Original Study

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The effect of unfractionated heparin on thrombelastographic analysis in healthy dogs

Jennifer R. Pittman, DVM; Amie Koenig, DVM, DACVIM, DACVECC and Benjamin M. Brainard, VMD, DACVA, DACVECC

Abstract

Objective – To determine the effect of single and multiple doses of SQ heparin (200 U/kg) on the thrombelastogram of healthy dogs.
Design – Prospective study.
Setting – University research facility.
Animals – Six random-source female dogs.
Interventions – Baseline parameters, including a CBC with platelet count, prothrombin time, activated partial thromboplastin time (aPTT), and antithrombin were performed. Thrombelastography (TEG) and aPTT were performed hourly for 12 hours after unfractionated heparin dosing (200 U/kg, SQ). Anti-Xa activity was assayed at 0, 3, 6, and 8 hours. Heparin was then administered every 8 hours for 3 days. The sampling protocol on Day 4 was identical to Day 1.
Measurements and Main Results – On Day 1, percentage change from baseline for TEG parameter *R*, as well as absolute values of *K*, angle, and maximum amplitude (MA) were evaluated. Statistically significant ($P < 0.01$) prolongation of the *R* time and a decrease in angle and MA was seen in all dogs by hour 3. *R* and MA were unmeasurable for most dogs between 3 and 5 hours. All TEG tracings returned to baseline by 12 hours. Day 4 TEG tracings mimicked those on Day 1. Only 1 dog achieved aPTT values outside the reference interval on both days. Anti-Xa activity levels increased on Day 4 but not on Day 1. Based on post hoc in vitro analysis, prolongation of *R* time occurred at plasma heparin levels as low as 0.075 U/mL, well below the lower limit of detection of the anti-Xa activity level assay.
Conclusions – Administration of SQ heparin results in progressive changes in the TEG tracing, with maximal change occurring 3–5 hours after dosing. The extensive prolongation of the *R* time also indicates that TEG may be too sensitive and limits its utility as a monitoring tool for unfractionated heparin therapy.

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Introduction

Hypercoagulability in dogs is associated with disease processes such as immune-mediated hemolytic anemia,[1] protein-losing nephropathy,[2] parvoviral enteritis,[3] or disseminated intravascular coagulation,[4] and may lead to life threatening venous or arterial thrombosis. In 1 study evaluating dogs that developed cranial vena caval thrombi, 6 of 17 dogs were receiving corticosteroids for treatment of presumed immune-mediated hematologic disease, 6 of 17 were septic, and 2 of 17 had protein-losing nephropathy.[5]

Heparin therapy is commonly used for prevention of thrombosis or thromboembolism in patients with hypercoagulability. Unfractionated heparin (UFH) exerts an anticoagulant effect by combining with antithrombin (AT) and inactivating clotting factors, predominantly FIIa and FXa.[6,7] Common dose recommendations for UFH in the dog range from 150 to 300 U/kg given SQ every 8 hours, although a wider dose range has also been published.[8,9,a]

Heparin therapy is traditionally monitored by evaluating the activated partial thromboplastin time

From the Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, University of Georgia, Athens, GA 30602.

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Address correspondence and reprint requests to
Dr. Benjamin Brainard, 501 DW Brooks Drive, Department of Small Animal Medicine and Surgery, University of Georgia College of Veterinary Medicine, Athens, GA 30602, USA.
Email: brainard@uga.edu
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(aPTT), with a goal to prolong the aPTT to 1.2–1.5 times the baseline.[10] A more accurate method of measuring the circulating level of heparin is the plasma anti-Xa activity.[7,11] This has been directly correlated to the blood levels of heparin, as well as the anticoagulant effects; however, this is a test that is not commonly available for same-day therapeutic adjustments.[12] While both of these assays may be an accurate assessment of the anticoagulant properties of heparin as it affects the traditional coagulation cascade, the global impact on coagulation is not assessed, providing for uncalculated effects on its antithrombotic efficacy.[13]

