Received: 12 April 2018 | Accepted: 26 February 2019

DOI: 10.1111/jvim.15478

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Therapeutic monitoring of rivaroxaban in dogs using thromboelastography and prothrombin time

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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.

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 also is used for the prophylaxis and treatment of deep vein thrombosis and pulmonary embolism.[1–4] Unfortunately, rivaroxaban has not been widely used 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.[13] Evaluating the effect of rivaroxaban in blood using TEG appears promising[11,14] 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.[15] 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, Leverkussen, 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 (Butophan 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 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 2000*g* 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 recalcification 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.[13,16–19] Briefly, 20 μL 0.2 M calcium chloride ($CaCl_2$) 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 0.2 M $CaCl_2$, 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 0.2 M $CaCl_2$ [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 0.2 M $CaCl_2$ 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.[20] A commercial chromogenic substrate test (Technochrome 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.[21]

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.[10,22] 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.[10,13] 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

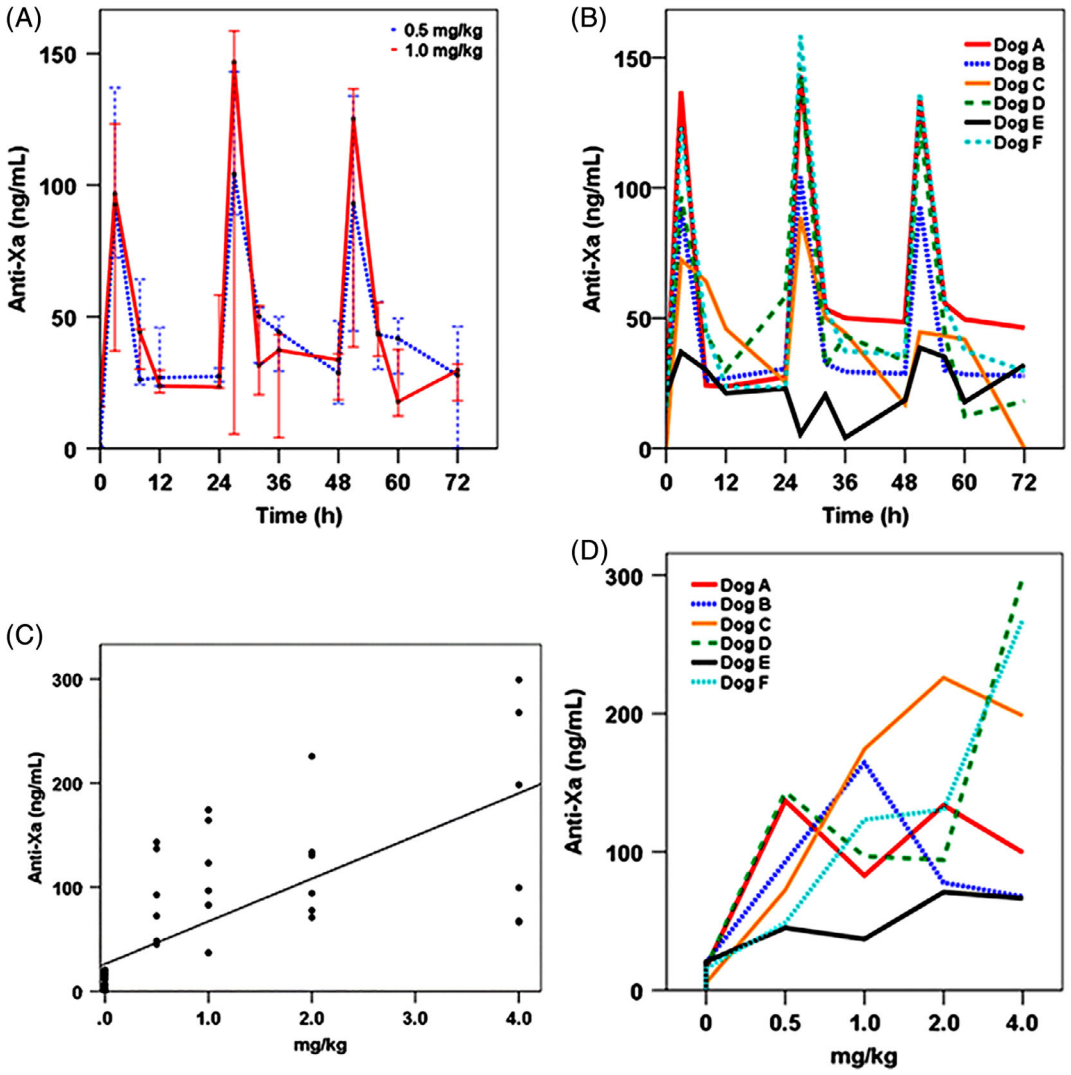

FIGURE 1 Plasma anti-Xa concentration in dogs with rivaroxaban administration. A, Plasma anti-Xa concentration after 3 consecutive days of rivaroxaban administration at different doses (0.5 mg/kg [n = 3] and 1 mg/kg [n = 3]). In order to determine peak plasma concentration of anti-Xa, only 2 different doses were challenged. Error bar shows 95% confidence intervals. B, Plasma anti-Xa concentration after administration for 3 consecutive days to different subject with 0.5 (Dog A-C) and 1 (Dog D-F) mg/kg. The