Evaluation of tranexamic acid and ε-aminocaproic acid concentrations required to inhibit fibrinolysis in plasma of dogs and humans

Daniel J. Fletcher, PhD, DVM; Kelly J. Blackstock, DVM; Kira Epstein, DVM; Benjamin M. Brainard, DVM

Objective—To determine minimum plasma concentrations of the antifibrinolytic agents tranexamic acid (TEA) and ε-aminocaproic acid (EACA) needed to completely inhibit fibrinolysis in canine and human plasma after induction of hyperfibrinolysis.

Samples—Pooled citrated plasma from 7 dogs and commercial pooled citrated human plasma.

Procedures—Concentrations of EACA from 0 µg/mL to 500 µg/mL and of TEA from 0 µg/mL to 160 µg/mL were added to pooled citrated canine and human plasma. Hyperfibrinolysis was induced with 1,000 units of tissue plasminogen activator/mL, and kaolin-activated thromboelastography was performed in duplicate. The minimum concentrations required to completely inhibit fibrinolysis 30 minutes after maximum amplitude of the thromboelastography tracing occurred were determined.

Results—Minimum plasma concentrations necessary for complete inhibition of fibrinolysis by EACA and TEA in pooled canine plasma were estimated as 511.7 µg/mL (95% confidence interval [CI], 433.2 to 590.3 µg/mL) and 144.7 µg/mL (95% CI, 125.2 to 164.2 µg/mL), respectively. Concentrations of EACA and TEA necessary for complete inhibition of fibrinolysis in pooled human plasma were estimated as 122.0 µg/mL (95% CI, 106.2 to 137.8 µg/mL) and 14.7 µg/mL (95% CI, 13.7 to 15.6 µg/mL), respectively.

Conclusions and Clinical Relevance—Results supported the concept that dogs are hyperfibrinolytic, compared with humans. Higher doses of EACA and TEA may be required to fully inhibit fibrinolysis in dogs. (*Am J Vet Res* 2014;75:731–738)

Hyperfibrinolysis has been well documented in human patients following shock, trauma, and invasive surgery, particularly in patients who require cardiac bypass.[1,2] Although the underlying mechanism of hyperfibrinolysis is not completely understood, patients with increased fibrinolysis are at higher risk for bleeding, requiring additional surgery because of bleeding, and death.[1] Antifibrinolytic drugs used to counteract this bleeding disturbance have included aprotinin, EACA, and TEA. Aprotinin, a plasmin and kallikrein inhibitor, was used extensively to treat bleeding during surgery with cardiopulmonary bypass until 2007, when it was associated with increased risk of myocardial infarction and death.[3] Unlike aprotinin, EACA and TEA inhibit fibrinolysis by blocking the lysine binding site of plasminogen. A recent review concluded that TEA and EACA are as effective as aprotinin in preventing surgical blood loss and decreasing transfusion need but are safer than aprotinin.[4] Tranexamic acid also decreased the risk of death from bleeding in a prospective randomized trial of 20,211 adult human trauma patients.[5]

Dogs have accelerated fibrinolysis, compared with humans. This was first observed in studies of pulmonary embolism in the 1960's, in which experimentally induced emboli in dogs resolved within hours to days,

Abbreviations

EACA	ε-Aminocaproic acid
EPL	Estimated percentage lysis
G	Elastic shear modulus
K	Time from the reaction time to a thromboelastogram tracing 10 mm wide
L	Total lysis
MA	Maximum amplitude
MRL	Maximum rate of lysis
MRTG	Maximum rate of thrombus generation
R	Reaction time
TEA	Tranexamic acid
TEG	Thromboelastography
TG	Total thrombus generation
TMRL	Time to maximum rate of lysis
TMRTG	Time to maximum rate of thrombus generation
tPA	Tissue plasminogen activator

Received March 10, 2013.
Accepted March 3, 2014.
From the Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853 (Fletcher, Blackstock); and the Departments of Large Animal Medicine (Epstein) and Small Animal Medicine and Surgery (Brainard), College of Veterinary Medicine, University of Georgia, Athens, GA 30602. Dr. Blackstock's present address is Fifth Avenue Veterinary Specialists, 1 West 15th St, New York, NY 10011.
Presented in abstract form at the International Veterinary Emergency and Critical Care Symposium, Nashville, Tenn, September 25, 2011.
Address correspondence to Dr. Fletcher (djf42@cornell.edu).

