Evaluation of effects of low-dose aspirin administration on urinary thromboxane metabolites in healthy dogs

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Objective—To evaluate markers of in vivo platelet function (urinary 11-dehydro-thromboxane B$_1$, 2,3-dinorTXB$_2$, and 11-dehydroTXB$_2$) and assess their response to administration of 2 commonly used dosages of aspirin in healthy dogs.

Animals—20 healthy dogs.

Procedures—Urine was collected prior to aspirin administration and on the morning following the last evening administration. Twenty dogs received aspirin (1 mg/kg, PO, q 24 h) for 7 consecutive doses. After a washout period of 5 months, 10 dogs received a single dose of aspirin (10 mg/kg, PO). Concentrations of urinary thromboxane metabolites 11-dehydroTXB$_2$, 2,3-dinorTXB$_2$, and 11-dehydroTXB$_2$ were measured via ELISA, and values were normalized to urine creatinine concentration.

Results—Median baseline 11-dehydroTXB$_2$ concentrations were 0.38 ng/mg of creatinine (range, 0.15 to 1.13 ng/mg). Mean ± SD baseline 2,3-dinorTXB$_2$ concentrations were 6.75 ± 2.77 ng/mg of creatinine. Administration of aspirin at a dosage of 1 mg/kg, PO, every 24 hours for 7 days did not significantly decrease urinary 11-dehydroTXB$_2$ concentration, but administration of the single aspirin dose of 10 mg/kg did significantly decrease 11-dehydroTXB$_2$, concentration by a median of 45.5% (range, 28.2% to 67.1%). Administration of the 1 mg/kg aspirin dosage significantly decreased urinary 2,3-dinorTXB$_2$, concentration by a mean ± SD of 33.0 ± 23.7%. Administration of the single aspirin dose of 10 mg/kg also significantly decreased 2,3-dinorTXB$_2$, concentration by a mean ± SD of 46.7 ± 12.6%.

Conclusions and Clinical Relevance—Aspirin administration (1 mg/kg/d) may be insufficient for reliable platelet inhibition in healthy dogs. (Am J Vet Res 2011;72:1038–1045)

A spirin is one of the most widely used drugs in human medicine because of its anti-inflammatory, antipyretic, and antiplatelet properties. The antiplatelet effects of aspirin therapy have been extensively evaluated for use with cardiovascular disease in humans. Extensive experimental$^{1-4}$ and clinical$^{5-8}$ evidence indicates that daily aspirin administration at approximately 1 mg/kg is effective for prevention of arterial occlusion (cardiovascular events) and is safer than anti-inflammatory dosages. When compared with higher dosages, low dosages of aspirin are thought to be safer and more efficacious because platelet cyclooxygenase function is reduced while sparing that of other systems, in particular cyclooxygenase used in endothelial prostaglandin production.$^5$ However, some human patients are inadequately responsive to aspirin at low dosages (termed aspirin resistant). Aspirin resistance is clearly associated with an increased likelihood of cardiovascular events.$^6$

Use of aspirin in dogs for its anti-inflammatory and analgesic properties has been widespread for decades. Pharmacokinetic and clinical studies$^{10-13}$ have indicated that dosages of 10 to 20 mg/kg are associated with substantial anti-inflammatory and antiplatelet effects, as well as the potential for gastrointestinal tract adverse effects. More recently, clinical use of much lower dosages of aspirin for thromboprophylaxis in dogs has been suggested.$^{14}$ Although outcome-based studies are lacking, use of aspirin in dogs has been recommended for prevention of thromboembolic complications of nephrotic syndrome and protein-losing enteropathy.$^{15}$ Recently, results of an uncontrolled retrospective study$^{16}$ of dogs with immune-mediated hemolytic anemia suggested that use of aspirin at 0.5 mg/kg (PO, q 24 h) might improve outcome because this disease may be associated with platelet activation.$^{17}$
In vitro assessments of the effect of low dosages of aspirin on canine platelets are limited. In 1 study, platelet aggregation was significantly reduced in healthy dogs in response to administration of 0.5 mg of aspirin/kg (PO, q 12 h). However, in a recent study, no effect of aspirin at this dosage was detected on platelet function measured via aggregometry and a shear-based function test. Instead, the authors reported 1 mg/kg to be the minimum dosage required to alter platelet function in healthy dogs when assessed with these methods.

