Therapeutic monitoring of rivaroxaban in dogs using thromboelastography and prothrombin time

Junwoo Bae1 | Hyunwoo Kim1 | Woosun Kim1 | Suhee Kim2 | Jinho Park3 | Dong-In Jung4 | Dohyeon Yu4

1College of Veterinary Medicine, Chonnam National University, Gwangju, Republic of Korea
2National Institute of Animal Science, Rural Development Administration, Wanju, Republic of Korea
3College of Veterinary Medicine, Chonbuk National University, Iksan, Republic of Korea
4College of Veterinary Medicine, Gyeongsang National University, Jinju, Republic of Korea

Correspondence
Dohyeon Yu, College of Veterinary Medicine, Gyeongsang National University, Jinju 52828, Republic of Korea.
Email: yudh@gnu.ac.kr

Abstract

Background: The chromogenic anti-Xa assay, the gold standard for monitoring the anti-Xa effect of rivaroxaban, is not available as a cage-side diagnostic test for use in a clinical setting.

Hypothesis/Objectives: To evaluate clinical modalities for measuring the anticoagulant effects of rivaroxaban using a point-of-care prothrombin time (PT) and thromboelastography (TEG).

Animals: Six healthy Beagle dogs.

Methods: Prospective, experimental study. Four different doses of rivaroxaban (0.5, 1, 2, and 4 mg/kg) were administered PO to dogs. Single PO and 3 consecutive dosing regimens also were assessed. Plasma rivaroxaban concentration was determined using a chromogenic anti-Xa assay, point-of-care PT, and TEG analysis with 4 activators (RapidTEG, 1:100 tissue factor [TF100], 1:3700 tissue factor [TF3700], and kaolin), and results were compared. Spearman correlation coefficients were calculated between ratios (peak to baseline PT; peak reaction time [R] of TEG to baseline [R] of TEG) and anti-Xa concentration.

Results: Anti-Xa concentration had a significant correlation with point-of-care PT (R = 0.82, P < .001) and RapidTEG-TEG, TF100-TEG, and TF3700-TEG (R = 0.76, P < .001; R = 0.82, P < .001; and R = 0.83, P < .001, respectively).

Conclusions and Clinical Importance: Overall, a 1.5-1.9 × delay in PT and R values of TEG 3 hours after rivaroxaban administration is required to achieve therapeutic anti-Xa concentrations of rivaroxaban in canine plasma. The R values of TEG, specifically using tissue factors (RapidTEG, TF100, TF3700) and point-of-care PT for rivaroxaban can be used practically for therapeutic monitoring of rivaroxaban in dogs.

KEYWORDS
anti-Xa, oral anticoagulant, point-of-care PT test, TEG

Abbreviations: ACT, activated clotting time; APTT, activated partial thromboplastin time; PT, prothrombin time; SP, split point; TEG, thromboelastography; TF100, tissue factor 1:100; TF3700, tissue factor 1:3700; TMRTG, time to maximum rate of thrombus generation.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2019 The Authors. Journal of Veterinary Internal Medicine published by Wiley Periodicals, Inc. on behalf of the American College of Veterinary Internal Medicine.
1 | INTRODUCTION

Despite the need for anticoagulants in companion animals, there have been some limitations with the use of anticoagulant agents in veterinary medicine. Agents such as warfarin, a PO administered anticoagulant, are rarely used in veterinary medicine because of safety issues, and heparin is available only in injectable form, which makes it difficult for animal owners to manage long-term treatment.

Recently, newly developed PO direct factor Xa anticoagulants were approved for the treatment of thrombosis in human patients. These anticoagulants have wide safety margins, making them safer for use than earlier anticoagulants. Rivaroxaban is a novel PO direct factor Xa anticoagulant that decreases the risk of stroke and systemic embolism in human patients with nonvalvular atrial fibrillation and is also used for the prophylaxis and treatment of deep vein thrombosis and pulmonary embolism.1–4 Unfortunately, rivaroxaban has not been used widely in clinical settings in veterinary medicine.2,4

Rivaroxaban exerts its anticoagulant effect by directly inhibiting activated factor Xa. Therefore, the assay to evaluate the activity of factor Xa can be used to evaluate plasma rivaroxaban concentration indirectly. The assay measures the amount of active Xa using a chromogenic substrate, and the absorbance is inversely proportional to the rivaroxaban concentration. This assay, commonly referred to as “chromogenic anti-Xa assay,” is considered a suitable method for monitoring the effect of rivaroxaban,5–7 but this test is impractical in veterinary medicine because of its limited availability.

