofen, meloxicam, and deracoxib (aspirin 10 mg/kg PO every 12 hours for 10 days, carprofen 2.2 mg/kg PO every 12 hours for 7 days, carprofen 4.4 mg/kg PO every 24 hours for 7 days, meloxicam 0.2 mg/kg first day then 0.1 mg/kg PO every 24 hours for 6 days, and deracoxib 2 mg/kg PO every 24 hours for 7 days).
* significantly decreased from baseline value ($p>0.05$).

Other Testing

No significant differences were seen in platelet count or PCV with regards to time or drug for any of the drugs evaluated. Due to a laboratory error, the baseline measurements for PCV and platelet count for the second rotation in the more COX-2 selective NSAIDs crossover trial (rotation B) were unavailable, so a baseline measurement was created by averaging the baseline measurements for the other three rotations (rotations A, C and D).

BMBT remained within normal limits (less than 3 minutes)[53] for all dogs at all time points.

DISCUSSION

Non-selective NSAIDs such as aspirin have long been considered to be contributing risk factors for peri-operative hemorrhage in both humans and dogs due to their effects on platelet function.[21,23,33,34,53] One of the perceived benefits of the newer more COX-2 selective NSAIDs is that they would be expected to have less effect on platelet function than drugs like aspirin. The primary purpose of our study was therefore to determine the effects of the newer more COX-2 selective NSAIDs on a comprehensive panel of tests of platelet function in the dog.

The clear and consistent response to aspirin among dogs in our study group as measured by PFA-100® (collagen/EPI) (Figure 1) and urinary 11-dTXB$_2$ (Figure 14) was both statistically significant and visually dramatic. Standard doses of the newer NSAIDs appear to have much the same effect, but to a lesser extent and with much wider individual dog variability.

All of the newer NSAIDs evaluated do appear to have a measurable effect on platelet function. As measured by PFA-100® (collagen/EPI), closure time was significantly prolonged from baseline to all other time points for all drugs with no significant difference between time points beyond baseline. Yet to be determined is whether this altered platelet function correlates with intra-operative hemorrhage. In human medicine, the finding that NSAIDs affect laboratory measures of platelet function has lead to the recommendation that, whenever possible, NSAIDs should be avoided prior to surgeries

where significant hemorrhage is expected.[32,33,34] In dogs, a significant but weak association has been made between a prolonged BMBT and blood loss intraoperatively,[54] and aspirin has been reported to significantly prolong BMBT.[53] Interestingly, in our study, for all dogs during administration of all drugs (including aspirin), the BMBT was within the normal range, however this is consistent with a number of other previous studies.[22,29,31] Based on our results, while we cannot conclude that the newer NSAIDs will predispose to clinical bleeding, we have clearly demonstrated that they do have an effect on laboratory measures of platelet function, and therefore should be used with caution in surgical patients that are at risk for bleeding.

While our study is very similar in overall design to two recent studies by Blois and others[55] and by Brainard and others,[27] our results and general conclusions are very different. Blois and others[55] evaluated the effects of one week of oral aspirin, carprofen, deracoxib, and meloxicam on platelet function in 10 healthy dogs using a comprehensive panel of tests including plasma thromboxane B_2 (TXB_2) and 6-keto prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$), platelet aggregometry using ADP and platelet-activating factor as agonists, and platelet function analysis using the PFA-100®, and concluded that these NSAIDS had minimal effect on platelet function. Interestingly, Blois and others[55] reported that oral aspirin at the same dose used in our study had no detectable effect on platelet function, which is in contrast to the findings in our study which documented a consistent and marked decrease in several measures of platelet function. We believe that these differing results are due to differences in the techniques used for measuring platelet function in the two studies: in particular, differences in the PFA-100® and thromboxane assays used. Firstly, Blois and others[55] measured platelet function with the PFA-100® using the