Thrombelastography (TEG) is a point-of-care test that is generating interest in the veterinary community for its ability to document both hypo- and hypercoagulable states.[14] TEG evaluation of coagulation has been performed in dogs,[3,15–17] cats,[18] and horses,[19] in addition to rabbits[20] and fish.[21] TEG produces a graph of the entire dynamic process of coagulation in whole blood, and consequently includes the contributions of soluble plasma factors as well as cellular (eg, platelet) components.[22] TEG is one of few methods available to document hypercoagulability in dogs.[3,17] TEG also allows for measurement of the degree of clot dissolution by fibrinolysis.[14]

Data obtained from each TEG tracing include the *R*-value, which represents the reaction time until the start of clot formation (initiation); *K* time, which represents the time for the growing clot to achieve a standard amplitude of 20 mm in the tracing; maximum amplitude (MA), a representation of the maximum clot strength; and α-angle, which is a measure of the rate at which the clot forms.[13,23]

TEG is a rapid test that is easy to perform, producing results in approximately 1 hour, which includes a 30-minute rest period for the blood sample. Depending on laboratory resources, the turn-around time for TEG may be more rapid and require fewer specialized resources than other measures of coagulation used for titration of heparin therapy (especially anti-Xa activity).

Normal values for TEG parameters in dogs and cats have been published.[14,18] The effect of both UFH and low-molecular-weight heparin on the TEG tracing of cats has been investigated.[18] While the effect of IV heparin administration in dogs has been investigated with similar technology,[15] this study aims to assess the more common clinical practice of SQ UFH administration at usually prescribed doses.[9] The objective of this study was to determine the effect of single and multiple doses of subcutaneous UFH on the thrombelastogram of healthy adult dogs. A secondary post hoc objective was to define the plasma heparin levels at which the *R* time became prolonged. Our hypothesis was that in healthy adult dogs administered single and multiple SQ UFH doses, the TEG tracing would show progressive prolongation of coagulation parameters that mirror changes in aPTT and plasma anti-Xa activity levels.

Materials and Methods

Baseline measurements

Six random-source female dogs determined to be healthy on the basis of physical examination, and a CBC and coagulation profile within reference intervals were used for this study. The study protocols were reviewed and approved by the Institutional Animal Care and Use Committee.

On Day 1, each dog was equipped with a sampling catheter[b] in the right jugular vein. An initial blood sample was collected to assess a complete baseline coagulation profile including platelet count, prothrombin time (PT),[c] aPTT,[d] AT,[e] and anti-Xa activity levels,[f] and TEG.[g] Blood was collected via the jugular catheter into a syringe and transferred into tubes[h] containing 3.2% sodium citrate at a final citrate:blood ratio of 1:9. One tube reserved for TEG analysis was kept at room temperature for 30 minutes before evaluation. The other citrate tube was centrifuged within 10 minutes of collection at $1500 \times g$ and 4°C (39.2°F), and the plasma portion was aliquoted into plastic vials and stored at −80°C until batch analysis. After sampling, the catheter was flushed with 1 mL of 50% dextrose solution every 8 hours to maintain patency.[24]

Single heparin dose protocol

UFH[i] (200 U/kg, SQ) was administered to each dog over the region of the right thorax. At 1-hour intervals for 12 hours after the initial heparin administration, a 3-mL waste sample was collected followed by a 3.6-mL blood sample via the jugular catheter. This blood was added to tubes containing 3.2% sodium citrate in a final citrate:blood ratio of 1:9. TEG was performed on 1 of these samples, and the other was immediately spun at $1500 \times g$ for 10 minutes at 4°C. After centrifugation, plasma was collected into plastic vials and stored at −80°C before batch analysis for coagulation times and AT levels. At the 0, 3, 6, and 8-hour time points, an additional 1.8 mL was collected and added to a citrate-containing tube as described above. Plasma from this tube was separated and stored as above for batched anti-Xa activity level analysis.

TEG samples were kept at room temperature for 30 minutes before analysis.[18] All TEG samples were performed in duplicate on 1 of 3 thromboelastographs,[g] with a single machine used for each dog for the duration of the study to minimize variation. For each sample, 340 μL of citrated blood was added to 20 μL of 0.2 M $CaCl_2$[j] and standard TEG performed using plain cups[k]

without additional activators.[18] All samples were allowed to run for a maximum of 1 hour. For each tracing, the variables *R* and *K* were reported in minutes, MA in millimeters, and α-angle (Ang) in degrees.

