The Effects of Cyclosporine and Aspirin on Platelet Function in Normal Dogs

J. Thomason, T. Archer, R. Wills, S. Press, and A. Mackin

Background: Cyclosporine increases thromboxane synthesis in dogs, potentially increasing the thrombogenic properties of platelets.

Hypothesis/Objectives: Our hypothesis was that the concurrent administration of low-dose aspirin and cyclosporine would inhibit cyclosporine-associated thromboxane synthesis without altering the antiplatelet effects of aspirin. The objective was to determine the effects of cyclosporine and aspirin on primary hemostasis.

Animals: Seven healthy dogs.

Methods: A randomized, crossover study utilized turbidimetric aggregometry and a platelet function analyzer to evaluate platelet function during the administration of low-dose aspirin (1 mg/kg PO q24h), high-dose aspirin (10 mg/kg PO q12h), cyclosporine (10 mg/kg PO q12h), and combined low-dose aspirin and cyclosporine. The urine 11-dehydro-thromboxane-B_2 (11-dTXB$_2$)-to-creatinine ratio also was determined.

Results: On days 3 and 7 of administration, there was no difference in the aggregometry amplitude or the platelet function analyzer closure time between the low-dose aspirin group and the combined low-dose aspirin and cyclosporine group. On day 7, there was a significant difference in amplitude and closure time between the cyclosporine group and the combined low-dose aspirin and cyclosporine group. High-dose aspirin consistently inhibited platelet function. On both days, there was a significant difference in the urinary 11-dTXB$_2$-to-creatinine ratio between the cyclosporine group and the combined low-dose aspirin and cyclosporine group. There was no difference in the urinary 11-dTXB$_2$-to-creatinine ratio among the low-dose aspirin, high-dose aspirin, and combined low-dose aspirin and cyclosporine groups.

Conclusions and Clinical Importance: Low-dose aspirin inhibits cyclosporine-induced thromboxane synthesis, and concurrent use of these medications does not alter the antiplatelet effects of aspirin.

Key words: Canine; Immune-mediated hemolytic anemia; Immunosuppression; Thromboxane.

Immune-mediated hemolytic anemia (IMHA) is a common cause of anemia in dogs.[1] Unfortunately, despite treatment efforts, mortality rates for IMHA in dogs are 50–70%.[2] One of the most common causes of death in dogs with IMHA is thromboembolic disease, particularly pulmonary thromboembolism (PTE). The incidence of PTE in dogs with IMHA may be as high as 50–80%.[3] The exact mechanisms leading to thrombus formation and PTE in dogs with IMHA are complicated, but one contributing factor is thought to be disruptions in normal platelet function.[2,4] Dogs with IMHA have been shown to have hyperactive platelets associated with platelet plasma membrane alterations.[4] Activated platelets release vasoactive molecules such as serotonin and thromboxane A_2, which may contribute to a hypercoagulable state and predisposition to PTE.[5]

Treatment of IMHA in dogs typically consists of immunosuppressive medications, drugs that inhibit hemostasis, and supportive care. Although glucocorticoids are the cornerstone of immunosuppressive therapy, additional immunosuppressive agents often are needed, and these drugs commonly are associated with adverse effects that can be deleterious to the patient, undesirable to owners or both. Cyclosporine has become a popular drug for the treatment of IMHA in dogs because of perceived safety and minimal adverse effects associated with its use.[6]

In humans, cyclosporine increases platelet synthesis of thromboxane A_2.[7,8] Thromboxane A_2 triggers vasoconstriction, causes platelet activation, and enhances platelet aggregation, all of which will decrease blood flow, increase blood stasis, and promote coagulation.[9] When compared to renal transplant recipients treated with azathioprine, human patients receiving cyclosporine have increased thromboxane A_2 production and

Abbreviations:

11-dTXB$_2$	11-dehydro-thromboxane B_2
ADP	adenosine diphosphate
COX	cyclooxygenase
IMHA	immune-mediated hemolytic anemia
PFA	platelet function analyzer®
PPP	platelet-poor plasma
PRP	platelet-rich plasma
PTE	pulmonary thromboembolism

increased rates of thrombus formation within the renal parenchyma.[8] In dogs, similar to humans, the administration of cyclosporine is associated with a significant increase in thromboxane synthesis, especially at immunosuppressive cyclosporine dosages and at times of peak blood drug concentrations.[10] This increase in thromboxane synthesis suggests that standard immunosuppressive dosages of cyclosporine might enhance platelet activation in dogs, potentially predisposing hypercoagulable patients to thromboembolic complications.

Drugs that inhibit hemostasis routinely are used prophylactically in dogs with IMHA in an attempt to prevent thromboembolism. Many of these drugs can only be given by injection, are cost prohibitive or both, leaving PO low-dose aspirin as one of the most affordable options for prophylactic therapy. In fact, the use of low-dose aspirin has been shown to improve survival rates in dogs diagnosed with IMHA.[11] Aspirin is a cyclooxygenase (COX) inhibitor that irreversibly inhibits platelet function. The COX enzyme is essential for the conversion of arachidonic acid to several biologically active prostaglandins, including thromboxane A_2, that are necessary for normal hemostasis.[12] By inhibiting COX, aspirin decreases platelet synthesis of thromboxane A_2, thereby inhibiting platelet function.[13]

Cyclosporine and low-dose aspirin often are used concurrently in dogs with IMHA. Currently, however, it is unknown if low-dose aspirin counteracts cyclosporine-associated thromboxane synthesis and, alternatively, if cyclosporine counteracts the antiplatelet effects of aspirin. Our study was designed to evaluate the effects of cyclosporine and aspirin (both low-dose and high-dose) given as a sole drug, and of concurrent administration of PO cyclosporine and low-dose aspirin, on platelet function and thromboxane synthesis in normal dogs.