collagen/ADP cartridge, whereas our study predominantly utilized the PFA-100® collagen/EPI cartridge. In people, Kundu and others[51] describe the use of the two different PFA-100® cartridges to allow differentiation of aspirin-induced platelet dysfunction from other congenital and acquired platelet function defects. If platelet dysfunction is due to aspirin, then PFA-100® collagen/EPI measurements will tend to be abnormal while collagen/ADP measurements will remain within the normal range, because the collagen/ADP cartridge appears to be less sensitive to aspirin-induced platelet dysfunction and therefore selects for more severe platelet disorders. Similarly, in dogs, Nielsen and others[49] found that administration of aspirin prolonged PFA-100® closure times when using collagen/EPI cartridges, but not when collagen/ADP cartridges were utilized. Our study results are very comparable to those of Neilsen and others[49], and strongly suggest that, in dogs as in people, prolongation of PFA-100® closure time utilizing the collagen/EPI cartridge is a sensitive indicator of NSAID-induced platelet dysfunction, whereas in contrast the collagen/ADP cartridge appears to be a very insensitive indicator. Secondly, Blois and others[55] measured the effects of NSAIDs on platelet thromboxane production by measuring plasma TXB_2 levels whereas, in our study, we utilized urinary 11-dTXB$_2$ as an indicator of platelet thromboxane production. In contrast to Blois and others[55], who found that aspirin had little effect on plasma TXB_2 levels in dogs, our study documented that aspirin induced a significant and consistent decrease in urine 11-dTXB$_2$ levels. The results of our study suggest that measurement of urinary TXB_2 metabolites is a much more sensitive indicator of NSAID-induced inhibition of platelet thromboxane production than is measurement of plasma TXB_2 levels, a finding that is supported by several other studies that conclude that urinary

TXB_2 metabolites are a more reliable indicator of the effects of NSAIDS, and less prone to sampling artifacts.[13,56,57,58,59,60] TXB_2 is metabolized at the tissue level into 11-dehydro-thromboxane B_2, which is then excreted in urine. The 11-dehydro-thromboxane B_2 assays are therefore not subject to sampling artifact related to in-vitro platelet activation.[61,62] Brainard and others[27] evaluated the effects of 10 days of oral aspirin (dosed at 5 mg/kg twice daily, half the dose used in our study), carprofen, deracoxib and meloxicam on platelet function in 8 dogs with osteoarthritis, and concluded that the more COX-2 selective NSAIDs had minimal and variable effects on most measures of hemostasis, though platelet aggregometry was significantly impaired in dogs receiving aspirin and carprofen. However, the panel of tests used by Brainard and others[27], which consisted of platelet TXB_2, plasma TXB_2, and 6-keto $PGF_{1\alpha}$, platelet aggregometry using collagen and ADP as agonists, and thromboelastography (a method of clot analysis comparable to viscoelastometry), lacked several of the more sensitive indicators of NSAID-induced platelet dysfunction such as urinary TXB_2 metabolites and utilization of epinephrine as a platelet agonist. In summary, the results of our study suggest that PFA-100® analysis using collagen/EPI cartridges and measurement of urinary 11-$dTXB_2$ may be more sensitive measures of NSAID-induced platelet dysfunction in dogs than many of the tests utilized in those previous studies that reported that aspirin and other NSAIDs had a lesser impact on platelet function.

Viscoelastometry via SonoClot® analyzer has been widely used in human medicine, including utilization in a number of disease conditions to predict hemorrhage,[63,64,65] and has become particularly important in cardiac surgery.[66,67,68,69,70] Viscoelastometry is considered a quick and simple method of evaluating clotting abnormalities in both