The 3 thromboelastographs used in this study are housed in the veterinary coagulation laboratory of the University of Georgia. They receive regular scheduled maintenance and regular quality control assessments. Two levels of quality control standards were run on each machine before each day of testing for this study, and were within acceptable limits. The University of Georgia has generated an institutional reference interval for canine whole blood assays on these machines.

Multiple heparin dose protocol

Beginning immediately after completion of the first phase of the study, the same dogs from the initial phase were administered UFH (200 U/kg, SQ, q 8 h) for 3 days to generate the data for the second phase of the study. Patency of the sampling catheter was maintained for the duration of treatment by flushing with 1 mL of 50% dextrose solution every 8 hours.[24] Dogs were monitored 3 times daily for signs of hemorrhage. Sampling procedures were not performed on Day 2 or 3.

On Day 4, blood was collected for an initial platelet count, PT, aPTT, AT, and anti-Xa activity levels, and TEG. Following this collection, a final dose of UFH (200 U/kg, SQ) was administered. As on Day 1, blood was sampled from the jugular catheter at 1-hour intervals for 12–14 hours after UFH administration and handled as described above to obtain hourly aPTT and TEG data. Based on the extensive prolongation of the *R* time during the single-dose UFH phase, no samples were taken at the 2, 4, 5, and 7 hours post administration time points. Sampling end points were determined by at least 2 consecutive samples within the reference interval for *R* time (2.1–11.0 s), or a maximum of 13 hours post administration. After the final TEG, the sampling catheters were removed.

Post hoc in vitro measurements

Based on the results from the in vivo study, a post hoc investigation was pursued to determine the effects of known heparin plasma concentrations on TEG parameters. Ten milliliters of blood was drawn from the jugular vein of 6 random source dogs and placed in 3.2% sodium citrate tubes to a final citrate:blood ratio of 1:9. Plasma volume of the samples was estimated by using the following equation: #mL plasma = #mL in tube–(PCV [%] × #mL in tube). UFH[i] was added to the whole blood samples to achieve final plasma heparin concentrations of 0.0, 0.025, 0.05, 0.075, and 0.1 U/mL. TEG was performed on each whole blood sample. Plasma samples from the lowest 2 concentrations were also evaluated by TEG to determine the effect of heparin on the plasma component of coagulation.

Statistical analysis

Data from paired TEG samples were averaged to obtain a single data point for each dog at each sampling time. Samples that were truncated at 1 hour were assigned an *R* time of 60 minutes, with no data available at these time points for *K*, MA, or Ang. Mean, standard deviation, maximum, and minimum values of TEG parameters were calculated.

Statistical analysis was performed using a commercial software program.[1] All data were tested for normality by the Kolmogorov-Smirnov test. All descriptive data are reported as median and range. A repeated measures model that recognized multiple observations as belonging to the same dog was used to test if the TEG parameters and aPTT values were significantly changed from baseline values on Day 1 and if Day 1 values were significantly different from Day 4 values. The model included a fixed factor of time and a random factor of dog for testing for changes from baseline on Day 1. The model included a continuous factor of time, a fixed factor of day, a day × time interaction term and a random factor of dog for day comparisons. In vitro *R* times were compared using an analysis of variance with correction for repeated measures. A *P*-value <0.05 was considered significant.

Results

Six adult (ages unknown) random-source female dogs, weighing an average of 25.7 kg (range: 18.5–41.2 kg) were used. None of the dogs used in the study became ill or exhibited signs of inappropriate hemostasis during the study period.

Baseline coagulation parameters consisting of platelet count, PT, aPTT, and AT activity were within reference intervals in all dogs (Table 1) except 2 who exhibited mild thrombocytopenia on Day 1 (174×10^9 and 122×10^9/L). The former dog remained mildly thrombocytopenic on Day 4 (162×10^9/L). There were no differences in platelet count as a result of UFH therapy ($P = 0.98$). Though AT activity was slightly lower on Day 4 than on Day 1, this was not statistically significant ($P = 0.1$).