In human medicine, prothrombin time (PT) can be an alternative measurement to plasma rivaroxaban concentration for monitoring treatment and is a more accurate strategy for assessing rivaroxaban than is activated partial thromboplastin time (aPTT).6,8–10 Variations in sensitivity were reported when different PT reagents were used. Modification of PT measurement has been proposed to enhance sensitivity and decrease reagent variability.6,7,11,12 Unlike in human medicine, alternative measurements of rivaroxaban concentration for therapeutic monitoring in veterinary medicine have not been reported.

Thromboelastography (TEG) is a viscoelastic hemostatic assay that measures the global viscoelastic properties of whole blood clot formation.11 In a previous study, TEG using different strong activators was used to monitor unfractionated heparin treatment in dogs.12 Evaluating the effect of rivaroxaban in blood using TEG appears promising11,14 but may yield variable results with different activators.

Rivaroxaban could be a more attractive long-term therapeutic option for outpatients with thrombotic diseases than is warfarin because it previously has been reported to have higher PO bioavailability (60%-86%) in dogs.5,13 However, guidelines for rivaroxaban use, including dosing regimens and monitoring methods in dogs with clinically relevant hypercoagulability, have not yet been established in veterinary medicine. Thus, our goal was to prospectively evaluate clinical methods (point-of-care PT and TEG with 4 different activators) for detecting the anticoagulant effect of rivaroxaban by measuring anti-Xa concentration in healthy dogs. We hypothesized that 1 of the variables of PT and TEG at peak time would correlate with serum anti-Xa concentration after administering rivaroxaban and could be applied as a cage-side diagnostic test.

2 | MATERIALS AND METHODS

2.1 | Animals

Six Beagle dogs (1-year-old intact males) underwent an adaptation period in a university-owned facility. The research protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC, YB-R-2017-05). The median weight was 9.95 kg (range, 9.7–12.3 kg). The dogs were deemed healthy based on physical examination, CBC (Procyte Dx hematology analyzer), serum biochemistry analysis (Catalyst Dx chemistry analyzer), coagulation profile (Coag Dx analyzer), urinalysis (VetLab UA analyzer, all IDEXX, Westbrook, Maine), and Doppler blood pressure measurement.

2.2 | Drug administration

Commercially available 20 mg rivaroxaban tablets (Xarelto 20 mg tablets; Bayer HealthCare AG, Leverkusen, Germany) were split using a pill cutter and then administered to the dogs. Single PO doses of rivaroxaban at 0.5, 1, 2, and 4 mg/kg were administered to 6 dogs with a 2-week washout period between the different dosages. To detect residual activity and cumulative effect of rivaroxaban, 0.5 and 1 mg/kg rivaroxaban were administered to 3 randomly selected dogs every 24 hours for 3 consecutive days. The dogs were not fasted and were evaluated twice daily during the study for any adverse effects. General physical examination and signs of bleeding were evaluated.

2.3 | Sample collection

For the single PO dose trials, blood samples were collected before and 3 hours after each dose administration. Six milliliters of blood were drawn from the jugular vein using 10-mL plastic syringes with 18G (38 mm) needles (Jungrim Medical, Korea). The blood was transferred into 3 3.2% sodium citrate tubes (Vacuette, 3.2% sodium citrate, 2-mL tubes; Greiner Bio-One, Kremsmünster, Austria) with a final citrate : blood ratio of 1 : 9.