Aspirin irreversibly inhibits cyclooxygenase activity, leading to a reduction in TXA₂, a potent platelet activator. Thromboxane A₂ has a half-life in canine blood of approximately 30 to 40 seconds and is rapidly converted to TXB₂, making direct study of TXA₂ impractical. Because TXB₂ is formed nonenzymatically in blood, concentrations can be affected by ex vivo factors such as platelet activation with sampling and sample manipulation, making blood evaluations less reliably useful. Thromboxane B₂ is further metabolized at the tissue level into 2,3-dinorTXB₂ and 11-dehydroTXB₂, which are both excreted in the urine. Because these metabolites are produced in tissues, they are not susceptible to in vitro platelet activation. This conversion to TXB₂ and urinary measurement of TXB₂ is therefore reflective of renal thromboxane synthesis rather than platelet thromboxane production.

In humans, 11-dehydroTXB₂ is an important metabolite associated with platelet thromboxane release. Measurement of urinary 11-dehydroTXB₂ concentrations in humans is becoming increasingly common as an indirect indicator of in vivo platelet activity and individual responsiveness to aspirin therapy. Aspirin resistance in humans. Typically, aspirin dosages of approximately 1 mg/kg/d are associated with > 80% reduction in urinary 11-dehydroTXB₂ concentration in humans. Furthermore, human studies have found a positive correlation between concentrations of 11-dehydroTXB₂ and risk of stroke or cardiac-related death, providing further evidence for its use as a measure of aspirin efficacy.

Several studies have suggested that platelet thromboxane release and metabolism are different in dogs than in other species. Concentrations of TXB₂ in serum of dogs following whole blood clotting are 8 to 375 times those in other species tested. In 1 study, infusion of TXB₂ in dogs somewhat increased blood concentrations of 11-dehydroTXB₂. In another study, infusion of TXB₂ failed to affect plasma concentrations of 11-dehydroTXB₂, whereas 2,3-dinorTXB₂ concentrations increased markedly, indicating that 2,3-dinorTXB₂ may be the major metabolite in dogs.

The purpose of the study reported here was to evaluate the effect of a commonly used dosage of aspirin on urinary thromboxane metabolites as indicators of aspirin efficacy in dogs by use of simple and readily available ELISA kits. As a control to confirm that these assays were capable of detecting aspirin-induced suppression of thromboxane production, urinary excretion of thromboxane metabolites in response to administration of an anti-inflammatory dosage of aspirin was also evaluated. We hypothesized that urinary concentrations of 11-dehydroTXB₂ and 2,3-dinorTXB₂ would decrease in response to administration of both dosages of aspirin, with the latter metabolite having the most pronounced reduction.

Materials and Methods

Study population—Privately owned apparently healthy dogs (n = 24) were volunteered by their owners for inclusion in the study. Routine laboratory screening (CBC, serum biochemical profile, prothrombin time, activated partial thromboplastin time, and urinalysis) was performed to rule out subclinical illness. Potentially relevant abnormalities were identified in 4 dogs, which were then excluded from further participation. The participating dogs (n = 20) were not receiving any medications other than routine heartworm and flea prophylaxis. The study was approved by the University of Illinois Institutional Animal Care and Use Committee.

Urine sample collection and storage—Urine samples were collected at baseline on day 1 (prior to aspirin administration) and again the morning following administration of the last aspirin dose (day 8 for the repeated low dosage). Urine samples were obtained via free catch whenever possible and with ultrasonographically guided cystocentesis when necessary. Additionally, to evaluate the normal day-to-day variation in concentrations of urinary thromboxane metabolites to ensure changes seen were not solely caused by this variation, free-catch urine samples were collected from 4 dogs that were not receiving aspirin on 4 days over a 1-week period. Aliquots of urine were stored at –80°C until batch analysis.