Materials and Methods

Study Design, Animals

Eight healthy intact Walker hound dogs, 7 females and 1 male, were used in this study. The dogs had not received any medications or vaccines for at least 1 month before initiation of the study. Normal health status was established by detection of no abnormalities on physical examination, CBC (including manual platelet count), serum biochemistry, urinalysis, and heartworm testing. However, during the early phases of the study, 1 female dog was diagnosed with hepatic lymphoma and removed from the study, and results from this dog were excluded from analysis. The median age of the remaining 7 dogs was 3.5 years (range, 2.5–10.5 years), and their median body weight was 25.8 kg (range, 19.4–31.4 kg). Body weight was obtained at the beginning of the study and used to calculate all subsequent drug doses. Animal use was approved by the Mississippi State University Institutional Animal Care and Use Committee and was in compliance with the requirements of a facility accredited by the American Association for Accreditation of Laboratory Animal Care.

In a 4-way, randomized, crossover study, the dogs were given either low-dose aspirin[a] (1.0 ± 0.03 mg/kg [mean ± SD] PO q24h), high-dose aspirin (9.9 ± 0.3 mg/kg PO q12h), cyclosporine[b] (9.9 ± 0.4 mg/kg PO q12h), or combined low-dose aspirin and

cyclosporine (1.0 ± 0.03 mg/kg PO q24h and 9.9 ± 0.4 mg/kg PO q12h, respectively). All drugs were administered PO for 7 days, followed by at least a 14 day washout period between dosing. In our previous low-dose aspirin and cyclosporine studies, platelet function had returned to baseline values within 2 weeks of discontinuing the medications.[10,14] After the 14-day washout period, the dogs switched groups, and the study was continued until all dogs had received each medication dose and combination.

Samples were collected for platelet function testing and urinary thromboxane analysis. For all medication groups, blood and urine samples were collected on day 0 (before drug administration) and again on days 3 and 7 of drug administration. For all medication groups, samples were collected at the time of estimated peak cyclosporine blood concentrations, which previously has been shown to be 2 hours after drug administration.[15] Blood samples were collected by jugular venipuncture with a 20-gauge needle directly into a 4.5-mL vacutainer tube containing 3.2% sodium citrate anticoagulant.[c] Each sample was collected with a ratio of 1:9 of citrate to blood to standardize the degree of anticoagulation. For urine samples, an ultrasound-guided cystocentesis using a 22-gauge 1.5 inch needle with a 6 mL syringe was performed. The volume of urine collected varied between 2 and 5 mL.

Platelet Function Analysis

Turbidimetric Platelet Aggregometry. A 2-channel platelet aggregometer[d] that allowed for 2 samples to be evaluated concurrently was used to analyze platelet aggregation in platelet-rich plasma (PRP). On each day of collection, samples were analyzed in duplicate, twice, for a total of 4 results, which were averaged to yield a single value at each time point. Aggregation was assessed using collagen as an agonist (final concentration, 10 μg/mL), a temperature of 37°C, and a stirring speed of 1,200 rpm. All samples were analyzed within 4 hours of collection.

To harvest PRP, whole blood collected into 3.2% sodium citrate was centrifuged at 1,200 rpm at room temperature for 3 minutes. Supernatant PRP was collected, and the remaining blood sample was centrifuged at 1,800 rpm at room temperature for 8 minutes to create platelet-poor plasma (PPP). Samples were analyzed based on the manufacturer's standard guidelines.[e] Briefly, 450 μL of PRP was transferred into a glass cuvette containing a siliconized magnetic stir bar, and 500 μL of PPP was placed into a cuvette without a stir bar. Samples were incubated, at 37°C, for 5 minutes, and placed into the aggregometer, and stable baseline values for minimal (PRP) and maximal (PPP) aggregation were obtained (assigned values of 0 and 100% aggregation, respectively). Collagen was added to the PRP, and aggregation was monitored for 12 minutes. The percentage aggregation at maximum amplitude was calculated and recorded using computer software.[f] The number of platelets within the PRP was not adjusted to a standardized count by dilution with PPP before analysis, based on recommendations published by the International Society of Thrombosis and Haemostasis Platelet Physiology and Scientific and Standardization Committee.[16–19] Before initiation of the study, all dogs were confirmed to have hematocrit and platelet counts within reference ranges.

Platelet Function Analyzer

A point-of-care platelet function analyzer (PFA-100)[g] that has been evaluated previously for use in dogs was used to analyze platelet function.[20–23] The PFA-100 assesses platelet function under high shear forces and after activation with several platelet agonists to measure the closure time, in seconds, needed to form a platelet plug and inhibit blood flow. The cutoff time for the instrument is >300 seconds.

The instrument was used according to the manufacturer's instructions. Briefly, blood samples were collected directly into blood collection tubes containing 3.2% sodium citrate and kept at room temperature without agitation until analysis. For analysis, 800 μl of whole blood was transferred into PFA-100 cartridges (collagen/ADP or collagen/epinephrine),[h,i] and analyzed. The collagen/ADP cartridge was utilized initially to establish normal platelet function, and the collagen/epinephrine cartridge then was used throughout the study to assess drug-associated platelet dysfunction. Two collagen/epinephrine cartridges were analyzed at each time point for all dogs, and the closure times were averaged. All samples were analyzed within 2 hours of collection.