One day before drug administration, 3 randomly selected dogs each in the 0.5 and 1 mg/kg dosage trial groups were given 0.5 mg/kg butorphanol (Butorphan Inj 2 mg/mL; Myungmoon Pharm, Korea) IV, and jugular venous catheters (18G, 20 cm single-lumen catheter; Sungwon Medical, Korea) were placed using a modified Seldinger technique for the 3 consecutive-day dosing trials. The catheters were irrigated with 0.9% normal saline (0.9% sodium chloride 100 mL; Daihan Pharm, Korea), and blood was collected using a 3-syringe technique to avoid hemodilution of the test sample. A purge sample of 6 mL was withdrawn from the catheter and then returned to the dog after obtaining a test sample. For 3 consecutive days during the dosing trials, 6 mL blood samples were collected before dosing (0 hours) and 3, 8, and 12 hours after PO dosing on days 1, 2, and 3. Final samples were obtained on day 4 at 0 hours (72 hours). Blood samples were
transferred into 3.2% sodium citrate tubes as described above. Additionally, blood for PCV, total protein concentration, platelet count, and fibrinogen concentration analyses was collected into tubes containing ethylenediaminetetraacetic acid (EDTA, 1.3 mL micro EDTA tubes; Sarstedt Inc, Germany) at the 0 and 12 hours time points on day 1 and 0 hour time point on day 4. Citrated whole-blood samples were submitted within 10 minutes of collection for PT (Coag Dx analyzer; IDEXX) and TEG (TEG 5000 Hemostasis Analyzer; Haemonetics Corp, Braintree, Massachusetts) analyses. The remaining citrated plasma was centrifuged at 2000g for 10 minutes, and the supernatant was stored at −80°C until the anti-Xa assay was performed.

2.4 | Analysis of blood samples

Citrated whole blood was used to perform PT and TEG analyses. The PT was evaluated within 2 hours of blood collection using a point-of-care device and cartridges for point-of-care PT devices were used after a 30-minute rest period at room temperature. Citrated whole blood samples were used for TEG after a 30-minute rest period at room temperature. The TEG analysis was performed within 2 hours of blood collection using the recalification method with 4 different activators (kaolin, RapidTEG [both Haemonetics Corp], tissue factor [Thromborel S, Siemens Healthcare Diagnostics Inc], 1 : 3700 [TF3700], and tissue factor 1 : 100 [TF100]). The 2 different dilution ratios of tissue factor (TF100 and TF3700) were chosen based on previous studies. Briefly, 20 µL of 0.2 M calcium chloride (CaCl₂) was added to disposable TEG cups for each test. The cups were preheated to 37°C before initiation. The TEG analyses using kaolin and RapidTEG as activators were performed according to the manufacturer's instructions. Samples for tissue factor as an activator contained an additional 10 µL of diluted tissue factor added to the reaction cup (340 µL of citrated whole blood was added to preheated cups containing 20 µL of 0.2 M CaCl₂, and 10 µL diluted tissue factor mixture). Diluted tissue factor was prepared within 24 hours of the analysis using normal saline. Then, 1 : 2.7 diluted tissue factor was added to perform the TF100-TEG (final dilution ratio with 340 µL blood and 20 µL of 0.2 M CaCl₂, [approximately 1 : 100]). Then, 1 : 100 diluted tissue factor was added to perform the TF3700-TEG (final dilution ratio with 340 µL blood and 20 µL of 0.2 M CaCl₂ was 1 : 3700).

When strong activators such as RapidTEG and TF100 were used, the R value results were obtained within minutes because these agents have strong activating properties. Thus, the R value results were set to seconds rather than minutes for precise analysis when RapidTEG and TF100 were used as activators. Besides conventional parameters of TEG (reaction time [R], clotting time [K], angle, and maximum amplitude), nonconventional parameters such as split point (SP), delta (R-SP), and time to maximum rate of thrombus generation (TMRTG) from the thrombus velocity curve were also calculated.

Citrated plasma was used to perform the anti-Xa assay according to the manufacturer's instructions as previously described. A commercial chromogenic substrate test (Technochrom anti-Xa) with the manufacturer's rivaroxaban calibrators (Technoview Rivaroxaban CAL Set) and control plasma (Technoview Rivaroxaban Control, all Technoclone, Vienna, Austria) was used. Samples with rivaroxaban concentration >150 ng/mL were reassessed at 1 : 20 dilution using the high calibrator set (Technoview Rivaroxaban CAL High Set). The assay was performed in 96-well microtiter plates in duplicate, and the absorbance was read at 405 nm with 620 nm as the reference wavelength using a microplate reader (VersaMax, Molecular Devices, Boston, Massachusetts). The detection limit for rivaroxaban in this assay was 10 ng/mL.

The EDTA blood samples were processed to obtain PCV, total protein concentration, platelet count, and fibrinogen concentration. Total protein concentrations were measured using a refractometer. Platelet counts were determined using an automated analyzer (Procyte Dx hematology analyzer). The fibrinogen concentration was measured using a semiquantitative heat precipitation method.