Aspirin preparation and administration—Aspirin was locally compounded into gelatin capsules at approximately 1 mg/kg or 10 mg/kg, depending on study dosage. A combination of pre-filled capsules was dispensed to result in doses of 5, 10, 20, and 25 mg. A combination of pre-filled capsules was dispensed to result in doses of approximately 1 mg/kg or 10 mg/kg, depending on study phase. Dispensed amounts of drug were based on body weight at the time of collection of samples for screening tests. Actual amounts administered ranged from 1.0 to 1.25 mg/kg for the 1 mg/kg dosage and 8.9 to 10.9 mg/kg for the 10 mg/kg dosage.

Repeated low-dosage phase

The dog’s owner was instructed to administer study-provided aspirin (1 mg/kg, q 24 h, PO) in the evenings with food for 7 consecutive doses. Owners were instructed to keep a daily drug administration log to evaluate compliance.

Single anti-inflammatory dose phase

As a control, some dogs (n = 10) additionally received a single anti-inflammatory dose (10 mg/kg) of aspirin 5 months after completion of the low-dosage portion of the study. A morning baseline free-catch urine sample was again collected, after which each dog received the single dose in the evening. Postadministration free-catch urine samples were collected the following morning.
Measurement of urinary thromboxane metabolite concentrations—Urine samples were thawed immediately prior to evaluation. Concentrations of urinary thromboxane metabolites were measured by use of commercially available ELISA kits according to the manufacturer's instructions. Use of the 11-dehydroTXB2 kit3 has been validated for canine urine.28 Use of the TXB2 kit and the 2,3-dinorTXB2 kit was validated locally as described. Samples were assayed at 2 dilutions and loaded in duplicate. Baseline and postadministration samples for each dog were assayed in the same test plate. Thromboxane metabolite values were corrected for urinary concentrating ability by use of measured urinary creatinine concentration to calculate a urinary thromboxane-to-creatinine ratio.

Results of ELISAs for urinary TXB2 and 2,3-dinorTXB2 were corrected for cross-reactivity, with calculations performed by use of manufacturer-reported cross-reactivity rates according to formulas:

$$\text{TXB}_2 \text{ELISA result } = 0.099 \times (\text{true TXB}_2) + \text{true 2,3-dinor TXB}_2$$

$$\text{2,3-dinorTXB2 ELISA result } = \text{true TXB}_2 + \text{true 2,3-dinor TXB}_2$$

Validation of the TXB2 and 2,3-dinorTXB2 assay kits—Neither TXB2 nor 2,3-dinorTXB2 is a species-specific compound,3 so documentation of antibody reactivity with canine-specific molecules was not necessary. In-house validation of the TXB2 kit was limited to determination of dilutional parallelism and intra-assay variation because this assay was not used for evaluation of the effect of aspirin administration. The manufacturer indicates that urine sample purification is not necessary for use with the TXB2 kit.

The manufacturer suggests purification of human urine samples for use with the 2,3-dinorTXB2 kit for 2 reasons: cross-reactivity for TXB2 is high for this kit, and concentrations of 2,3-dinorTXB2 in human urine are generally low in comparison to TXB2 (which would be removed in the purification process). Because canine urine contains much higher concentrations of 2,3-dinorTXB2 than human urine, removal of the cross-reacting molecule is not necessary. Purification may possibly be needed to remove other interfering substances; however, previous assessments of the 11-dehydroTXB2 kit indicated that canine urine does not contain interfering substances that would require sample purification prior to performance of ELISAs for urinary thromboxanes.28 Samples were consequently not purified prior to performance of the 2,3-dinorTXB2 ELISA.