Day 1 results

Prolongation of the *R* time and a decrease in Ang and MA was seen in all dogs after UFH administration on Day 1. The maximal UFH effect, as evidenced by the most prolonged *R* time, was present between 3 and 5 hours after administration, with a steady progression back to baseline by 12 hours post administration

Table 1: Initial ($T = 0$) hemostatic parameters on Days 1 and 4 ($n = 6$)

		Day 1			Day 4		
Dog	Reference interval	Platelet count ($\times 10^9$/L) ($>200 \times 10^9$/L)	Prothrombin time (s) (5.8–9.8 s)	Antithrombin activity (%) (108–146%)	Platelet count ($\times 10^9$/L) ($>200 \times 10^9$/L)	Prothrombin time (s) (5.8–9.8 s)	Antithrombin activity (%) (108–146%)
1		291	6.9	117	243	7.2	95
2		251	6.6	116	256	6.7	89
3		255	6.8	142	260	7.4	92
4		174	9.2	128	162	8.7	101
5		390	7.2	113	304	7.2	79
6		122	6.9	135	253	7.7	97

(Figure 1). The aPTT remained within the laboratory reference interval for all dogs. Anti-Xa activity levels were below the limit of detection (0.1 U/mL) in all but 3 dogs, even when the thrombelastogram showed significant prolongation of the *R* time (Table 2).

Day 4 results

The results of the TEG tracings on Day 4 mimicked those on Day 1. UFH administration again produced maximal prolongation of *R* time at 3–5 hours post administration, which returned to being within the reference interval between 10 and 12 hours after UFH dosing (Table 3). Initial values for *R* were significantly prolonged ($P = 0.02$) on Day 4 compared with Day 1. Four dogs on Day 4 had their initial ($T = 0$ h) *R* times truncated at 60 minutes. Because of the number of samples that failed to generate an *R* value, insufficient MA values were generated for statistical comparison on Day 4.

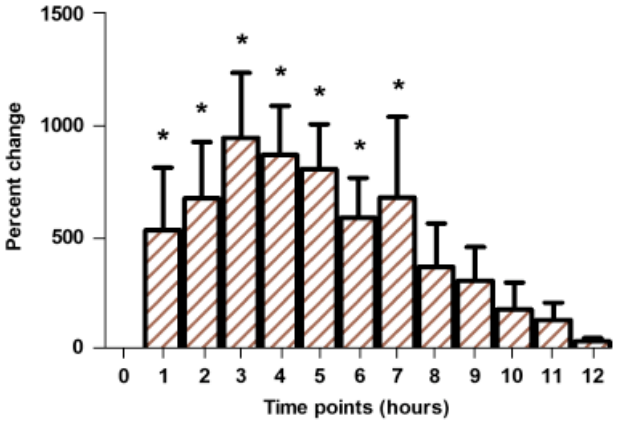

Figure 1: The shaded regions represent the mean percentage change in *R* time from baseline for all 6 dogs on Day 1, showing maximal prolongation between 3 and 5 hours post-heparin dose. The bars indicate standard deviation. Asterisks indicate statistically significant values ($P < 0.05$).

The degree of prolongation in the *R* time after dosing of UFH was significantly higher on Day 4 than Day 1 ($P = 0.006$) (Table 3). Decreases in Ang were significantly larger on Day 1 compared with Day 4 ($P < 0.001$). Changes in MA after dosing were not significantly different between days. Many tracings were unable to be evaluated for Ang and MA due to prolonged *R* time, which necessitated discontinuation of each analysis at 60 minutes to allow the next samples to be run (ie, the *R* time was at least 60 min). These missing data points were not included in the statistical analysis.

As on Day 1, patient response to multiple doses of UFH was variable, as noted in the initial thrombelastographs on Day 4. Two dogs had returned to a tracing within reference intervals at Day 4, ($T = 0$ h), which was 8 hours after their previous dose, while the remaining 4 still had TEG tracings consistent with hypocoagulability at this time point. Regardless of the starting data point, all 6 dogs returned to baseline values by 12 hours after their final dose of UFH.

Only 1 dog achieved aPTT values of 1.5 times baseline on Day 4, with a concurrent anti-Xa activity level of 0.55 U/mL and maximally prolonged *R* time on the TEG. Anti-Xa activity levels on Day 4 confirmed heparin plasma levels of >0.3 U/mL for at least 1 time point in 5 dogs, and levels ≥ 0.5 U/mL in 2 dogs (Figure 2).