2.5 | Statistical methods

The commercially available statistical package for the social sciences (SPSS) software (version 23.0; SPSS, Chicago, Illinois) was used to generate descriptive statistics. The normality of the data was tested using the Kolmogorov-Smirnov test. The parametric data were presented as the means ± SD, whereas the nonparametric data were presented as medians (range). The normally distributed (parametric) data were analyzed using a 1-way analysis of variance when there were ≥3 groups. When the data distribution was skewed (nonparametric), the Kruskal-Wallis test (≥3 group comparison) or Mann Whitney U test (2 group comparison) was used. Spearman correlation coefficients were calculated to compare plasma anti-Xa concentration with other coagulation variables and doses. Peak time was regarded at 3 hours after drug administration based on previous reports. Spearman correlation coefficients were calculated between ratios (peak time PT, R value of TEG/baseline PT, R value of TEG) and anti-Xa concentration. This analysis included data of 0 (baseline) and 3 hours (peak) time points of the single dose trial and 0, 3, 24, 27, 48, and 51 hours time points of the 3-day consecutive dose trial. Scatter plots with regression lines were constructed to show the linear relationship between ratios (PT, R value of TEG) and anti-Xa concentration. The proposed therapeutic range that corresponded to plasma anti-Xa concentrations of 140 to 260 ng/mL was derived as the ordinate value on the regression line according to clinical studies in humans. A P < .05 was considered statistically significant.

3 | RESULTS

3.1 | Rivaroxaban concentration determined using chromogenic anti-Xa assay and its adverse effects

Rivaroxaban increased peak plasma anti-Xa concentration (averaged) at 3 hours after drug administration (P < .001 versus baseline, Figure 1A), and the increment in plasma anti-Xa concentration was dose-dependent (Spearman's correlation coefficient R = 0.84; Figure 1C and Table 1). However, the dose-effect relationship was not proportional among dogs, and interindividual differences in drug effects were significant (Figures 1B, D). The between-subject coefficients of variation were 39.5% (0.5 mg/kg), 51.8% (1 mg/kg), 46.7% (2 mg/kg), and 61.9% (4 mg/kg). There was no evidence of cumulative effects
of rivaroxaban (Figure 1B), but median plasma anti-Xa concentrations at 3 hours after rivaroxaban administration (0.5 mg/kg [n = 3] and 1 mg/kg [n = 3]) were not significantly different among the dosages. No clinical signs of minor or major hemorrhage or adverse gastrointestinal effects were observed in any dosing groups. Total protein concentration, PCV, platelet count, and fibrinogen concentration were within reference intervals at 12 and 72 hours (Table 2).

### 3.2 | Effect of rivaroxaban on PT and TEG parameters

At 3 hours (peak time) after rivaroxaban administration, increments in anti-Xa concentrations and PT and TEG parameters with 4 different activators were observed: PT showed a strong correlation with anti-Xa activity ($R = 0.79, P < .001$). However, only R and SP showed strong correlation with anti-Xa among TEG parameters when RapidTEG, TF100, and TF3700 were used. The $R$ values in RapidTEG-TEG ($R = 0.70$), TF100-TEG ($R = 0.78$), and TF3700-TEG ($R = 0.76$) showed a strong correlation with rivaroxaban concentration, as measured with the anti-Xa assay, as well as with SP in RapidTEG-TEG ($R = 0.73$), TF100-TEG ($R = 0.75$), and TF3700-TEG ($R = 0.67$; Table 3).

At peak time (3 hours), anti-Xa-based determination of rivaroxaban concentration had strong correlation with the ratio (peak time result/baseline result) of parameters ($R = 0.82$ for PT ratio; $0.76$ for RapidTEG-R ratio; $0.82$ for TF100-R ratio; $0.83$ for TF3700-R ratio; Table 4). For SP ratio, Spearman's correlation values with anti-Xa-based determination of...
rivaroxaban concentration were $R = 0.81$ (RapidTEG-SP ratio), 0.76 (TF100-SP ratio), and 0.66 (TF3700-SP ratio). However, the $R$ ratio ($R = 0.52$) and SP ratio ($R = 0.51$) of kaolin-TEG showed poor correlation with plasma anti-Xa concentration.

Proposed therapeutic ranges of the ratio values were calculated using linear regression ($PT$ ratio $[y = 0.00353x + 0.97]$, RapidTEG-TEG $R$ ratio $[y = 0.00311x + 1.03]$, TF100-TEG $R$ ratio $[y = 0.00337x + 1.01]$, TF3700-TEG $R$ ratio $[y = 0.00338x + 1.02]$, and kaolin-TEG $R$ ratio $[y = 0.0047x + 1.03]$) that corresponded to a plasma anti-Xa concentration range of 140-260 ng/mL (Figure 2).