Statistical analysis—Data were evaluated for normality by use of a Kolmogorov-Smirnov test. Correlation between platelet count and urinary thromboxane metabolite concentrations was evaluated via Pearson product moment correlation analysis. Baseline values for urinary thromboxane metabolite concentrations were compared between low dosage and anti-inflammatory dosage aspirin administration by use of a paired t test. Values for urinary thromboxane metabolite concentrations in baseline and postadministration samples were compared by use of repeated-measures ANOVA. When data were normally distributed, a Tukey test was used to determine P values. When data were not normally distributed, repeated-measures ANOVA on ranks was performed. Tests were performed by use of statistical software. A P value < 0.05 was considered significant.

Results

Sample population—Of the original 24 dogs, 20 (7 spayed females, 1 sexually intact male, and 12 castrated males) met the inclusion criteria for the study. Study participants ranged from 6.3 to 35 kg (mean ± SD, 21.3 ± 9.1 kg) in body weight and 2 to 9 years (mean ± SD, 2.7 ± 3.2 years) of age. Ten of the dogs were of mixed breeds, and 10 were purebred, including 1 of each of the following: French Bulldog, Border Collie, Bichon Frise, Miniature Poodle, German Shorthaired Pointer, Australian Shepherd, Boston Terrier, Boxer, Catahoula Leopard Dog, and Shetland Sheepdog. Three of the excluded dogs were mildly thrombocytopenic (platelet count < 175,000 platelets/μL). Although the thromboxypenia in these 3 dogs was mild and unlikely to be clinically relevant, they were excluded because of their potential effect on global platelet function indicators. The remaining excluded dog had markedly high serum alkaline phosphatase activity. Urine samples from 2 of the dogs in the repeated low-dose aspirin study were acquired via ultrasonographically guided cystocentesis. Free-catch collection was used for all remaining urine samples. All 20 of the included dogs received each of the provided aspirin dosages on the basis of the drug administration logs. A few dogs received aspirin doses at unplanned times during the repeated low-dosage protocol (6 doses were 12 hours late and 2 doses were 12 hours early of 140 total doses administered). No adverse effects were reported for any of the dogs receiving the repeated low doses of aspirin. All 10 dogs received the anti-inflammatory dose of aspirin at the appropriate times. Five hours after receiving the anti-inflammatory dose of aspirin, 1 dog vomited and promptly consumed the vomitus in its entirety with no further adverse effects reported.

Assay validation—For the TXB2 kit, the manufacturer reports 9.9% cross-reactivity with 2,3-dinorTXB2 and 0.42% with 11-dehydroTXB2. The reported lower limit of detection is 11 pg/mL. The working standard range on in-house assays was 15 to 400 pg/mL. In-house validation indicated good dilutional parallelism for 5 samples assayed at 4 dilutions (8-, 15-, 30-, and 60-fold), with the range of observed and expected ratios of 82% to 127% for samples being within the working standard range. The median intra-assay coefficient of variation was 3.4% (range, 0.1% to 27%) for 10 samples assayed in duplicate. Study samples were subsequently diluted 15- and 60-fold for analysis.

For the 2,3-dinorTXB2 kit, the manufacturer reports 100% cross-reactivity with TXB2 and 1.5% with 11-dehydroTXB2. The reported lower limit of detection is 7 pg/mL. The working standard range on in-house assays was 10 to 575 pg/mL. In-house validation indicated strong dilutional parallelism for 5 samples assayed at 5 dilutions (30-, 60-, 90-, 120-, and 240-fold) with the range of observed and expected ratios of 84%
to 104% for samples with concentrations within the working standard range. Cold spiking with manufacturer-provided 2,3-dinorTXB2 of 2 and 5 ng/mL into a sample containing 13.8 ng/mL resulted in 106% and 101% recovery, respectively. The median intra-assay coefficient of variation was 2.9% (range, 0.1% to 11%) for 10 samples assayed in duplicate on the same plate. The mean ± SD interassay coefficient of variation was 10.9 ± 8.4% for 14 samples assayed on 2 plates. Study samples were thereafter diluted 60- and 120-fold for analysis, with occasional samples requiring 240-fold dilution to be within the acceptable standard range.