Urinary Thromboxane

Urinary 11-dehydro-thromboxane B_2 (11-dTXB$_2$) concentration was analyzed using an enzyme-linked immunosorbent assay kit[j] that has been validated previously in dogs.[24] Urine samples were collected by cystocentesis and stored at −80°C for later analysis. Before analysis, samples were thawed to room temperature and diluted with the assay buffer. A correction factor was applied to account for the dilution. Samples were analyzed in triplicate according to the manufacturer's instructions and reported in picograms per milliliter of urine. Briefly, a 96-well plate was prepared by adding the dilute sample, 11-dTXB$_2$ monoclonal acetylcholinesterase tracer and 11-dTXB$_2$ monoclonal antibody to each well, and then incubated at room temperature, in the dark and on an orbital shaker. Ellman's reagent was added to each well, and the plate was incubated again at room temperature, in the dark on an orbital shaker. The plate was analyzed at a wavelength of 412 nm using a plate reader.[k] Urine creatinine concentration was measured using a biochemistry analyzer[l] and used to calculate a urinary 11-TXB$_2$-to-creatinine ratio.

Low-Dose Aspirin Response

Based on previously published criteria used for dogs,[25] for both the low-dose aspirin group and the combined low-dose aspirin and cyclosporine group, each dog was, on both days, classified as either an aspirin responder or an aspirin nonresponder. For turbidimetric aggregometry, a dog was considered to be an aspirin responder if there was a >25% decrease in the percentage aggregation at maximum amplitude compared to day 0 values. For the PFA-100, a dog was considered to be an aspirin responder if the closure time was >300 seconds.

Statistical Analysis

Histograms were used to visually assess if the measured outcomes were normally distributed using a statistical computer program.[m] The distributions of the outcome measures were not consistently normally distributed. Accordingly, additional diagnostic procedures were conducted to ensure that the proper statistical methods were utilized. To determine the effect of day on each outcome measured within a treatment group, a mixed model analysis was conducted. For each marker of platelet function measured within a treatment group, a model was fit with fixed effects of day, period, and day-by-period interaction. Dog was included as a random effect. The repeated measures of dog within period were accounted for in a repeated statement. The distribution of the conditional residuals was evaluated for each marker to ensure the assumptions of the statistical method had been met. Mixed models also were fit that included day, period, group, day-by-period interaction, day-by-group interaction, and group-by-period interaction terms as fixed effects. Differences in least squares means with Tukey adjustment for multiple comparisons were determined for markers with significant main effect or interaction terms. An alpha level of 0.05 was used to determine statistical significance for all methods.

Results

Turbidimetric Aggregometry

The median turbidimetric aggregometry maximum amplitude for the low-dose aspirin group on day 0 was 48.3% (range, 35.3–63.5%), whereas the median amplitude on day 3 and day 7 was 38% (range, 2–53.8%) and 10.3% (range, 1–67.8%), respectively. When compared to day 0, there was a significant decrease in amplitude on day 7 ($P = .0261$). There was no significant difference in amplitude between days 0 and 3 and days 3 and 7 (Fig 1A). The median maximum amplitude for the high-dose aspirin group on day 0 was 53.3% (range, 46–75.3%), whereas the median amplitude on day 3 and day 7 was 1.8% (range, 0.3–2.3%) and 1.5% (range, 0.5–2.3%), respectively. When compared to day 0, there was a significant decrease in amplitude on both day 3 ($P < .0001$) and day 7 ($P < .0001$). There was no significant difference in amplitude between days 3 and 7 (Fig 1B).

The median maximum amplitude for the cyclosporine group on day 0 was 63.5% (range, 46–76.5%), whereas the median amplitude on day 3 and day 7 was 58.3% (range, 21.3–68%) and 56% (range, 47.5–75.5%), respectively. There was no significant difference in amplitude among days 0, 3, and 7 (Fig 1C). The median maximum amplitude for the combined low-aspirin and cyclosporine group on day 0 was 51.8% (range, 39.8–75%), whereas the median amplitude on day 3 and day 7 was 38.5% (range, 1.3–63.8%) and 3% (range, 1–55.5%), respectively. When compared to day 0, there was a significant decrease in amplitude on day 7 ($P = .0243$). There was no significant difference in amplitude between days 0 and 3 and days 3 and 7 (Fig 1D).

There was no difference in the median maximum amplitude on day 0 for any of the 4 medication groups. However, on day 3, when compared to the 3 other medication groups, there was a significant decrease in amplitude in the high-dose aspirin group ($P < .0001$). There was no difference in amplitude among the cyclosporine group, the low-dose aspirin group, and the combined low-dose aspirin and cyclosporine group. On day 7, there was a significant difference between the low-dose and high-dose aspirin groups ($P = .012$) and between the high-dose aspirin and cyclosporine groups ($P < .0001$). Additionally, on day 7, there was a significant ($P < .0001$) difference between amplitudes in the cyclosporine group and the combined low-dose aspirin and cyclosporine group. However, there was no difference between the low-dose aspirin group and the combined low-dose aspirin and cyclosporine group.