**DISCUSSION**

We showed that PO rivaroxaban administration has predictable anticoagulant effects with high interindividual variability. Anti-Xa concentration peaked at 3 hours after drug administration. The statistical correlations between the anti-Xa concentrations peaked at 3 hours after drug administration, and TEG and PT results showed that values and ratios of PT, $R$, and SP of TEG were strongly correlated with anti-Xa concentration when RapidTEG, TF100, and TF3700 were used. Rivaroxaban was well tolerated by healthy dogs, and no adverse effects were noted.

Rivaroxaban shows predictable drug effects in humans, thus monitoring drug effects in general is not necessary. However, interindividuum variability still is a factor, and some studies have recommended monitoring drug effects in humans.23–27 Although average peak anti-Xa concentration at 3 hours after drug administration was observed, interindividual variability was observed despite the small number of animals in our study. One dog (dog E in Figure 1) showed relatively
TABLE 3  Spearman’s correlation coefficients between plasma anti-Xa concentration and thromboelastography (TEG) parameters using 4 different activators: RapidTEG; TF100; TF3700, and kaolin

| Parameters     | RapidTEG | TF100 | TF3700 | Kaolin |
|----------------|----------|-------|--------|--------|
| TEG-ACT        | 0.70***  | ...   | ...    | ...    |
| R              | 0.70***  | 0.78***| 0.76***| 0.53***|
| K              | −0.44*** | −0.22 | 0.05   | 0.27*  |
| Angle          | 0.29*    | 0.10  | −0.18  | −0.34**|
| MA             | 0.20     | 0.07  | 0.07   | 0.15   |
| SP             | 0.73***  | 0.75***| 0.67***| 0.54***|
| Delta (R-SP)   | 0.02     | −0.01 | 0.44***| 0.41***|
| TMRTG          | 0.26*    | 0.75***| 0.69***| 0.55***|

Abbreviations: MA, maximum amplitude; SP, split point; TEG-ACT, activated using 4 different activators: RapidTEG; TF100; TF3700, and kaolin anti-Xa concentration and thromboelastography (TEG) parameters

TABLE 4  Spearman’s correlation coefficients between plasma anti-Xa concentration and ratio (peak time result/baseline result) values at peak time (3 hours)

|              | PT ratio | RapidTEG-R ratio | TF100-R ratio | TF3700-R ratio | Kaolin-R ratio |
|--------------|----------|------------------|---------------|----------------|---------------|
| Correlation  | 0.82*    | 0.76*            | 0.82*         | 0.83*          | 0.52*         |
| Therapeutic range (times of baseline) | 1.46-1.89 | 1.47-1.84 | 1.48-1.89 | 1.49-1.92 | 1.69-2.25 |

Therapeutic ranges for ratio values were determined using linear regression equation corresponding to plasma anti-Xa concentration of 140–260 ng/mL. *P < .001.
after administration. Therefore, twice daily dosing is appropriate to produce 24-hour anticoagulant effects. Although there was no overall advantage of the twice daily dosing compared to once daily dosing in a study of humans, further studies are required to determine whether once-daily and subtherapeutic dosing could exert adequate prophylaxis and treatment effects for thrombosis in dogs.

Thromboelastography analysis can be performed using various activators to facilitate blood coagulation times and decrease variations in each test. Conventional TEG uses kaolin as the activator, but we used RapidTEG and tissue factor as additional activators. RapidTEG is a combination of kaolin and tissue factor supplied by the manufacturer for faster TEG results. Tissue factor–activated TEG showed stronger correlation with plasma anti-Xa concentration in comparison to the RapidTEG-activated TEG in our study. This result may have been a consequence of different tissue factor sources of activators. Kaolin-activated TEG showed the weakest correlation with plasma anti-Xa concentration. These results were thought to have occurred because PT analysis using tissue factor as a reagent showed a stronger correlation than that shown by aPTT analysis using surface activators as reagents. Among the various TEG parameters, R, SP, TMRTG, and TEG activated clotting time (ACT) were sensitive, with R being the most sensitive. The R value is a major parameter for detecting coagulation cascade abnormality, and R may have been the most sensitive because rivaroxaban directly inhibits coagulation factor X. The novel TEG parameter delta (difference between R and SP), which allows the differentiation of enzymatic and platelet hypercoagulability, also was calculated for each test. However, delta showed poor correlation with the plasma anti-Xa concentration (Table 3). The TEG-ACT also was calculated when RapidTEG was used as an activator, and this parameter showed a strong correlation with conventional ACT devices. However, TEG-ACT did not show superior results to those of the R value in our study (Table 3).