**Urinary 11-dehydroTXB2—**Baseline urinary 11-dehydroTXB2 concentrations were low with a median of 0.38 ng/mg of creatinine (range, 0.15 to 1.13 ng/mg of creatinine) prior to repeated administration of a low dosage of aspirin (n = 20 dogs) and 0.64 ng/mg of creatinine prior to administration of a single anti-inflammatory dosage (10). Baseline urinary 11-dehydroTXB2 concentration was significantly (P = 0.040) positively correlated with platelet count (r = 0.462; Figure 1). Baseline values were significantly (P = 0.019) different between the 2 dosage protocols (measured 5 months apart) for the 10 dogs that received both dosages, with a median of coefficients of variation between the 2 baseline samples of 2.7% (range, 6.2% to 32.4%).

Urinary 11-dehydroTXB2 concentrations were not significantly different from baseline values following repeated low-dosage aspirin administration (median, 0.29 ng/mg of creatinine [range, 0.15 to 1.80 ng/mg of creatinine]; P = 0.37). Median change in response to repeated low dosage was –12.2% (range, –69.1% to 120.7%; Figure 2). In 8 of the 20 dogs, urinary 11-dehydroTXB2 concentrations were higher following repeated low-dosage aspirin administration than at baseline.

Urinary 11-dehydroTXB2 concentrations were significantly different from baseline values following administration of the single anti-inflammatory aspirin dosage (median, 0.27 ng/mg of creatinine [range, 0.15 to 0.50 ng/mg of creatinine]; P < 0.001). Median reduction of 11-dehydroTXB2 concentration in response to administration of the single anti-inflammatory dosage was 45.5% (range, 28.2% to 67.1%; Figure 2). The 11-dehydroTXB2 concentration decreased in all 10 dogs, and the magnitude of the decrease in response to administration of a single anti-inflammatory aspirin dosage was larger than that which occurred in response to repeated administration of a low dosage of aspirin for all dogs.

**Urinary TXB2—**Baseline urinary TXB2 concentrations were measured in 20 samples to evaluate the potential effect of cross-reactivity. Obtained values for TXB2 concentration were low, with a mean ± SD of 0.40 ± 0.19 ng/mg of creatinine. Correction of measured TXB2 concentrations for cross-reactivity with 2,3-dinorTXB2 resulted in a mean ± SD baseline value of 0.26 ± 0.14 ng/mg of creatinine.

**Urinary 2,3-dinorTXB2—**Baseline urinary 2,3-dinorTXB2 concentrations were markedly higher than 11-dehydroTXB2 concentrations and TXB2 concentrations at a mean ± SD of 6.75 ± 2.77 ng/mg of creatinine prior to repeated administration of a low dosage of aspirin (n = 20) and 2.84 ± 1.1 ng/mg of creatinine prior to single administration of an anti-inflammatory dosage of aspirin (n = 10). Baseline urinary 2,3-dinorTXB2 concentration was significantly (P = 0.006) positively correlated with platelet count (r = 0.593; Figure 1). Baseline values were significantly (P = 0.001) different between the 2 dosage protocols (measured 5 months apart) for the 10 dogs that received both dosages, with mean ± SD coefficients of variations between the 2 baseline samples of 35.9 ± 25.2%. Because urinary concentrations of TXB2 were low in comparison with those of 2,3-dinorTXB2, correction of the ELISA results for cross-reactivity had a negligible effect on measured 2,3-dinor concentrations. Mean ± SD decrease in 2,3-dinorTXB2 when corrected for cross-reactivity for TXB2 was 10 ± 5% in the 20 samples tested. The remaining reported data were consequently not corrected for cross-reactivity.

**Urinary 2,3-dinorTXB2 concentrations were significantly different from baseline values following repeated administration of the low dosage of aspirin (mean ± SD, 4.29 ± 1.8 ng/mg of creatinine; P < 0.001). Mean ± SD reduction of 2,3-dinorTXB2 in response to repeated administration was 33.0 ± 23.7% (Figure 3). In 3 of the 20 dogs, urinary 2,3-dinorTXB2 concentrations were.
higher following repeated administration of low doses of aspirin, compared with baseline.