primary and secondary hemostasis, and can be performed intraoperatively or bedside. The viscoelastometric signature curve can be evaluated visually or via computer-generated algorithms to determine the source of the abnormality based on changes in curve shape. General platelet function is believed to be represented by the time to peak.[71] Platelet function is also thought to correspond with the shape of the peak, with more active platelets resulting in a sharper peak.[72] There are, however, well documented limitations to the use of viscoelastometry to evaluate platelet function, including variability in results related to age, gender, and platelet count,[73] and subjectivity associated with visual curve evaluation. In addition, viscoelastometry has been proven to be ineffective in detecting aspirin-induced platelet dysfunction in humans.[74] Our study is one of the first to investigate the use of SonoClot® viscoelastometry in veterinary medicine. Although the validity of viscoelastometry for assessing platelet function in dogs has not been established, we chose to use the SonoClot® based on its acceptance in human medicine for the detection of platelet abnormalities. As has been documented in people, no viscoelastometreic abnormalities were detected after administration of aspirin to the dogs in our study. However, it is interesting that the SonoClot® ACT was prolonged after treatment with carprofen 4.4 mg/kg PO q 24 h, and that both carprofen dosing regimens resulted in a faster CR. While these SonoClot®values are not thought to reflect platelet function, platelets do provide procoagulant factors, so altered platelet function could, in theory, affect all aspect of viscoelastometry, including ACT and CR.

In dogs, the newer so-called 'COX-2 selective' NSAIDS are in fact not completely selective for inhibition of COX-2 alone.[8,11,12,16,75] The degree of COX-2 selectivity varies among the newer NSAIDS, with many also having some degree of documented COX-1

inhibition.[11] Since aspirin is known to impair platelet function via inhibition of platelet COX-1, it would seem likely that the more a newer NSAID inhibits COX-1, the more likely it is to also impair platelet function. Carprofen, for example, was thought to have little to no effect on platelet function since it was believed to spare COX-1 activity,[26] however the degree of carprofen COX-2 selectivity has not been definitively established. While one study reported that the potency of carprofen for COX-2 was more than 100-fold greater than for COX-1 in dogs,[76] another study reported carprofen to be only 1.75 times more selective for COX-2 than COX-1.[11] Meloxicam reportedly inhibits canine COX-2 activity 12 times more effectively than COX-1 activity.[11] Since even very low doses of aspirin are known to impair platelet function in many people via inhibition of platelet COX-1, it is possible that even if a newer NSAID is responsible for only a small amount of COX-1 inhibition compared to a relatively much greater COX-2 inhibition, that small degree of COX-1 inhibition may be enough to significantly impair platelet function. Our study certainly strongly suggests that all of the newer NSAIDs that we evaluated have the potential to significantly impair platelet function in individual dogs. While the results of our study may appear at first glance to contradict the findings of other recent and superficially similar studies, a close examination of the individual studies reveals a tremendous variability in the methodologies employed to evaluate platelet function. Apparently conflicting findings may therefore be to a large extent due to the use of measures of platelet function that vary widely in their sensitivity to the effects of aspirin. However, even when two studies use very comparable drug doses and hemostatic testing methodologies, conflicting results are occasionally reported. For example, a previous study by Gaal and others evaluating the effects of carprofen on

platelet function found no difference in PFA-100® closure times between control and carprofen-treated dogs with either collagen/EPI or collagen/ADP agonist cartridges,[77] a finding that contrasts with the observation in our study that carprofen significantly prolongs collagen/EPI PFA-100® closure times. Published studies also describe conflicting results with regards to the effects of meloxicam on bleeding times. Although Luna and others[21] reported prolonged bleeding times in dogs within 7 days of administration of meloxicam compared to control dogs, our study and another study by Mathews and others reported that the administration of meloxicam to dogs did not result in a prolonged BMBT.[28,30]

Variability in the platelet responses of the individual dogs in our study to the newer NSAIDs, and perhaps even apparent discrepancies in the results of different published studies, may potentially be explained by dog-to-dog variability in NSAID responsiveness. In our study, it was certainly interesting that while all dogs responded to standard anti-inflammatory doses of aspirin in a predictable fashion (particularly by prolongation of PFA-100® collagen/EPI closure times and decreased levels of urine 11-dTXB$_2$), individual dog responses to the other newer NSAIDs were much more variable. This variable responsiveness to the newer NSAIDS may be comparable to the variable responsiveness to low dose aspirin that is well documented in people. In humans, while high doses of aspirin typically have predictable effects on platelet function via inhibition of COX-1, platelet responsiveness to low dose aspirin varies widely. Although many possible etiologies for this variability have been investigated, the mechanism or mechanisms by which some patients are 'resistant' to low dose aspirin remain unknown.[16,48,75] Potentially, the mechanism in dogs that confers variable platelet responsiveness to

the newer NSAIDS despite a predictable responsiveness to anti-inflammatory doses of aspirin may be comparable to the mechanism that causes aspirin resistance in people.