For the low-dose aspirin group, 3 dogs were considered to be aspirin responders on day 3, whereas 5 dogs were aspirin responders on day 7. All 3 dogs that were considered aspirin responders on day 3 also were
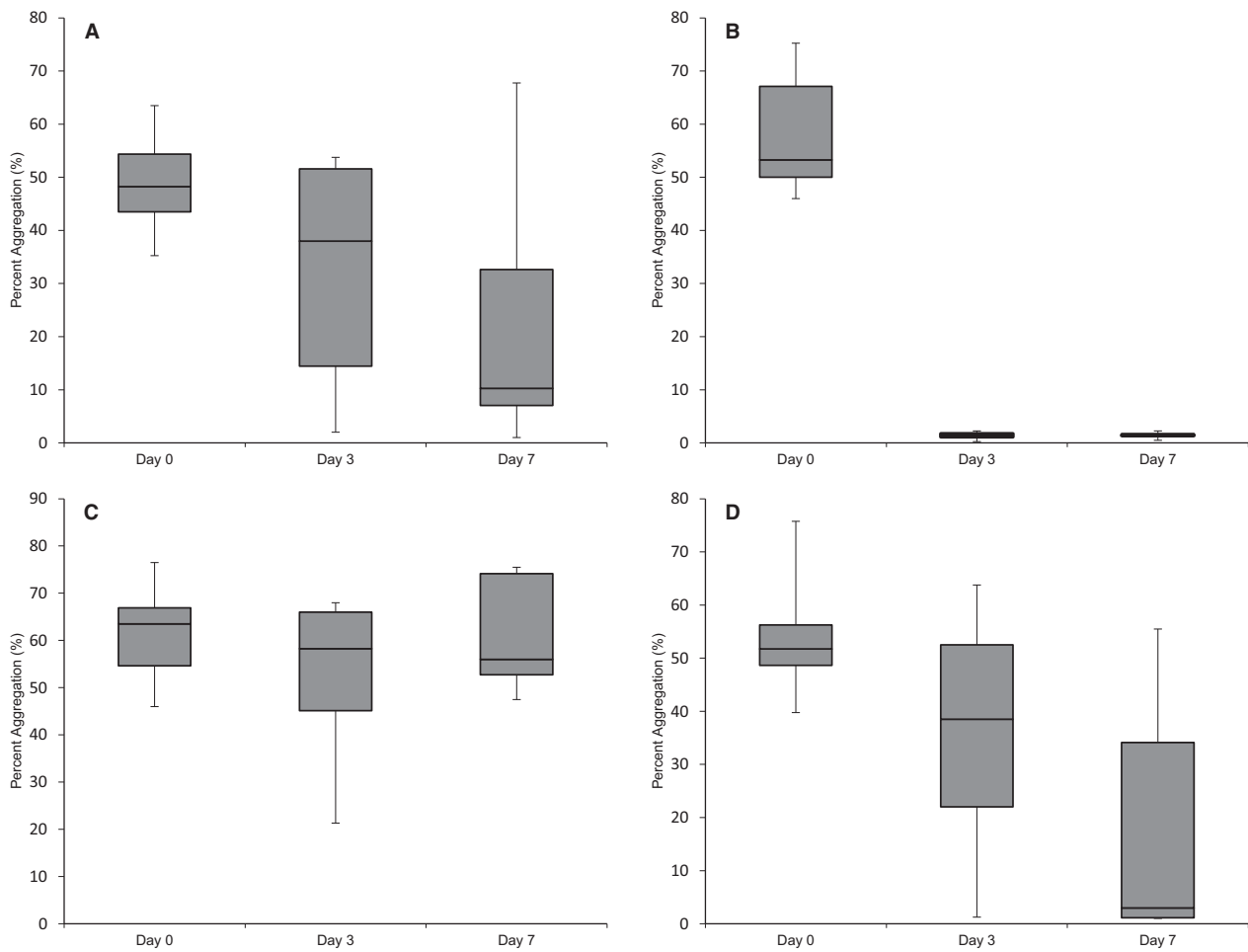
Fig 1. Maximum amplitude (percentage) of aggregation via turbidimetric aggregometry in dogs treated with (**A**) low-dose aspirin (1 mg/ kg, PO, q24h), (**B**) high-dose aspirin (10 mg/kg, PO, q12h), (**C**) cyclosporine (10 mg/kg, PO, q12h), and (**D**) low-dose aspirin and cyclosporine. The box and whiskers plot demonstrates the median (line), interquartile range (box), and total range (whiskers).

classified as aspirin responders on day 7. For the combined low-dose aspirin and cyclosporine group, 4 dogs were aspirin responders on day 3, and 5 dogs were aspirin responders on day 7. The 4 dogs classified as aspirin responders on day 3 also were considered aspirin responders on day 7. On day 3, the 3 dogs that were classified as aspirin responders in the low-dose aspirin group also were considered aspirin responders in the combined low-dose aspirin and cyclosporine group. Similarly, on day 7, the 4 dogs that were classified as aspirin responders in the low-dose aspirin group also were considered aspirin responders in the combined low-dose aspirin and cyclosporine group.

Platelet Function Analysis

The median PFA-100 closure time using the collagen/ ADP cartridge was 65 seconds (range, 52–83 seconds). The median PFA-100 closure time using the collagen/ epinephrine cartridge for the low-dose aspirin group on day 0 was 112.5 seconds (range, 66–243 seconds), whereas the median closure time on day 3 and day 7 was 132.5 seconds (range, 86–254.5 seconds) and

243.5 seconds (range, 89–300 seconds), respectively. When compared to day 0, there was a significant increase in closure time on day 7 ($P = .0428$). There was no significant difference in closure times between days 0 and 3 and days 3 and 7 (Fig 2A). The median closure time for the high-dose aspirin group on day 0 was 110 seconds (range, 75–155 seconds), whereas the median closure time on day 3 and day 7 was 300 seconds (range, 237–300 seconds) and 300 seconds (range, 186–300), respectively. When compared to day 0, there was a significant increase in closure time on both day 3 ($P = .0003$) and day 7 ($P = .0003$). There was no significant difference in closure time between days 3 and 7 (Fig 2B).