Urinary 2,3-dinorTXB₂ concentrations were also significantly different from baseline values following administration of the single anti-inflammatory dosage of aspirin (mean ± SD, 1.46 ± 0.61 ng/mg of creatinine; P < 0.001). Mean ± SD reduction of 2,3-dinorTXB₂ in response to administration of the single anti-inflammatory aspirin dosage was 46.7% ± 12.6% (Figure 3). The 2,3-dinorTXB₂ concentration decreased in all 10 dogs, and in 8 of 10 dogs, the magnitude of the decrease in response to administration of a single anti-inflammatory dosage of aspirin was larger than that which occurred in response to repeated administrations of low dosages of aspirin.

Normal day-to-day variability in concentrations of urinary 2,3-dinorTXB₂ within a 7-day period for 4 dogs not receiving aspirin was minor, with a range of changes from baseline of −20.6% to 38.9%. Interday variability was characterized by coefficients of variation of 9.7% to 22.4%.

Discussion

The ideal method for evaluating the effect of aspirin administration on platelet function has not been determined. Bleeding time assessments, although they do evaluate platelet function in vivo, are insensitive to the effect of aspirin administration and are subject to marked variability. Platelet function can be assessed in vitro by use of aggregometry with whole blood, platelet-rich plasma, or washed platelets. Light transmission aggregometry following stimulation of platelet-rich plasma with a platelet agonist is considered the gold standard for evaluation of aspirin resistance and has predictive value for clinical outcomes in human patients. This approach has limited clinical use because it requires specialized laboratories and technical skill.

Use of newer specific point-of-care tools such as a platelet function analyzer and platelet mapping with thromboelastography has recently increased. These in vitro tests can be adversely affected by platelet activation during sample collection and require specialized reagents and equipment. Several reports describe measurement of TXB₂ or its metabolites in canine plasma, serum, or urine, but this approach has the potential to be influenced by ex vivo factors. Plasma thromboxane concentrations may be artifactually influenced by platelet activation during sample collection, and serum thromboxane concentration is indicative of the total capacity of platelet responses. Because TXA₂ is transformed to TXB₂ in blood and then metabolized to 11-dehydroTXB₂ and 2,3-dinorTXB₂ by tissues, urinary concentrations of these metabolites are thought to provide an indication of ongoing whole-body in vivo platelet thromboxane release. In contrast, urinary TXB₂ is primarily of renal origin rather than an indicator of platelet function. Potential advantages of measuring concentrations of urinary thromboxane metabolites over other tests include ease of sample acquisition (particularly in the outpatient setting), lack of need for specialized equipment or expertise (other than a standard microplate reader), and insensitivity to ex vivo factors.

When the different function methods have been compared with human platelets, there has been inconsistent agreement in defining aspirin resistance. Measuring urinary 11-dehydroTXB₂ to determine platelet responsiveness to aspirin is clinically relevant because concentrations of this metabolite are correlated with risk of stroke or cardiac death in humans.

Both thromboxane metabolites measured in the present study have the potential to be useful indicators of canine platelet function. Plasma 11-dehydroTXB₂ increases in dogs in response to infusion of platelet activating factor, and this change is blocked by prednimustidine of a high dosage (approx 45 mg/kg) of aspirin. Urinary 2,3-dinorTXB₂, increases with induction of coronary thrombosis in a model in dogs, and the increase is prevented by administration of a thromboxane synthetase inhibitor. Indomethacin (a cyclooxygenase inhibitor) decreases urinary excretion of 2,3-dinorTXB₂ in a dose-dependent manner in dogs. Urinary 2,3-dinorTXB₂ concentration is significantly reduced in dogs receiving aspirin at approximately 6 to 12.5 mg/kg, compared with controls in a cardiopulmonary bypass model.