In conclusion, aspirin and all of the newer more COX-2 selective NSAIDS evaluated in our study caused significantly prolonged PFA-100® closure times measured using collagen/EPI cartridges, indicating NSAID-induced platelet dysfunction. When evaluated with collagen/ADP cartridges, however, there was no change in PFA-100® closure time, even with aspirin, confirming that NSAID-induced platelet dysfunction cannot be accurately assessed using collagen/ADP cartridges. Significant decreases in urinary 11-dTXB$_2$ after treatment with aspirin also confirmed aspirin-induced platelet dysfunction, and although the decrease in 11-dTXB$_2$ did not reach statistical significance, there was a consistent drop below baseline for all drugs. Failure of the SonoClot® to demonstrate platelet dysfunction after treatment with aspirin suggests that, as in humans, this methodology is not appropriate for the detection of aspirin-induced platelet dysfunction in dogs. Although our study also revealed a prolonged ACT and faster CR after treatment with carprofen, this result was somewhat unexpected, and should be further investigated before conclusions are drawn. Our study demonstrated that oral administration of the commonly used COX-2 selective NSAIDS at standard doses resulted in significantly decreased platelet function. While we did not undertake to demonstrate a correlation between the results of laboratory findings and clinical bleeding, based on the results of our study, hemostatic evaluation of surgical patients known to be exposed to NSAIDS may be warranted in order to predict and potentially reduce the incidence of perioperative hemorrhage. Caution may be advised in the administration of these drugs prior to surgery, particularly in the presence of other risk factors for bleeding.

a. PFA-100®, Siemens Healthcare Diagnostics, Deerfield, IL

b. SonoClot®, Sienco Inc, Arvada, CO

c. Luminex® 11-dehydro Thromboxane B2 Kit, Cayman Chemical Co, Ann Arbor, MI

d. Abbott Cell-Dyn® 3700, Abbott Laboratories, Abbott Park, IL

e. PFA Collagen/ADP Test Cartridge, Siemens Healthcare Diagnostics, Duluth, GA

f. PFA Collagen/EPI Test Cartridge, Siemens Healthcare Diagnostics, Duluth, GA

g. SonACT® Kit, Sienco, Inc, Arvada, Colorado

h. Signature Viewer™ Program, Sienco, Inc, Arvada, Colorado

i. ACE Alera® Clinical Chemistry System, Alfa Wasserman, Inc., West Caldwell, NJ

j. Triplett Bleeding Time Device®, Helena Laboratories Corp, Beaumont, TX

k. HemoHeel®, Helena Laboratories Corp, Beaumont, TX

l. Aspirin, Major Pharmaceuticals, Livonia, MI

m. Rimadyl, Pfizer Animal Health, NY, NY

n. Metacam suspension (0.5%), Boehringer Ingelheim Vetmedica, St Joseph, MO

o. Deramaxx, Novartis Animal Health, Greensboro, NC

p. SAS for Windows version 9.2, SAS Institute, Cary, NC, 2008

q. Sucralfate, Teva Pharmaceuticals, Sellersvillle, PA

CHAPTER III

SUMMARY AND CONCLUSIONS

The goal of this project was to investigate the effects of commonly used COX-2 selective NSAIDS on platelet function in the dog. This study found that all of the COX-2 selective NSAIDS evaluated significantly affected platelet function. This finding is noteworthy because these more selective NSAIDS were developed to help reduce the side effects caused by COX-1 inhibition, including alterations in platelet function.