line represented individuals. C, Spearman correlation coefficient showing linear correlation between the dose and plasma anti-Xa concentration at 3 hours time point with baseline values of 0 mg/kg dosage group (R = 0.84, $P < .001$). However, 3 hours plasma anti-Xa concentrations of each dosage (0.5, 1, 2, and 4 mg/kg [n = 6]) of rivaroxaban were not significantly different among doses. D, Plasma anti-Xa concentration after a single dose of rivaroxaban administered after 3 hours to dogs. The line represented individuals

of rivaroxaban (Figure 1B), but median plasma anti-Xa concentrations at 3 hours after rivaroxaban administration (0.5, 1, 2, and 4 mg/kg) 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

TABLE 1 Anti-Xa concentration, prothrombin time (PT), and reaction time (R) value of thromboelastography (TEG) at baseline (0 hours) and peak time (3 hours) in dogs treated with 4 different doses of rivaroxaban

		Dose (mg/kg)			
	Time	0.5	1	2	4
Anti-Xa (ng/mL)	Baseline	2.1 (0.7-16.4)	16.0 (5.5-20.7)	0 (0-2.5)	0.1 (0-18.4)
	Peak	82.6* (45.1-143.3)	110.0* (37.0-174.3)	112.4* (70.9-225.8)	149.0* (66.4-299.3)
PT (s)	Baseline	12.0 (10.0-12.0)	13.0 (12.0-15.0)	12.0 (12.0-14.0)	12.0 (12.0-14.0)
	Peak	15.5* (12.0-19.0)	17.5* (14.0-22.0)	19.0* (15.0-24.0)	18.5* (17.0-26.0)
TEG					
RapidTEG-R (s)	Baseline	35.0 (25.0-40.0)	40.0 (35.0-40.0)	40.0 (40.0-50.0)	42.5 (35.0-50.0)
	Peak	47.5* (40.0-55.0)	57.5* (40.0-65.0)	60.0* (55.0-75.0)	60.0* (55.0-60.0)
TF100-R (s)	Baseline	40.0 (30.0-40.0)	37.5 (35.0–40.0)	35.0 (35.0-40.0)	37.5 (30.0-45.0)
	Peak	55.0* (40.0-60.0)	57.5* (40.0–65.0)	55.0* (50.0-70.0)	52.5* (45.0-60.0)
TF3700-R (min)	Baseline	1.9 (1.5-2.6)	1.8 (1.7-2.2)	2.0 (2.0-2.2)	2.0 (1.8-2.1)
	Peak	2.2 (2.1-2.9)	2.8* (2.2-3.1)	2.9* (2.3-3.1)	2.8* (2.2-4.2)
Kaolin-R (min)	Baseline	2.5 (1.7-4.3)	2.8 (1.7-3.5)	3.3 (2.4-4.2)	2.8 (1.9-4.3)
	Peak	3.8* (3.4-6.0)	4.0 (3.4-6.0)	4.1 (2.8-5.5)	4.5 (2.2-6.8)

*Statistically significant differences ($P < .05$) between baseline and peak time were noted. RapidTEG, TF100, TF3700, and kaolin are activators of TEG. Results are presented as median (range).

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 [0.0047x + 1.03]) that corresponded to a plasma anti-Xa concentration range of 140-260 ng/mL (Figure 2).