The median closure time using the collagen/epinephrine cartridge for the cyclosporine group on day 0 was 130.5 seconds (range, 98–195 seconds), whereas the median closure time on day 3 and day 7 was 117 seconds (range, 91.5–191 seconds) and 126.5 seconds (range, 92.5–138 seconds), respectively. There was no significant difference in closure times among days 0, 3, and 7 (Fig 2C). The median closure time for the combined low-dose aspirin and cyclosporine group on day 0
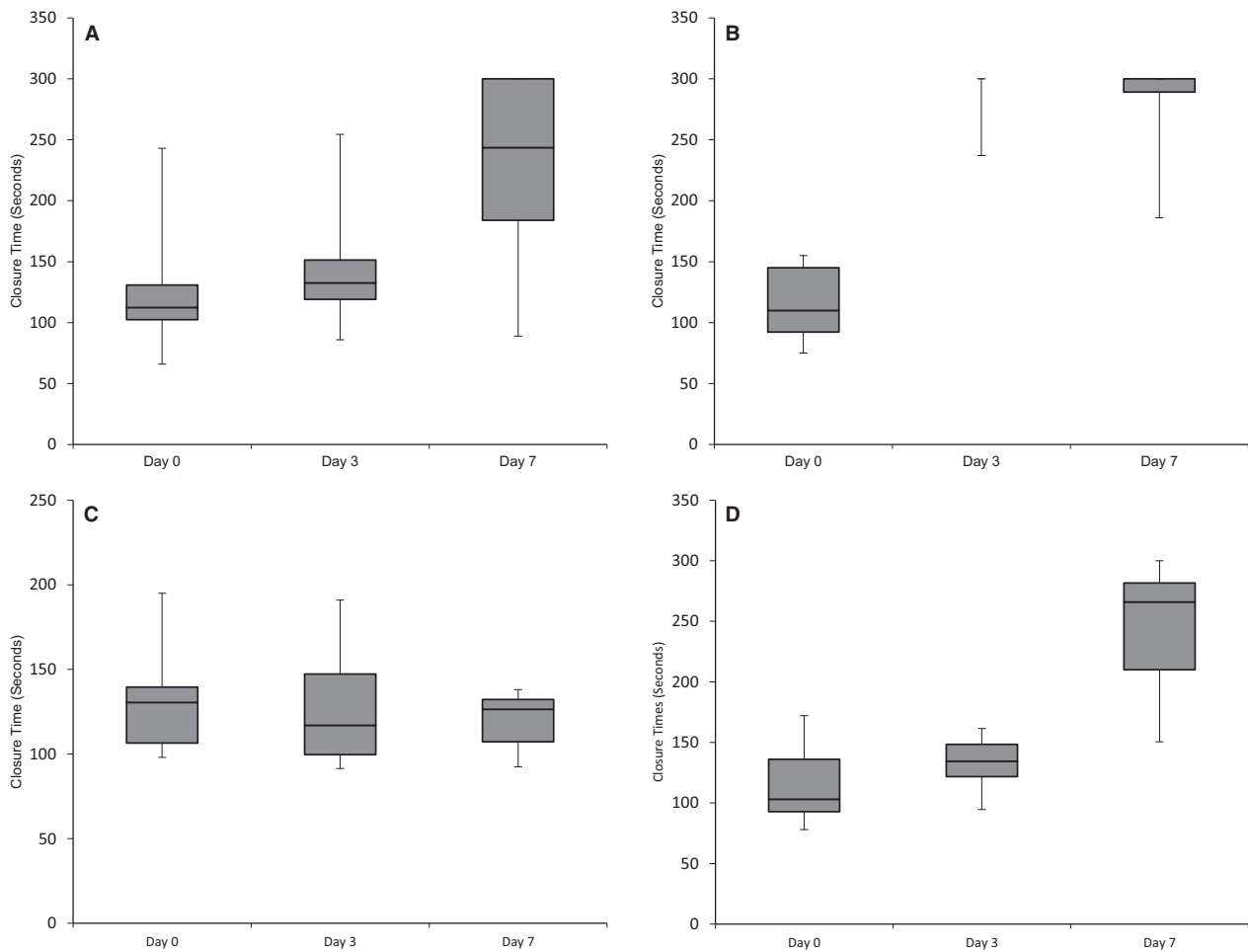
Fig 2. PFA-100® closure times (collagen/epinephrine cartridge) in dogs treated with (**A**) low-dose aspirin, (**B**) high-dose aspirin, (**C**) cyclosporine, and (**D**) low-dose aspirin and cyclosporine. The box and whiskers plot demonstrates the median (line), interquartile range (box), and total range (whiskers).

was 103 seconds (range, 78–172 seconds), whereas the median closure time on day 3 and day 7 was 134.5 seconds (range, 94.5–161.5 seconds) and 266 seconds (range, 150.5–300 seconds), respectively. When compared to day 0, there was no difference in closure time on day 3, but there was a significant increase in closure time on day 7 ($P = .0012$). There was a significant difference ($P = .0021$) in closure time between days 3 and 7. (Fig 2D).

There was no difference in the median closure time on day 0 for any of the 4 medication groups. On day 3, when compared to the 3 other medication groups, there was a significant increase in the median closure time in the high-dose aspirin group. There was no difference in the closure time among the cyclosporine group, the low-dose aspirin group, and the combined low-dose aspirin and cyclosporine group. On day 7, there was a significant difference in closure time between the low-dose and high-dose aspirin groups ($P = .0043$) and between the low-dose aspirin and cyclosporine groups ($P < .0001$). Additionally, there was a significant ($P < .0001$) difference between the closure time in the cyclosporine group and the combined low-dose aspirin

and cyclosporine group. However, there was no difference between the low-dose aspirin group and the combined low-dose aspirin and cyclosporine group.

On day 3, all dogs in both the low-dose aspirin and the combined low-dose aspirin and cyclosporine groups were considered to be aspirin nonresponders. On day 7, 3 dogs were considered to be aspirin responders in the low-dose aspirin group and 2 dogs were considered to be aspirin responders in the combined low-dose aspirin and cyclosporine group. However, only 1 dog on day 7 was considered to be a responder in both the low-dose aspirin group and the combined low-dose aspirin and cyclosporine group.

Urinary Thromboxane

The median 11-dTXB$_2$-to-creatinine ratio for the low-dose aspirin group on day 0 was 6.3 (range, 2.6–32.2), whereas the median ratio on day 3 and day 7 was 3.0 (range, 1–6.6) and 3.2 (range, 1–4.5), respectively (Fig 3A). The median 11-dTXB$_2$-to-creatinine ratio for the high-dose aspirin group on day 0 was 4.5 (range, 1.8–13.1), whereas the median ratio on day 3 and day 7

was 2.7 (range, 1.4–6.9) and 3.0 (range, 0.6–17.9), respectively (Fig 3B). For both the low-dose aspirin group and the high-dose aspirin group, there were no significant differences in 11-dTXB$_2$-to-creatinine ratio among days 0, 3, and 7.