The correlation analysis included baseline (0, 24, and 48 hours) and peak (3, 27, and 51 hours) time points of the 3-day consecutive dosing trial. However, the Spearman correlation significantly decreased when we included the 8 and 12 hours time points (8, 12, 32, 36, 56, and 60 hours) of the 3-day consecutive dosing trial. This result was thought to be a consequence of decreases in plasma rivaroxaban concentration below the detection limit of the PT and TEG devices. Thus, a comparison of baseline and peak time point is reasonable for clinical monitoring of rivaroxaban.

The advantages of clinical therapeutic monitoring of rivaroxaban include the following: (i) optimal and effective dose regimens can be determined for each individual immediately; (ii) rivaroxaban is mainly excreted by renal and biliary routes, and patients with decreased renal function or cholestasis, and those that are critically ill may benefit from therapeutic drug monitoring for safety; and, (iii) administration of crushed tablets with other medications could cause drug-drug interactions and clinical monitoring could be used to assess the effectiveness of anticoagulation in such situations.

Our study had some limitations. First, a small number of dogs per group was used. Second, rivaroxaban was only tested in healthy Beagle dogs. Third, the therapeutic ranges of plasma anti-Xa concentration were extrapolated from studies of humans. Further research...
using larger numbers of dogs and clinical cases should be conducted in the future.

In conclusion, individual differences in drug effects were identified. Thus, rivaroxaban treatment in dogs may be improved by clinical monitoring methods. Prolongation of PT and R value in TEG using a tissue factor or RapidTEG as an activator showed significant correlations with anti-Xa concentration of rivaroxaban. Furthermore, 1.5-1.9 × delayed results of the PT and R value of TEG 3 hours after administration would be required to achieve the proposed therapeutic anti-Xa concentrations based on studies in humans. This method is practical and can be used for individual dosage adjustment to decrease adverse effects and achieve proper anticoagulant effects. Further studies are warranted in critically ill patients to determine whether clinical outcome is different when therapeutic plasma concentrations of rivaroxaban are achieved using point-of-care PT and TEG activated with tissue factor or RapidTEG as a monitoring method.

ACKNOWLEDGMENTS
This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2017R1D1A1B03034904). Presented, in part, at the International Veterinary Emergency and Critical Care Symposium, Nashville, 2017. Presented, in part, at the 2018 American College of Veterinary Internal Medicine Forum, Seattle, Washington.

CONFLICT OF INTEREST DECLARATION
Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION
Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION
Chonnam National University Institutional Animal Care and Use Committee (IACUC, YB-R-2017-05).

HUMAN ETHICS APPROVAL DECLARATION
Authors declare human ethics approval was not needed for this study.