TABLE 2 PCV, total protein, platelets, and fibrinogen concentration at baseline, 12 hours, and final time point

Time (h)	PCV (%)	Total protein (g/dL)	Platelets ($\times 10^3/\mu L$)	Fibrinogen (mg/dL)
0	45.0 (42.0-47.0)	6.6 ± 0.3	257.2 ± 75.2	300 (100-400)
12	45.5 (44.0-48.0)	6.6 ± 0.3	278.0 ± 84.8	250 (100-300)
72	42.5 (39.0-46.0)	6.6 ± 0.5	218.2 ± 67.8	200 (200-300)

Results are presented as mean ± SD if data distribution was parametric or median (range) if data distribution was skewed.

4 | 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, interindividual 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 clotting time; TMRTG, time to maximum rate of thrombus generation.
*P < .05, **P < .01, and ***P < .001.

low increments in PT and TEG parameters as well as anti-Xa concentration after drug administration. These results are similar to those of a previous study that reported that interindividual variability was moderate to large in 24 healthy Beagles.[14] We speculate that individual monitoring of rivaroxaban in dogs could be beneficial, unlike in humans, and appropriate individual dosage adjustment could be achieved by PT and TEG monitoring according to the study.

Previous studies in dogs have reported a mild to moderate interindividual variability, and the dosage could be appropriately adjusted by interpreting the PT results in a clinical setting.[4,14] Although the exact reason is not known, interindividual variability also was detected in our study. However, the same individual showed similar anticoagulant effects with the same repeated dosing in the 3-day consecutive dosing trial. Thus, dosage adjustments may be required to achieve similar anticoagulant effects in individual animals. This variation may have been affected by gastrointestinal drug absorption and genetic polymorphism in drug metabolism.[23] Rivaroxaban has a lower affinity for canine factor Xa than for human factor Xa, and this difference may affect interindividual variability.[28]

In terms of peak time, peak plasma activity of rivaroxaban was reached 2-4 hours after administration in humans.[29] We selected 3 hours post-administration as the peak time point according to previous studies in humans.[10,22] A recent study reported a peak time point of 1.5-2 hours after dosing in dogs.[14] However, the dogs in our study were not allowed to fast during the study period; thus, the peak time may have been slightly delayed because food can interfere with the absorption of rivaroxaban, leading to delayed peak times of 0.5-1.5 hours compared to those in the fasted state.[24] We attempted to simulate actual situations of clinical drug administration and therefore the dogs were not fasted. In addition, the drug effect was detected 3 hours after dosing in various tests but not 8 or 12 hours after dosing using the point-of-care PT and TEG devices. This result was similar to results of a previous study.[14] Three dogs each in the 0.5 and 1 mg/kg dosing groups were given rivaroxaban once daily for 3 consecutive days and were monitored. No cumulative effect was observed at these dosages, but statistical power was low because of the small numbers of animals per group.

To monitor responses to the rivaroxaban administration, the relationship between ratio values (PT and TEG) and anti-Xa concentration was plotted, followed by calculation of therapeutic ranges of ratio values using the least square method according to previous reports of unfractionated heparin.[13,30] The plasma anti-Xa concentration range of 140-260 ng/mL was considered an effective thromboprophylactic anti-Xa concentration range of rivaroxaban in studies of humans.[1,3,10,27,31,32] As a result, strong statistical correlations were observed between anti-Xa concentration and PT as well as TEG activated with tissue factor and RapidTEG. We used ratio values (measured values/baseline values) when determining therapeutic ranges of each test because doing so can decrease the effect of individual variation of baseline values. Similar to studies in humans, the chromogenic anti-Xa assay was the most sensitive in detecting plasma rivaroxaban concentration.[12] However, this assay is limited in clinical settings because it is only available in large diagnostic laboratories. Various commercial chromogenic anti-Xa kits have been tested in veterinary medicine, and plasma anti-Xa activity in dogs can be monitored accurately.[10,33–36] Several chromogenic assays have been validated in humans and small animals for the evaluation of anti-Xa activity for the quantification of rivaroxaban plasma concentration.[12,28,29] A chromogenic assay was used in our study based on the methodology previously described.[20] Nonetheless, a standard curve was generated using frozen dried human plasma, not canine plasma, from the commercial chromogenic anti-Xa kit that we used. Thus, this could be a major limitation of our study.