Two recently published studies reported different results on the effects of COX-2 selective NSAIDS on platelet function in dogs.[27,55] These studies evaluated a similar number of dogs but used different measurements of platelet function than used in our study. The study by Blois utilized the PFA-100® collagen/ADP cartridges, but not the collagen/EPI cartridges, which is likely the reason for their lack of detecting changes in platelet function. The study by Brainard assessed platelet function by using platelet aggregation assays, thromboelastography, and measurement of lipopolysaccharide-stimulated prostaglandin E_2, platelet thromboxane B_2 (TXB_2), and free serum TXB_2 and 6-keto-prostaglandin F(PGF)-1α concentrations. Platelet aggregation decreased after treatment with aspirin and carprofen, and clot strength decreased after treatment with carprofen and increased with deracoxib. Testing methods in Brainard's study were much different from the methods we used. Testing results may have been affected by the fact

that the patients were osteoarthritic and client-owned, both of which have the potential to drastically affect results.

Neither of these published reports assessed platelet function using the PFA-100® collagen/EPI cartridge or measurement of urinary 11-dTXB$_2$ as used in our study. Both of these measures of platelet function have proven to be effective methods of determining platelet dysfunction.[13,49,51,56,57,58,59,60] Based on studies in both humans[51] and dogs,[49] prolongation of PFA-100® collagen/EPI closure time has been found to be a sensitive indicator of platelet dysfunction due to NSAID administration. In addition, multiple studies in humans and one in dogs[13,56,57,58,59,60] have determined measurements of urinary TXB$_2$ metabolites to be a more reliable indicator of NSAID affects on platelets than measures of TXB$_2$ metabolites in other locations, with less sampling artifacts. In our study, both tests clearly demonstrated their ability to determine platelet dysfunction due to NSAID administration. Initial evaluation with the PFA-100® collagen/EPI cartridge after aspirin administration proved it to be a very clear and consistent method of detecting platelet dysfunction, as there was a significant and obvious alteration in platelet function with the collagen/EPI cartridge, but not with the collagen/ADP cartridge. Evaluation of platelet function using the PFA-100® collagen/EPI cartridge after administration of each of the other NSAIDS in our study also showed a significant alteration in platelet function, although platelet dysfunction in individual dogs was not as consistent as that seen with aspirin. While measurement of urinary TXB$_2$ metabolites was significantly decreased only after aspirin administration, there was a non-significant decline in urinary TXB$_2$ after administration of all the NSAIDS.

When evaluated using PFA-100® collagen/EPI cartridges, platelet function was more variable in dogs treated with COX-2 selective NSAIDS than when these same dogs were treated with aspirin. This may be due to interindividual variability, similar to the unpredictable response seen in humans to aspirin. The fact that platelet dysfunction, based on PFA-100® collagen/EPI, after treatment with COX-2 selective NSAIDS was less pronounced than after aspirin treatment is encouraging, since alterations in platelet function and other side effects shouldn't be as severe. However, because treatment with COX-2 selective NSAIDS resulted in a more variable response than aspirin (as measured with the PFA-100® collagen/EPI cartridge), severity of potential side effects cannot be predicted. The cause of this variability is yet undetermined.

The results of the study reported here did not identify a variable response to aspirin in dogs as has been reported in humans.[38,39] All dogs responded quickly and similarly to aspirin treatment at standard anti-inflammatory doses. However, a larger low dose aspirin study may need to be performed to detect variations in response to aspirin. It remains unclear if dogs might have a consistent response to aspirin and a variable response to COX-2 selective NSAIDS, and additional research is needed for clarification.

Future research should focus on determining if there is a correlation between the NSAID affects on platelet function described in this study and prolonged bleeding clinically. The challenge will be to develop an accurate method of objectively quantifying intraoperative hemorrhage. Methods recently researched to quantify hemorrhage include measuring irrigation fluid, weighing surgical sponges, using gravimetric measurements, and quantification using spectrophotometric analysis of hemoglobin content, all of which were accurate and correlated with amount of blood lost.[78] However, gravimetric

measurements and quantification of hemoglobin content are not practical methods of quantifying blood loss clinically. BMBT has been weakly but significantly correlated with intraoperative blood loss[78] and is a quick, easy, and inexpensive method of preoperative evaluation of patients to rule-out major clotting abnormalities. BMBT has been noted to be significantly prolonged in dogs within 1 day of treatment with aspirin.[53] However, in our study, there was no prolongation of BMBT beyond what is considered normal.