At the end of Day 4, between 10 and 12 hours post administration, 4 dogs displayed a shortened *R* time and increased MA compared with their baseline tracings. This was a transient effect, and not statistically significant ($P = 0.27$ and 0.13, respectively). The remaining 2 dogs did not have TEG performed because the blood sample clotted before anticoagulation by the citrate in the tube. By 14 hours, all dogs had TEG tracings that were statistically similar to their baseline traces.

Post hoc in vitro results

TEG evaluation of in vitro addition of UFH to whole blood revealed prolongation of the *R* time from baseline at a heparin dose of 0.075 U/mL ($P = 0.051$), which was

Table 2: Thrombelastography, activated partial thromboplastin time, and anti-Xa activity results from Day 1 ($n = 6$)

Time (hours)	*R* (min) (2.1–11.0 min)	*K* (min) (1.2–4.6 min)	Angle (degrees) (39–74°)	MA (mm) (44.5–61.7 mm)	aPTT (s) (9.4–15.1 s)	Anti-Xa (U/mL) (0.3–0.7 U/mL)
0	4.4 (2.5–9.3)	2.3 (2.0–5.2)	60.0 (38.8–63.3)	55.0 (40.8–58.1)	11.5 (9.7–13.8)	
1	9.5 (3.30–60)	2.3 (1.8–9.5)	57.5 (2.3–66.8)	53.7 (41.2–58.9)	11.8 (11.0–14.0)	
2	20.9 (10.5–60)	12.2 (8.0–17.8)	18.2 (14.0–26.5)	38.0 (37.2–38.8)	13.2 (10.9–14.9)	
3	44.2 (22.6–60)*	20.1 (16.0–24.2)	12.4 (4.0–17.3)	†	12.9 (11.0–14.8)	0.1 (<0.1–0.5)
4	46.8 (25.7–60)*	17.4 (17.1–17.7)	12.7 (3.6–17.7)	†	13.1 (11.4–14.8)	
5	60.0 (14.2–60)*	17.3 (10.8–23.8)	12.9 (4.2–21.6)	†	13.2 (11.6–15.1)	
6	29.3 (8.15–60)	14.7 (5.7–26.1)	11.5 (1.9–34.0)	40.6 (39.1–42.2)	12.9 (11.2–174.2)	0.1 (<0.1–0.15)
7	24.9 (2.85–60)	11.9 (2.5–21.0)	26.2 (9.9–57.8)	48.9 (46.7–51.1)	12.3 (10.5–14.1)	
8	13. (2.75–60)	4.0 (2.2–20.3)	45.3 (12.3–61.9)	51.0 (37.7–56.8)	13.1 (11.9–14.6)	<0.1 (<0.1–0.1)
9	13.3 (3.0–41.3)	9.5 (2.8–21.4)	28.3 (10.7–56.9)	489 (32.6–50.2)	12.4 (11.6–14.8)	
10	9.6 (2.85–21.75)	7.3 (2.3–15.3)	35.4 (14.7–59.4)	50.7 (38.5–57.7)	12.3 (11.4–14.8)	
11	6.8 (2.6–19.9)	2.7 (2.1–17.1)	56.6 (12.9–62.2)	52.8 (32.9–58.0)	12.7 (11.3–14.8)	
12	5.2 (2.7–12.2)	2.3 (1.9–7.6)	60.8 (27.4–64.6)	54.4 (43.3–58.3)	12.6 (11.6–14.4)	

Data are shown as median (range).
*Indicates statistically significant values ($P < 0.01$).
†No MA data are available for 3, 4, or 5 hours post administration due to extensive prolongation of R time.
MA, maximum amplitude; aPTT, activated partial thromboplastin time.

further prolonged and statistically significant at a heparin concentration of 0.1 U/mL ($P < 0.001$) (Figure 3). When whole blood TEG tracings were compared with those performed using plasma, prolongation of the *R* time was evident at concentrations as low as 0.025 U/mL; however, this was not significantly different from the baseline tracing ($P = 0.07$).

Discussion

Administration of UFH to healthy dogs caused a consistent prolongation of TEG parameters, specifically the *R* time. Surprisingly, at the time of peak effect as demonstrated by TEG (between 3 and 5 h for most dogs), only minimal prolongation of aPTT was observed. Another surprising finding was that even when *R* time was significantly prolonged, anti-Xa activity levels did not rise into the proposed therapeutic range in any dogs on Day 1, and in many dogs on Day 4.