In our study, dosages above 2 mg/kg were appropriate for healthy dogs to achieve an anti-Xa concentration >140 ng/mL at the peak time. This dosage was similar to the appropriate dosage regimen suggested in a previous report.[14] Choosing the lowest dosage to achieve therapeutic ranges of plasma rivaroxaban concentrations could decrease the total cost of drug required for treatment. This would be beneficial for long-term management because some dogs in the 0.5 or 1 mg/kg group showed anti-Xa concentrations in the therapeutic range. The clinical devices could not detect the anticoagulant effect of rivaroxaban 8 hours

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.

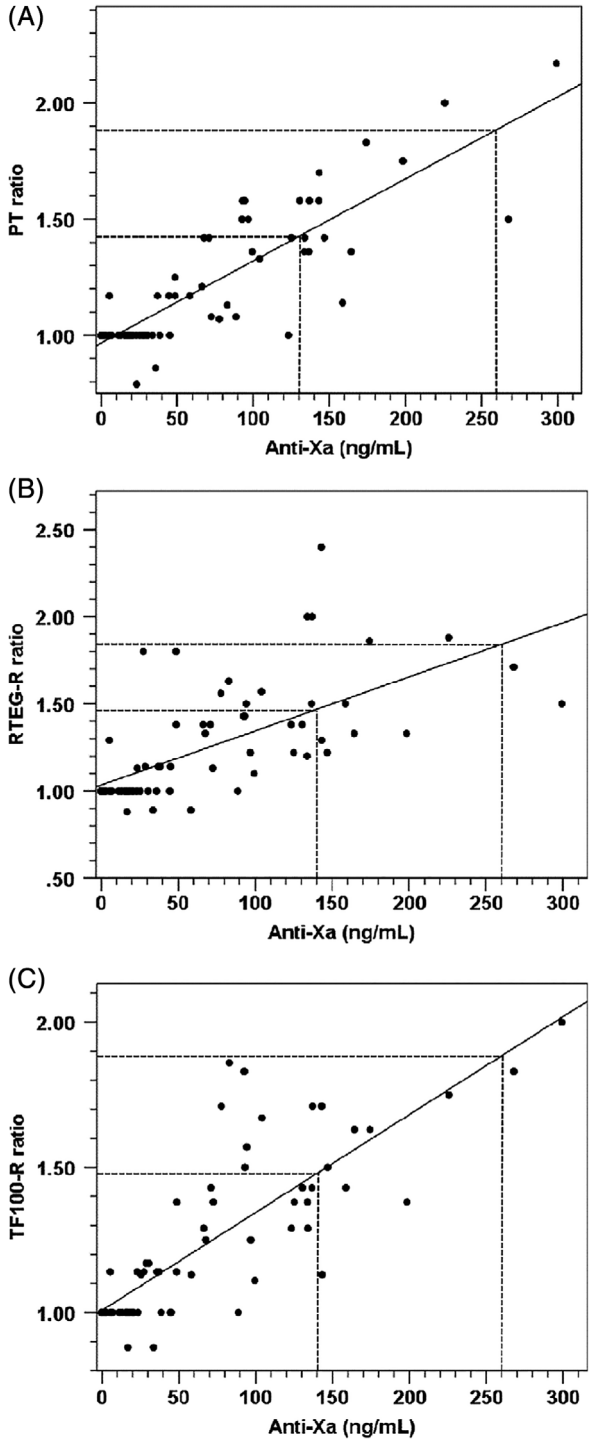

FIGURE 2 Scatter plot shows a strong, positive linear association between plasma anti-Xa concentration and ratio values (peak time result/baseline result). (A) Prothrombin time (PT) ratio, (B) RapidTEG-TEG R ratio, (C) TF100-TEG R ratio. Ratio values were calculated by dividing results at peak time (3 hours) to baseline. Scatter plot shows based on anti-Xa concentration after 4 different dosages (0.5, 1, 2, and 4 mg/kg) of rivaroxaban. Therapeutic range of ratio values (dashed line) was calculated by using linear regression equation that corresponds to plasma anti-Xa concentration of 140-260 ng/mL, which is an effective thromboprophylactic anti-Xa concentration range in humans. Thus, the dashed lines represent the targeted plasma anti-Xa concentration and the corresponding PT ratio. TEG, thromboelastography

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,[37] 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.[38,39] 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.[40] 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,[15] and patients with decreased renal function or cholestasis, and those that are critically ill may benefit from therapeutic drug monitoring for safety[4,41–43]; 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.[43]

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.

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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