In the present study, both ELISAs were easy to implement and performed consistently well with canine urine. Both urinary thromboxane metabolites were significantly positively correlated with platelet count, although the 2,3-dinorTXB₂, correlation was markedly better. This finding, which is consistent with data from healthy and thrombocythemic humans, supports the concept that urinary thromboxane metabolites are a time-averaged indicator of in vivo biosynthetic activity for platelet-mediated thromboxane release. Direct comparison of our results for 11-dehydroTXB₂-to-creatinine ratios with those reported previously from dogs is difficult because of variations in reporting methods. One study of experimentally induced glomerulonephritis in healthy dogs reported baseline urinary excretion rates of 11-dehydroTXB₂, measured via ELISA as approximately 1,200 pg/min/kg. Another report describing use of the 11-dehydroTXB₂ ELISA for dog urine reported a median value for the ratio to creatinine.
of 9.83, but exact units were not given, so direct comparison was not possible. Concentrations of 11-dehydroTXB₂ in dog urine in the present study were similar to those obtained with the ELISA method for human urine.³⁶,³⁷,³⁸ Comparison of the 2 sets of pre–aspirin administration baseline urine samples collected 5 months apart indicated some variability over this time frame. This apparent variance could be an artifact associated with the low concentrations of this metabolite in canine urine, a function of normal time-related intrapatient variability in platelet release of thromboxane, or a result associated with changes over time in platelet count. In humans, aspirin responsiveness is generally evaluated by use of a percentage decrease in response to treatment or, more commonly, by comparison of postadministration concentrations with a clinically validated cutoff value. Because a validated maximum postadministration metabolite concentration has yet to be determined for dogs, we used the percentage decrease approach. To eliminate any possible effects of interday variability for the assay, baseline and postadministration samples were always assessed together on the same ELISA plate.

The baseline 2,3-dinorTXB₂ concentrations were similar to or slightly higher than those in other studies¹³,³⁶,³⁷,³⁸ that used other methods for analysis of canine urine. As for 11-dehydroTXB₂, baseline concentrations were quite different between urine samples collected prior to the 2 dosages that were administered 5 months apart. Concentrations of 2,3-dinorTXB₂ in human urine are typically markedly lower (approx 0.1 ng/mg of creatinine).³⁶,³⁷ The lower concentrations of 2,3-dinorTXB₂ in human urine make cross-reactivity with TXB₂ more of an issue, which may be 1 reason that 11-dehydroTXB₂ is a preferred marker of responsiveness to aspirin in this species.

In the present study, both urinary thromboxane metabolite concentrations decreased by approximately 45% in response to administration of a single aspirin dose of 10 mg/kg. Results of most studies³⁴,³⁵,³¹,³³ using other methodologies indicate that this dosage of aspirin affects canine platelet function. In the dogs reported here, the magnitude of the observed decrease for 11-dehydroTXB₂ concentration in response to a single administration of 10 mg/kg was lower than that reported for humans.⁴¹

There was no consistent effect of repeated low-dosage aspirin administration on urinary 11-dehydroTXB₂ concentration in this study. Although concentrations decreased in some dogs, they increased in other dogs. The majority (70%) of dogs were more responsive to a single anti-inflammatory administration than to the repeated low-dosage administration protocol. Repeated administration of doses of aspirin ranging from 75 to 100 mg/d in humans generally suppresses urinary 11-dehydroTXB₂ concentration by approximately 80%.⁴⁵–⁴⁸ No dogs in the present study reached the magnitude of decrease generally observed in most humans.

Although the concentrations of 2,3-dinorTXB₂ measured after repeated administrations of low dosages of aspirin were significantly different from baseline, the clinical relevance of the observed changes was uncertain. Concentrations decreased in most dogs but increased in a few dogs. Repeated administration of aspirin in humans at dosages from 30 to 100 mg/d suppresses urinary 2,3-dinorTXB₂ concentration by approximately 80%.³⁵,³⁸ In no dogs in the present study did the magnitude of the decrease approach that generally observed in response to similar doses for most humans. Furthermore, all dogs were more responsive to administration of a single anti-inflammatory dosage than to the repeated administration dosage protocol. Lastly, the degree of changes observed in response to repeated administration of low dosages of aspirin was not markedly different from those observed with normal day-to-day variation in dogs not receiving aspirin. Therefore, it is possible the changes seen with the low dosages of aspirin were attributable to the expected day-to-day variation in 2,3-dinorTXB₂ excretion rather than a direct drug effect.