Despite significant differences in baseline parameters between Days 1 and 4 (which corresponded to 8 h after the previous subcutaneous dose), all dogs' tracings returned to the Day 1 baseline within 14 hours after their final UFH dose.

UFH consists of a population of negatively charged molecules of different sizes that interact with many plasma proteins as well as cellular components. When UFH is administered subcutaneously, differences in absorption and adherence may contribute to a delayed action on the measured parameter of anti-Xa activity. The relation between anti-Xa activity and TEG was not demonstrated clearly in this study. Given that, in the

Table 3: Thrombelastography, activated partial thromboplastin time, and anti-Xa activity results from Day 4 ($n = 6$)

Time (hours)	*R* (min) (2.1–11.0 min)	*K* (min) (1.2–4.6 min)	Angle (degrees) (39–74°)	MA (mm) (44.5–61.7 mm)	aPTT (s) (9.4–15.1 s)	Anti-Xa (U/mL) (0.3–0.7 U/mL)
0	60.0 (3.35–60.0)*		46.4 (1.8–54.2)		12.3 (10.5–15.9)	0.2 (<0.1–0.75)
1	60.0 (5.85–60.0)*				13.1 (11.2–17.0)	
3	60.0 (46.8–60.0)*				15.3 (14.2–22.8)	0.4 (0.2–0.65)
6	60.0 (40.6–60.0)*				14.8 (13.4–19.8)	0.3 (<0.1–0.5)
8	60.0 (6.7–60.0)		18.8 (7.9–29.6)		13.2 (11.0–15.9)	0.2 (<0.1–0.35)
9	48.6 (3.8–60.0)	8.3 (2.5–24.0)	18.8 (3.7–56.9)	47.7 (43.7–51.8)	12.5 (11.1–23.2)	
10	5.9 (3.5–60.0)	3.5 (2.2–5.6)	45.8 (2.6–61.4)	51.0 (45.0–58.4)	12.0 (11.4–14.3)	
11	6.4 (2.0–13.2)	3.9 (1.9–26.3)	45.5 (10.0–64.6)	51.1 (38.4–61.1)	12.3 (10.8–14.1)	
12	4.53 (3.3–15.5)	3.1 (2.4–9.3)	52.5 (22.4–59.0)	52.5 (38.6–57.2)	12.0 (10.9–14.1)	

Data shown as median (range).
No data were collected at the 2, 4, 5, and 7 hours post administration time points based on the extensive prolongation of the R time during the single dose heparin phase. For the same reason, accurate values were unable to be obtained for *K*, angle and MA for all dogs before 9 hours post administration.
*Indicates statistically significant values ($P < 0.01$).
MA, maximum amplitude; aPTT, activated partial thromboplastin time.

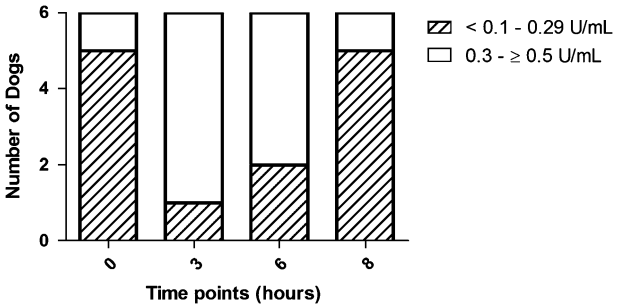

Figure 2: Anti-Xa activity level of all 6 dogs on Day 4. Shaded areas indicate subtherapeutic plasma heparin levels, while the non-shaded regions represent the number of dogs that did achieve appropriate anti-Xa activity levels (0.3–0.7 U/mL). Only 3 values were above 0.5 U/mL (1 dog at 0 hours and 2 dogs at 3 hours).

presence of excessive TEG changes, we did not meet other criteria for adequate heparin effects (ie, anti-Xa activity levels in the therapeutic range), either therapeutic heparin levels in the dog are lower than those proposed for humans, or the TEG is not a useful tool for evaluating the results of UFH therapy in dogs because it is too sensitive to low concentrations of UFH. Our TEG results parallel a study in cats in which hypocoagulable TEG tracings (characterized by a prolonged *R* time) were seen 4 hours after subcutaneous administration (250 IU/kg) of UFH.[18] However, in contrast to the current study, all cats evaluated had reached target anti-Xa activity levels at the same time as the hypocoagulable tracings. This is likely due to variation in pharmacokinetics and pharmacodynamics between species.[18] Administration of an IV loading dose of