The median 11-dTXB$_2$-to-creatinine ratio for the cyclosporine group on day 0 was 4.2 (range, 3.3–18.1), whereas the median ratio on day 3 and day 7 was 8.6 (range, 3.8–37.9) and 7.6 (range, 4.9–73.5), respectively. When compared to day 0, there was a significant increase in the median 11-dTXB$_2$-to-creatinine ratio on day 3 ($P = .0064$) and on day 7 ($P < .0001$). There was also a significant difference in the 11-dTXB$_2$-to-creatinine ratio between days 3 and 7 ($P = .0003$); (Fig 3C). The median 11-dTXB$_2$-to-creatinine ratio for the combined low-dose aspirin and cyclosporine group on day 0 was 5.2 (range, 3.1–11.5), whereas the median ratio on day 3 and day 7 was 6.5 (range, 3.2–10.6) and 3.9 (range, 1.6–7.2), respectively. There were no significant differences in the 11-dTXB$_2$-to-creatinine ratio among days 0, 3, and 7 (Fig 3D).

There was no difference in the median 11-dTXB$_2$-to-creatinine ratio on day 0 for any of the 4 medication groups. There was a significant difference between the cyclosporine group and the combined low-dose aspirin and cyclosporine group on day 3 ($P = .0268$) and day 7 ($P < .0001$). On both days, there was no difference among the high-dose aspirin group, the low-dose aspirin group, and the combined low-dose aspirin and cyclosporine group.

Discussion

Cyclosporine is a commonly used immunosuppressive agent in dogs, partly because of the perception of relatively minimal adverse effects.[6,26] However, several studies in humans have indicated that cyclosporine can increase the risk of thromboembolic complications by increasing synthesis of thromboxane A$_2$.[7,8,27–29] Recently, similar to humans, it has been shown that cyclosporine, especially at the time of peak blood drug concentrations, increases synthesis of thromboxane in
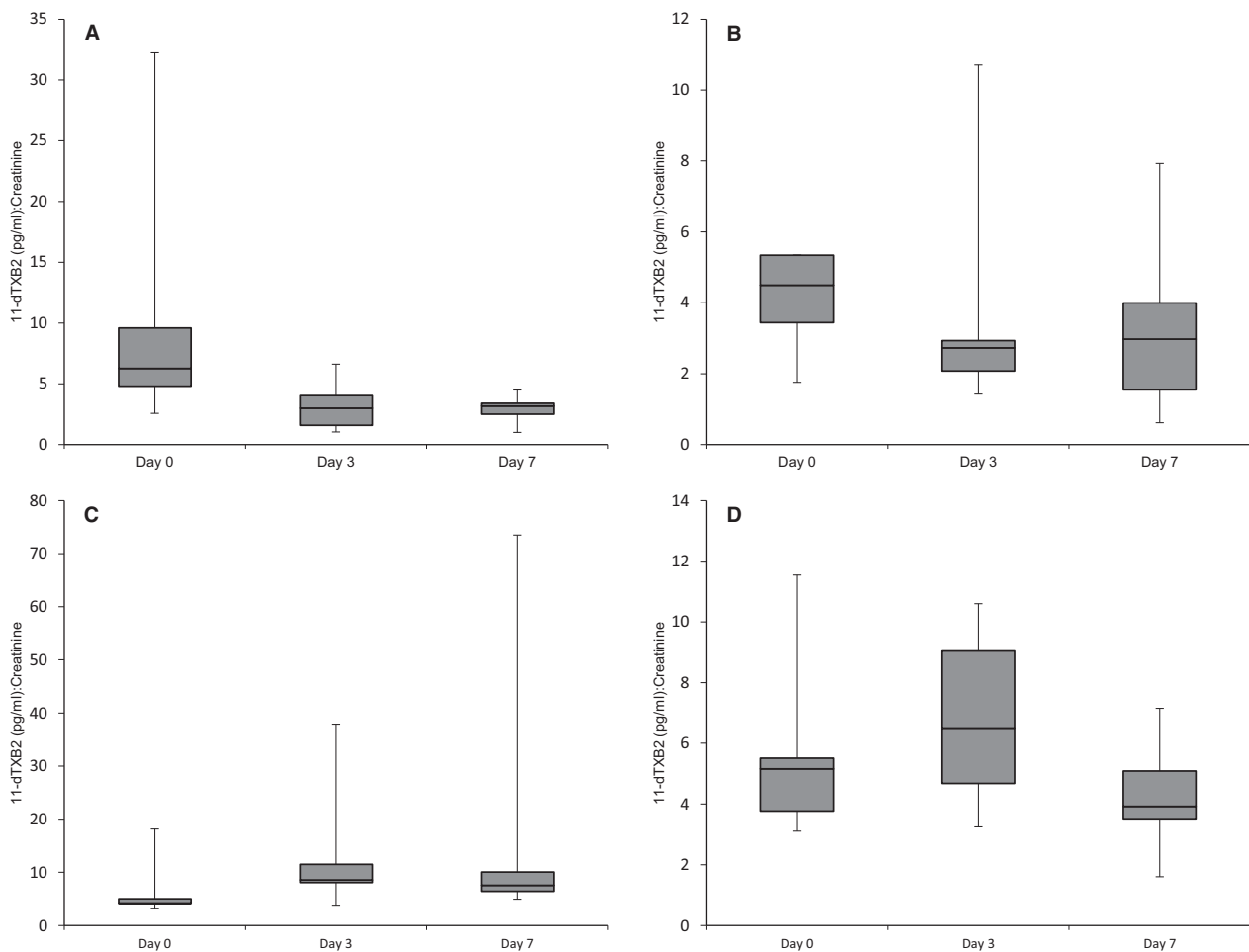

Fig 3. Urine 11-dehydro-TXB$_2$:creatinine ratio seen in dogs treated with (**A**) low-dose aspirin, (**B**) high-dose aspirin, (**C**) cyclosporine, and (**D**) low-dose aspirin and cyclosporine. The box and whiskers plot demonstrates the median (line), interquartile range (box), and total range (whiskers).

dogs in a dose-dependent manner.[10] The results of our current study confirm that, when cyclosporine is administered to dogs at an immunosuppressive dosage, there is a significant increase in thromboxane synthesis. The exact mechanism for the increase in thromboxane is unknown.