ORCID
Dohyeon Yu https://orcid.org/0000-0001-7645-6926

REFERENCES
1. Patel MR, Mahaffey KW, Garg J, et al. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. N Engl J Med. 2011;365:883-891.
2. Morassi A, Bianco D, Park E, Nakamura RK, White GA. Evaluation of the safety and tolerability of rivaroxaban in dogs with presumed primary immune-mediated hemolytic anemia. J Vet Emerg Crit Care. 2016;26:488-494.
3. Bausersachs R, Berkowitz SD, Brenner B, et al. Oral rivaroxaban for symptomatic venous thromboembolism. N Engl J Med. 2010;363:2499-2510.
4. Yang VK, Cunningham SM, Rush JE, de Laforcade A. The use of rivaroxaban for the treatment of thrombotic complications in four dogs. J Vet Emerg Crit Care. 2016;26:729-736.
5. Cuker A, Siegal DM, Crowther MA, Garcia DA. Laboratory measurement of the anticoagulant activity of the non-vitamin K oral anticoagulants. J Am Coll Cardiol. 2014;64:1128-1139.
6. Miyares MA, Davis K. Newer oral anticoagulants: a review of laboratory monitoring options and reversal agents in the hemorrhagic patient. Am J Health Syst Pharm. 2012;69:1473-1484.
7. Samama MM, Contant G, Spiro TE, et al. Laboratory assessment of rivaroxaban: a review. Thromb J. 2013;11:11.
8. Samama MM, Martinoli JL, LeFlem L, et al. Assessment of laboratory assays to measure rivaroxaban—an oral, direct factor Xa inhibitor. Thromb Haemost. 2010;103:815-825.
9. Perzborn E, Strasserburger J, Wilmen A, et al. In vitro and in vivo studies of the novel antithrombotic agent BAY 59-7939—an oral, direct factor Xa inhibitor. J Thromb Haemost. 2005;3:514-521.
10. Dixon-Jimenez AC, Brainard BM, Brooks MB, et al. Pharmacokinetic and pharmacodynamic evaluation of oral rivaroxaban in healthy adult cats. J Vet Emerg Crit Care. 2016;26:619-629.
11. Brinkman HJ. Global assays and the management of oral anticoagulation. Thromb J. 2015;13:9.
12. Douxfils J, Mullier F, Loosen C, Chatelain C, Chatelain B, Dogné JM. Assessment of the impact of rivaroxaban on coagulation assays: laboratory recommendations for the monitoring of rivaroxaban and review of the literature. Thromb Res. 2012;130:956-966.
13. McLaughlin CM, Marks SL, Dorman DC, et al. Thromboelastographic monitoring of the effect of unfractionated heparin in healthy dogs. J Vet Emerg Crit Care. 2017;27:71-81.
14. Convery B, Blais MC, Dunn M, Gara-Boivin C, del Castillo JRE. Anticoagulant activity of oral rivaroxaban in healthy dogs. Vet J. 2017;223:5-11.
15. Weinz C, Buettehorn U, Daehler HP, et al. Pharmacokinetics of BAY 59-7939—an oral, direct factor Xa inhibitor. J Thromb Haemost. 2005;3:891-910.
16. Banerjee A, Blois SL, Wood RD. Comparing citrated native, kaolin-activated, and tissue factor-activated samples and determining intraindividual variability for feline thromboelastography. J Vet Diagn Invest. 2011;23:1109-1113.
17. Epstein KL, Brainard BM, Lopes MA, et al. Thrombelastography in 26 healthy horses with and without activation by recombinant human tissue factor. J Vet Emerg Crit Care (San Antonio). 2009;19:96-101.
18. Kristensen AT, Winberg B, Jessen LR, Andreasen E, Jensen AL. Evaluation of human recombinant tissue factor-activated thromboelastography in 49 dogs with neoplasia. J Vet Intern Med. 2008;22:140-147.
19. Winberg B, Jensen AL, Rokjaer R, Johansson P, Kjeldgaard-Hansen M, Kristensen AT. Validation of human recombinant tissue factor-activated thromboelastography on citrated whole blood from clinically healthy dogs. Vet Clin Pathol. 2005;34:389-393.
20. Harenberg J, Kramer R, Giese C, et al. Determination of rivaroxaban by different factor Xa specific chromogenic substrate assays: reduction of interassay variability. J Thromb Thrombolysis. 2011;32:267-271.
21. Millar HR, Simpson JG, Stalker AL. An evaluation of the heat precipitation method for plasma fibrinogen estimation. J Clin Pathol. 1971;24:827-830.
22. Rodriguez-Pozo ML, Armengou L, Monreal L, et al. Evaluation of an oral direct factor Xa inhibitor anticoagulant in healthy adult horses. J Vet Emerg Crit Care. 2017;27:82-88.
23. Mismetti P, Laporte S. New oral antithrombotics: a need for laboratory monitoring. For J Thromb Haemost. 2010;8:621-626.