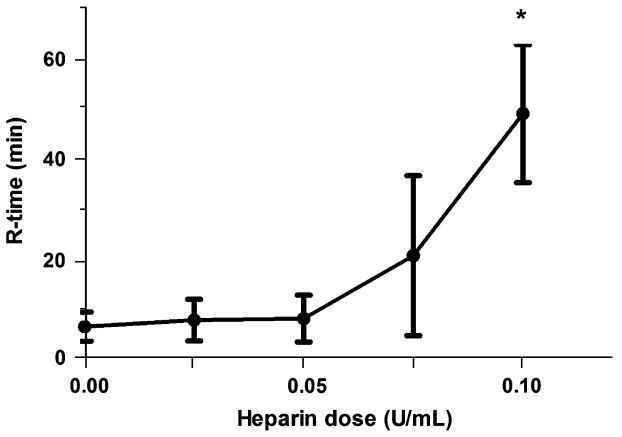

Figure 3: Mean and standard deviation of *R* time after in vitro addition of heparin in whole blood. Prolongation was initially noted at 0.075 U/mL; however, this prolongation was not statistically significant ($P < 0.05$, indicated by asterisk) until a concentration of 0.1 U/mL was reached.

UFH before initiating subcutaneous UFH therapy has been recommended in humans, and may result in a faster rise to therapeutic anti-Xa activity levels.[7] The results of the in vitro portion of this study suggest that when analyzing whole blood samples, the *R* time is prolonged at plasma heparin levels of 0.075 U/mL or less, depending on the dog. These values are significantly below what is thought to be the therapeutic threshold for anticoagulation,[12] and this sensitivity may limit the utility for TEG monitoring of UFH therapy in dogs. The fact that the *R* time was prolonged at even lower in vitro heparin levels (0.025 U/mL) when analyzing plasma samples indicates that the increased sensitivity of the test is not due to a single component (eg, cellular binding of heparin or inactivation of coagulation factors) alone.[7]

While plasma heparin concentrations of 0.3–0.7 anti-Xa U/mL have previously been correlated with a 120–160% prolongation of aPTT,[12] similar results were not demonstrated in this study. Possible causes of this discrepancy include changes to aPTT measurements as a result of storage; however, samples were run within 2 months of acquisition and previous studies suggest that storage -80°C for up to 4 months does not have a significant effect on aPTT.[25]

The testing methodology may also be a reason that prolonged *R* time was not accompanied by a prolongation in aPTT. TEG requires a clot to form between a rotating cup and a stationary pin such that when they begin to move in concert they create the deflections seen as a thrombelastogram. If samples with UFH are unable to form a strong enough clot to link the cup and the pin, the tracing is unable to start. The aPTT requires only sufficient clot formation to slow the motion of a small metal ball within the assay, and thus shear stress does not contribute to assessment of aPTT, possibly resulting in the dichotomous results between the 2 methods. Modifications to viscoelastic coagulation techniques such as the addition of an activator (tissue factor or glass beads) to enhance thrombin formation may be a more accurate way to evaluate canine patients who are receiving UFH.

Anti-Xa activity is the gold standard for monitoring heparin therapy, and the human literature gives a target plasma concentration of 0.3–0.7 U/mL for adequate anticoagulation.[26] The chromogenic assay used in this study has a limit of quantification of 0.1 U/mL.[11] Significant prolongations of TEG parameters, especially *R* time, were noted even when plasma heparin concentrations were below this lower limit of detection. It is unclear how much lower than this limit the anti-Xa activity levels from the dogs in this study fell. In vitro TEG analysis of plasma heparin concentrations indicated significant prolongations of *R* time at a

concentration of 0.075 U/mL, explaining why we found significant changes in the TEG parameters of treated dogs when anti-Xa activity levels confirmed heparin concentrations were not in the proposed therapeutic range.