Another interesting direction for future research would be evaluation of platelet function using the PFA-100 in patients receiving COX-2 selective NSAIDS for an existing medical condition (i.e. osteoarthritis or acute musculoskeletal trauma). It is not known if these types of conditions may induce platelet activation and to what degree, if they specifically alter platelet function in some manner, or if they alter the effects of COX-2 selective NSAIDS on platelet function.

One of the most exciting possibilities for future research is based on the recent evidence that COX-2 is expressed by human platelets.[79,80] If COX-2 is present in canine platelets, it may help explain the variable response to different COX-2 selective NSAIDS in our study. Studies investigating whether canine platelets express COX-2 would be of great interest and would impact research, drug development, and clinical use of NSAIDS. It would also encourage interest in the development of pure COX-2 selective NSAIDS and influence our clinical use of "COX-2 selective" NSAIDS. In fact, a very recent study has found that when platelets are permeabilized, COX-2 is detected intracellularly.[81] There are many more questions to be answered: Why has COX-2 not been previously detected on canine platelets? Is it only expressed when platelets are activated? What

percentage of platelets must be activated for clinical effects? What insults would cause this level of activation – Trauma? Osteoarthritis? Drug administration? What are the effects of "COX-2 selective" NSAID administration in dogs with other existing conditions that may or may not induce platelet function?

Continued research of the effects of COX-2 selective NSAIDS on canine platelet function would not only expand our understanding and knowledge of the topic, but would also be applicable clinically. More thorough preparation could be made for dogs on these drugs undergoing surgery, resulting in less risk to the patient and less morbidity.

There are several limitations to our study. Firstly, only eight dogs were evaluated in a cross-over study design. This number was determined to be the minimum number needed to evaluate for the presence of aspirin responders and non-responders, and to insure that minor variations in individual dog measurements would not alter our overall findings. This number is consistent with common recommendations for pharmacokinetic studies of this type,[82] and is extrapolated from a recent publication[27] describing a similar study in which the same design and animal numbers were used. The crossover design allowed for the complete evaluation of four different NSAIDS/dosages while utilizing only 8 dogs. Fortunately, eight dogs was, in our study, sufficient to detect significant alterations in platelet function with all of the NSAIDS evaluated, although it is possible that there were not enough dogs to provide sufficient power to detect alterations in urinary TXB_2 levels with the more COX-2 selective NSAIDS.

Another limitation is that both collagen/EPI and collagen/ADP cartridges were not used at all time points in the study. It could be argued that since closure time for both collagen/EPI and collagen/ADP cartridges must be evaluated to rule out more severe

platelet function disorders, both cartridges should have been used at all time points. However, because there was such a large difference between the two cartridges when used with the initial aspirin treatment of these dogs, we determined it was unnecessary to obtain measurements with both cartridges at each time point. In addition, we determined prior to the study that all of the dogs were free of diseases known to cause platelet and clotting dysfunction. Also, based on descriptions of the PFA-100®[51,83] and previous studies evaluating its use,[84,85] the collagen/ADP cartridge is only beneficial in detecting disorders of primary hemostasis such as von Willebrand disease and thrombopathia, while the collagen/EPI cartridge detects aspirin-like defects.

An additional limiting factor may be the use of intact female dogs. Several dogs were observed to go through heat cycles during the study, which could theoretically alter platelet count and PCV. However, we measured those values at peak administration of all drugs with no difference detected from baseline values.

In conclusion, with the expanding use of NSAIDS in veterinary medicine, their affects on the body, particularly hemostasis, need to be better understood. For many conditions currently treated with NSAIDS, elective surgical options are available and even recommended. If it is determined that certain NSAIDS, particularly the more commonly used COX-2 selective NSAIDS, cause increased intraoperative hemorrhage due to platelet dysfunction, steps can be taken preoperatively to prevent excessive surgical hemorrhage and other potentially life-threatening complications that may result. At this point, based on the results of this study, caution should be exercised when administering cox-2 selective NSAIDS to dogs, particularly those with other hematologic abnormalities and those that may need surgery.

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