24. Kubitz D, Becka M, Zuehlsdorf M, Mueck W. Effect of food, an antacid, and the H2 antagonist ranitidine on the absorption of BAY 59-7939 (rivaroxaban), an oral, direct factor Xa inhibitor, in healthy subjects. J Clin Pharmacol. 2006;46:549-558.
25. Mani H, Kasper A, Lindhoff-Last E. Measuring the anticoagulant effects of target specific oral anticoagulants-reasons, methods and current limitations. J Thromb Thrombolysis. 2013;36:187-194.
26. Kreutz R. Pharmacodynamic and pharmacokinetic basics of rivaroxaban. Fundam Clin Pharmacol. 2012;26:27-32.
27. Freyburger G, Macouillard G, Labrouche S, Sztark F. Coagulation parameters in patients receiving dabigatran etexilate or rivaroxaban: two observational studies in patients undergoing total hip or total knee replacement. Thromb Res. 2011;127:457-465.
28. Conversy B, Blais MC, Dunn M, Gara-Boivin C, Carioto L, del Castillo JRE. Rivaroxaban demonstrates in vitro anticoagulant effects in canine plasma. Vet J. 2013;198:437-443.
29. Baglin T, Hillarp A, Tripodi A, Elalamy I, Buller H, Ageno W. Measuring oral direct inhibitors (ODIs) of thrombin and factor Xa: a recommendation from the Subcommittee on Control of Anticoagulation of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. J Thromb Haemost. 2013;11:756-760.
30. Brill-Edwards P, Ginsberg JS, Johnston M, Hirsh J. Establishing a therapeutic range for heparin therapy. Ann Intern Med. 1993;119:104-109.
31. Moore KT, Plotnikov AN, Thyssen A, Vaccaro N, Ariyawansa J, Burton PB. Effect of multiple doses of omeprazole on the pharmacokinetics, pharmacodynamics, and safety of a single dose of rivaroxaban. J Cardiovasc Pharmacol. 2011;58:581-588.
32. Samama MM, Guinet C, Le Flem L, et al. Measurement of dabigatran and rivaroxaban in primary prevention of venous thromboembolism in 106 patients, who have undergone major orthopedic surgery: an observational study. J Thromb Thrombolysis. 2013;35:140-146.
33. Lunsford KV, Mackin AJ, Langston VC, Brooks M. Pharmacokinetics of subcutaneous low molecular weight heparin (enoxaparin) in dogs. J Am Anim Hosp Assoc. 2009;45:261-267.
34. Mischke R, Schmitt J, Wolken S, Böhm C, Wolf P, Kietzmann M. Pharmacokinetics of the low molecular weight heparin dalteparin in cats. Vet J. 2012;192:299-303.
35. Brooks MB. Evaluation of a chromogenic assay to measure the factor Xa inhibitory activity of unfractionated heparin in canine plasma. Vet Clin Pathol. 2004;33:208-214.
36. Lynch AM, deLaforcade AM, Sharp CR. Clinical experience of anti-Xa monitoring in critically ill dogs receiving dalteparin. J Vet Emerg Crit Care (San Antonio). 2014;24:421-428.
37. Kreutz R. A clinical and pharmacologic assessment of once-daily versus twice-daily dosing for rivaroxaban. J Thromb Thrombolysis. 2014;38:137-149.
38. Gonzalez E, Kashuk JL, Moore EE, Silliman CC. Differentiation of enzymatic from platelet hypercoagulability using the novel thromboelastography parameter delta (delta). J Surg Res. 2010;163:96-101.
39. Hamzioupour N, Chan DL. Thromboelastographic assessment of the contribution of platelets and clotting proteases to the hypercoagulable state of dogs with immune-mediated hemolytic anemia. J Vet Emerg Crit Care (San Antonio). 2016;26:295-299.
40. Chavez JJ, Foley DE, Snider CC, et al. A novel thrombelastograph tissue factor/kaolin assay of activated clotting times for monitoring heparin anticoagulation during cardiopulmonary bypass. Anesth Analg. 2004;99:1290-1294.
41. Douxfils J, Tamigniau A, Chatelain B, et al. Comparison of calibrated chromogenic anti-Xa assay and PT tests with LC-MS/MS for the therapeutic monitoring of patients treated with rivaroxaban. Thromb Haemost. 2013;110:723-731.
42. Lindhoff-Last E, Ansell J, Spiro T, Samama MM. Laboratory testing of rivaroxaban in routine clinical practice: when, how, and which assays. Ann Med. 2013;45:423-429.
43. Ikeda K, Tachibana H. Clinical implication of monitoring rivaroxaban and apixaban by using anti-factor Xa assay in patients with nonvalvular atrial fibrillation. J Arrhythm. 2016;32:42-50.

How to cite this article: Bae J, Kim H, Kim W, et al. Therapeutic monitoring of rivaroxaban in dogs using thromboelastography and prothrombin time. J Vet Intern Med. 2019;33:1322-1330. https://doi.org/10.1111/jvim.15478