Concurrent decreases in Ang and MA were seen in this study, although none of these parameters were measurable at the peak of the heparin effect, so it is difficult to draw conclusions. This is consistent with the decrease in clot strength and rate of formation that is expected with UFH therapy; however, we cannot conclude more about the utility of these parameters for monitoring UFH anticoagulation due to many missing data points. As the UFH dose effect was waning, however, a return to the baseline clot strength was noted, with some dogs actually exceeding their initial values (ie, displaying a shortened *R* time) for a period of 2 hours.

The presence of a rebound hypercoagulability in the dog is previously unrecognized. The trend toward hypercoagulability noted in 4 of the dogs in this study at the end of Day 4 indicates that this is a phenomenon that may occur in dogs if UFH therapy is rapidly withdrawn. A state of transient hypercoagulability has been reported in human patients in whom UFH therapy is abruptly discontinued,[27–29] and thus it has been recommended to taper UFH doses over several days in dogs, although no primary investigation of this phenomenon exists. Further investigation of serial TEG analysis after discontinuation of UFH therapy may give additional insight into the phenomenon of hypercoagulability after abrupt discontinuation of UFH, and may provide guidelines for the most appropriate way to taper the dose.

The maximum anticoagulant effect, as defined by the maximal prolongation of *R* time, was seen in this study between 3 and 5 hours after administration, which is consistent with prior studies in dogs.[12] TEG is very sensitive for the effect of UFH in whole blood; however, it is too sensitive to be used as an indicator of excessive anticoagulation. Based on the findings reported here, patient monitoring via TEG should ideally occur 3–5 hours after UFH administration to identify its maximal effect. While the presence of a prolonged *R* time does not confirm adequate anticoagulation, a lack of prolongation indicates subtherapeutic UFH levels, and merits an increase in the dose. Caution is also merited when evaluating TEG tracings performed on blood that was obtained from indwelling catheter that has been flushed with UFH-containing solutions. Because even small amounts of UFH may change the appearance of the tracing, it is wise to use direct venipuncture rather than acquiring blood for TEG analysis from a heparinized sampling catheter.

The study presented here had several limitations. Ideally, anti-Xa activity levels would have been performed on samples from the in vitro portion of the study to confirm plasma heparin concentrations. Another potential limitation involves using each dog as its own control. Although this should be accounted for in the repeated measures analysis, some dogs did not return to their initial baseline range before beginning analysis on Day 4.

In conclusion, TEG and aPTT should be used together in guiding and assessing UFH therapy in dogs. The lack of definitive TEG endpoints and presence of markedly prolonged *R* time despite negligible alterations in aPTT or anti-Xa activity suggests TEG may be too sensitive to identify adequate or excessive anticoagulation. The aPTT is a more accurate test to demonstrate excessive anticoagulation, as it produces a measurable value in states of hypocoagulability, while on TEG tracings this is indicated only by a straight line. Because the only parameter in the dog shown to correlate with adequate anticoagulation is aPTT,[15] it is difficult to determine if the anticoagulation measured using TEG is consistent with *adequate* anticoagulation, or just the start of an effect. Until true outcome studies are completed, it is difficult to make definitive conclusions on the utility of TEG for therapeutic UFH monitoring. In the interim, lack of *R* prolongation measured 3–5 hours after UFH administration likely indicates a need to increase the UFH dose.

Footnotes

[a] Kellerman DL, Lewis DC, Bruyette DS, et al. Determination and monitoring of a therapeutic heparin dosage in the dog (abstr.) J Vet Intern Med 1995; 9:187.
[b] Central Venous Catheterization Set, Arrow International, Reading, PA.
[c] Thrombo-MAX by AMAX, Trinity Biotech, Berkeley Heights, NJ.
[d] Alexin by AMAX, Trinity Biotech.
[e] ACCUCOLOR by AMAX, Trinity Biotech.
[f] Modified Rotachrom Heparin, Diagnostica Stago, Parsippany, NJ, performed at the Animal Health Diagnostic Center, Cornell University, Ithaca, NY.
[g] TEG 5000, Haemoscope, Niles, IL.
[h] Citrate tubes, Becton Dickenson, Franklin Lakes, NJ.
[i] Heparin sodium, 1000 U/mL, Baxter Healthcare Corporation, Deerfield, IL.
[j] $CaCl_2$, 0.2 M, Haemoscope.
[k] Unactivated cups and pins, Haemoscope.
[l] SAS V 9.1, SAS Institute Inc, Cary, NC.

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