Once synthesized, thromboxane A_2 triggers vasoconstriction, causes platelet activation, and enhances platelet aggregation, all of which will increase blood stasis and promote thrombogenesis. After synthesis, thromboxane A_2 is converted rapidly to several metabolites that then are eliminated in the urine.[24] Measurement of urine concentrations of a stable thromboxane metabolite such as 11-dTXB$_2$ is considered a reliable marker of systemic thromboxane A_2 production.[30,31] Although platelets generate substantial amounts of thromboxane A_2, other cells, such as leukocytes and endothelial cells, are capable of synthesizing this eicosanoid.[31] Some of the urinary 11-dTXB$_2$ measured in our study therefore may have been derived these additional cells and not solely platelets. Regardless of the source of thromboxane, our study confirms that cyclosporine administration in dogs is associated with increased thromboxane synthesis. Increased thromboxane synthesis would be expected to enhance platelet reactivity in some dogs. Only about 30% of dogs, however, have platelets that are sensitive to the effects of thromboxane,[32–34] suggesting that drug-induced increases in thromboxane synthesis may not reliably increase platelet reactivity in all dogs. Even if platelets do not consistently respond to thromboxane stimulation in dogs, thromboxane still may contribute to vasoconstriction, which may in turn increase the risk of thrombus formation.

Because IMHA in dogs enhances the risk of thromboembolism, a clinically relevant increase in thromboxane synthesis could predispose these patients to excessive thrombus formation. Antiplatelet therapy such as low-dose aspirin is commonly administered to IMHA patients to inhibit platelet function in an effort to prevent thromboembolism. Aspirin irreversibly inhibits platelet function by preventing the synthesis of thromboxane. Low-dose aspirin (1 mg/kg PO q24h) in dogs significantly decreased urinary 11-dTXB$_2$-to-creatinine ratios within 3 days of starting therapy.[35] In our study, we also identified a numerical decrease in the 11-dTXB$_2$-to-creatinine ratio after administration of low-dose aspirin, but the decrease was not significant between day 0 and days 3 or 7. One likely explanation for the discrepancy between these 2 studies is that our study enrolled a smaller number of dogs compared to the previous study and therefore may have lacked sufficient power to detect a difference between pre- and post-treatment results. Measurement of urinary 2,3-dinor thromboxane B$_2$, a thromboxane metabolite that is more sensitive to the effects of aspirin in dogs than is 11-dTXB$_2$, may have permitted the detection of more subtle decreases in thromboxane synthesis, but unfortunately this assay was not available at the time of our study.[24]

In our study, there was no difference between the 11-dTXB$_2$-to-creatinine ratio results in the low-dose aspirin group and the combined low-dose aspirin and cyclosporine group. Additionally, when compared to the administration of cyclosporine alone, the concurrent use of cyclosporine with low-dose aspirin caused a significant decrease in the 11-dTXB$_2$-to-creatinine ratio, suggesting that low-dose aspirin will prevent cyclosporine-induced thromboxane synthesis. The results of our study suggest that when these 2 drugs are used concurrently, the pharmacological effects of low-dose aspirin overcome cyclosporine-induced thromboxane synthesis and potentially decrease the risk of thromboembolism created by cyclosporine therapy. A similar phenomenon has been observed in rats that were exposed to both drugs concurrently.[28]

Anti-inflammatory dosages of aspirin (typically, 10 mg/kg PO q12h) reliably inhibit platelet function in dogs, as confirmed by our study, but potentially can cause gastrointestinal ulceration and acute renal failure.[14] In our study, 1 dog receiving high-dose aspirin developed mild, self-limiting hematochezia. Much lower dosages (0.5–1 mg/kg PO q24h) of aspirin typically are recommended to maintain antiplatelet effects while decreasing the risk of adverse effects.[36] In our study, we used an aspirin dosage of 1 mg/kg PO q24h, a standard antiplatelet dosage of aspirin in dogs.[24] Low dosages of aspirin, however, do not consistently inhibit platelet function in all dogs.[25,35] In humans, thrombus formation, insufficient or inconsistent inhibition of platelet function or both despite appropriate antiplatelet dosages of aspirin is known as "aspirin resistance". Based on turbidimetric aggregometry, approximately 80% of dogs treated with low dosages of aspirin were considered responsive to aspirin after 3 and 7 days of therapy.[25] In our study, 43% of dogs were classified as aspirin responders based on turbidimetric aggregometry results after 3 days of therapy. However, after 7 days of aspirin therapy, 71% of dogs in our study were considered aspirin responders. These results suggest that after 1 week of low-dose aspirin therapy, the dogs in our study had an aspirin response percentage similar to that of previous studies, but that the dogs in our study required several additional days to become responsive to aspirin. In human and veterinary medicine, both the incidence and mechanism of aspirin resistance is unknown. Unfortunately, there are no established pre-treatment diagnostic tests that can reliably predict which dogs will respond to the antiplatelet effects of low-dose aspirin therapy. Although the dosage of aspirin in our study was effective at negating cyclosporine-induced thromboxane synthesis, it is possible that hypercoagulable patients with hyperactive platelets could require higher doses of aspirin to achieve the same level of thromboxane inhibition.