It is not yet clear which metabolite would be the better measure of in vivo platelet response to aspirin for dogs. A major disadvantage of use of 11-dehydroTXB₂ in canine urine is that concentrations are low, so samples must be minimally diluted, creating the potential for interference. It is also not entirely clear from published experimental models in dogs that 11-dehydroTXB₂ is a relevant metabolite of platelet TXA₂ release in dogs.⁶,²⁷ One of the potential advantages of using 11-dehydroTXB₂ is the extrapolation of response targets from the extensive supply of outcome-based data in humans that has been published. However, the data from humans are primarily from a population with cardiovascular disease (arterial thrombosis), whereas one of the major target populations for thromboprophylaxis in dogs is affected primarily by venous thrombosis and thromboembolism. The potential usefulness of antiplatelet treatment in this clinical canine population remains to be proven.

The advantages of using 2,3-dinorTXB₂ are that concentrations in canine urine are high, making them easily detectable with marked dilution, and that experimental models confirm this metabolite is an indicator of platelet function in dogs.¹³,³⁶,³⁷ There are also several possible disadvantages of using urinary 2,3-dinorTXB₂ concentration to evaluate responses to aspirin in dogs. Because this metabolite is rarely measured in human clinical populations, expected response versus outcome data are minimal. An additional concern is the cross-reactivity of the ELISA with TXB₂. In the healthy dog population of the present study, TXB₂ concentrations were markedly lower than 2,3-dinorTXB₂ concentrations and responded similarly to administration of aspirin. As a consequence, the cross-reactivity had minimal to no effect on interpretation of the 2,3-dinorTXB₂ ELISA results. It is possible that renal production of TXB₂ might be altered in disease states so that cross-reactivity could have a larger or more unpredictable effect on 2,3-dinorTXB₂ concentrations as measured via ELISA in samples from clinical populations. This problem could be overcome with routine purification of the urine samples, but such a procedure greatly increases the technical difficulty of performing the assay. Lastly, the shorter half-life of this metabolite could potentially result in more episodic fluctuations in urinary concentrations in response to aspirin administration.
As an assessment of the effect of low dosages of aspirin on canine platelets, our study had several relevant limitations. We did not perform checks of owner compliance other than the provided daily drug administration logs, so the lack of adequate response to repeated low-dosage administration of aspirin may have been influenced by missed doses. Additionally, several of the doses in a few of the dogs were given at improper times for the study, which could have influenced results. It is possible that neither metabolite is responsive to aspirin administration in dogs, but the consistent decreases in both metabolites in response to the higher dosage suggest that this is not true. We elected to not evaluate aspirin responsiveness by use of other methods for comparison. Previous reports\(^{1,7,10,11}\) evaluating the effect of low dosages by use of other methods have indicated inconsistent results, with some indicating decreased platelet function as measured via aggregometry and others indicating no effect on platelet function.

Neither 11-dehydroTXB\(_2\), nor 2,3-dinorTXB\(_2\) concentrations indicated consistent suppression of platelet function with daily administration of 1 mg of aspirin/kg in clinically normal dogs. Results of the present study suggest that daily dosages of 1 mg/kg or less may not consistently affect platelet-mediated thrombosis and thromboembolism on the basis of criteria from human medicine. Direct comparison of urinary metabolites to other methods of assessing platelet function such as aggregometry or a commercial platelet function analyzer may be warranted, and outcome-based, appropriately controlled studies of low-dose aspirin administration are needed before similar dosages can be recommended for clinical use.

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References