In our study, platelet function was evaluated using both turbidimetric aggregometry and the PFA-100 platelet function analyzer (collagen/epinephrine cartridge). In both human and veterinary medicine, platelet aggregometry is considered to be the gold standard method for evaluation of drug-induced platelet dysfunction.[22,37] Aggregometry assesses platelet function using agonists to activate platelets in either platelet-rich plasma or

whole blood and measures platelet ability to aggregate.[37] The PFA-100 uses whole blood under high shear forces to assess platelet function and is more practical than aggregometry for the clinical setting. In humans, the collagen/epinephrine cartridge has been shown to be more likely to be influenced by medications, whereas the collagen/ADP cartridge is considered to be a more general indicator of platelet function.[22,38] However, in dogs, there is some degree of test-to-test variability in closure times generated by the collagen/epinephrine cartridge, suggesting that this cartridge is not a precise indicator of drug-induced platelet dysfunction.[20,21] To compensate for this variability, 2 samples per dog per time point were analyzed and averaged. The results of our platelet function analysis suggest that there is no detectable change in platelet function during treatment with cyclosporine. Our results were similar to those of a previous study that evaluated the effects of cyclosporine on canine platelet function,[10] except that our study also utilized platelet aggregometry to provide an additional assessment of platelet function.

Hypercoagulable patients with hyperactive platelets may require a higher dose of aspirin to consistently inhibit platelet function. Even though our study demonstrated that cyclosporine-induced thromboxane synthesis did not enhance platelet aggregation and that the combination of cyclosporine with low-dose aspirin did not counteract the antiplatelet effects of aspirin, both the low-dose aspirin group and the combined low-dose aspirin and cyclosporine group had variable dog-to-dog inhibition of platelet function that is not seen with higher aspirin doses. Based on our aggregometry results on day 3, all of the dogs that were classified as responders with low-dose aspirin also were considered aspirin responders when low-dose aspirin was combined with cyclosporine. Similarly, on day 7, 4 of the 5 dogs that were considered aspirin responders during low-dose aspirin therapy also were responders when low-dose aspirin was combined with cyclosporine. The 1 dog that was classified as an aspirin responder when treated with low-dose aspirin, and classified as a nonresponder when cyclosporine was added to the aspirin therapy, had a decrease in the percentage aggregation at maximum amplitude of 23% when receiving combined low-dose aspirin and cyclosporine. In our study, to be considered an aspirin responder, the percentage aggregation at maximum amplitude must have decreased by 25% from the initial baseline. Therefore, this dog may be considered an aspirin responder with a few additional days of aspirin therapy, or a slightly increased aspirin dose. A higher dose of aspirin, but not as high as the anti-inflammatory dose used in this study, may provide a more consistent inhibition of platelet function, while still decreasing cyclosporine-induced thromboxane synthesis. The ideal antiplatelet dosage of aspirin which provides consistent inhibition of thromboxane synthesis and platelet function, while minimizing any adverse drug reactions, has not been determined in dogs.

Our study had several limitations. Firstly, this study was conducted in healthy dogs and not hypercoagulable or IMHA patients. It is possible that the thromboxane concentration in hypercoagulable patients treated with cyclosporine would be higher than that reported in this study, in which case a 1 mg/kg daily dosage of aspirin might be insufficient to adequately decrease thromboxane synthesis. Continued thromboxane synthesis, along with a decrease in the antiplatelet benefits of aspirin, still could predispose hypercoagulable patients to thromboembolism and necessitate higher doses of anticoagulant and antiplatelet drugs. Secondly, despite a sample size calculation indicating that this study had appropriate power if 8 dogs were enrolled, a smaller number of dogs than expected completed the study. Unfortunately, 1 dog had to be removed from the study because of unexpected illness, which could explain why the study failed to detect a statistically significant decrease in thromboxane synthesis during low-dose aspirin administration.

Recently, cyclosporine has been shown to increase thromboxane synthesis in dogs, suggesting that the use of this medication could increase the risk of thromboembolism, especially in hypercoagulable patients. Our study indicates that, in normal dogs, low-dose aspirin is capable of inhibiting cyclosporine-induced thromboxane synthesis and that the concurrent use of these 2 medications does not significantly alter the antiplatelet effects of aspirin. Therefore, if cyclosporine is used in patients that may be at risk for thromboembolism, the concurrent use of low-dose aspirin may be advisable.

Footnotes

[a] Aspirin, Major Pharmaceuticals, Livonia, MI
[b] Atopica, Novartis Animal Health, Greensboro, NC
[c] 3.2% sodium citrate, Vacutainer tube, Becton Dickinson, Franklin Lakes, NJ
[d] Chronolog 700 Whole Blood/Optical Lumi-Aggregometer, Chronolog Corporation, Haverton, PA
[e] Chronolog 700 Manual; Chronolog Corporation
[f] AGGRO/LINK 8; Chronolog Corporation
[g] PFA-100®, Siemens Healthcare Diagnostics, Deerfield, IL
[h] PFA Collagen/ADP Test Cartridge; Siemens Healthcare Diagnostics, Duluth, GA
[i] PFA Collagen/EPI Test Cartridge; Siemens Healthcare Diagnostics
[j] 11-dehydro-thromboxane B_2 EIA kit-Monoclonal, Cayman Chemical Co, Ann Arbor, MI
[k] SpectraMax M5 Multi-Mode Microplate Reader, Molecular Devices, Sunnyvale, CA
[l] ACE Alera® Clinical Chemistry System, Alfa Wasserman, Inc., West Caldwell, NJ
[m] SAS for Windows version 9.2, SAS Institute, Cary, NC, 2008

Acknowledgments

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