rather than the months to years typically seen in human patients.[6,7] Greater fibrinolysis in dogs, compared with humans, can be detected by use of the euglobulin method.[8] Lang et al[9] attributed this specifically to greater secretion of tPA by the pulmonary endothelium, greater plasminogen activator activity (tPA and other plasminogen activators), and greater platelet thrombolytic activity. Chromogenic substrate assays confirm this finding; there is 25% more tPA in canine plasma than in human plasma.[10] Creation of an experimental model of chronic pulmonary embolism in dogs was only possible by use of treatment with TEA.[7] Antifibrinolytic drugs have been used clinically in veterinary medicine to reduce postoperative hemorrhage in Greyhounds, a breed with a high prevalence of postoperative bleeding complications.[11,12] Despite their clinical use, effective plasma concentrations of antifibrinolytic agents in dogs are unknown, and administration regimens have been extrapolated from pharmacodynamic data in humans. Given the strong evidence that dogs have a stronger fibrinolytic response than humans, it is likely that these drugs have been administered in inadequate doses in dogs, potentially limiting their efficacy.

Thromboelastography has been used in the diagnosis of disorders of coagulation and fibrinolysis in humans. Previous studies reveal strong associations between TEG measures of fibrinolysis and in vivo serum markers of fibrinolysis.[13,14] In addition, TEG studies in adult and neonatal humans reveal a dose-dependent enhancement of fibrinolysis in vitro with both tPA and urokinase.[13,15] Recently, TEG was used in an in vitro study of hyperfibrinolysis to investigate therapeutic concentrations of EACA in pooled plasma from adult and neonatal humans.[13] Hyperfibrinolysis was induced with the addition of 1,000 units of tPA/mL to pooled citrated plasma. That study[13] corroborated previous findings defining therapeutic plasma concentrations of EACA in human adults (130 µg/mL) and established that the therapeutic plasma concentration of EACA in neonates (40 µg/mL) is less than in adults.[13] Although this concentration of tPA is 3 times that of blood concentrations of tPA measured in hyperfibrinolytic patients during cardiopulmonary bypass, there is strong evidence that local endothelial production of tPA greatly increases (> 100-fold) and that systemic tPA concentrations can severely underestimate local concentrations.[16] To our knowledge, TEG has not been used to evaluate effective concentrations of TEA in humans or dogs; however, the proposed therapeutic plasma concentration of TEA in humans is 10 µg/mL[17,18]

The objective of the study reported here was to determine minimum plasma concentrations of the antifibrinolytic agents TEA and EACA needed to completely inhibit fibrinolysis in canine blood after induction of hyperfibrinolysis and to compare these concentrations between dogs and humans. We hypothesized that the minimum required concentrations of TEA and EACA to inhibit this hyperfibrinolytic state would be higher in dog plasma than in human plasma.

Materials and Methods

Samples—Blood sample collection for this study was approved by the University of Georgia Institutional Animal Care and Use Committee. Seven random-source mixed-breed dogs, deemed healthy on the basis of results of physical examination and CBC were used. From each dog, 9 mL of blood was collected via jugular venipuncture into a 12-mL polypropylene syringe containing 1 mL of 3.2% sodium citrate, for final citrate:blood ratio of 1:9. After 30 to 40 minutes, the tubes were centrifuged at 1500 × *g* for 10 minutes, and 5 mL of citrated plasma was used to create 35 mL of pooled plasma, which was then stored in aliquots of 5 mL at –80°C until time of use (within 2 months of collection). Coagulation testing was completed on

Table 1—Mean (coefficient of variation) values determined by use of thromboelastography for clotting variables in pooled canine and human plasma after addition of various concentrations of tPA.

tPA (U/mL)	Species	R (min)	K (min)	α-Angle (°)	MA (mm)	EPL (%)	G (dyn/cm²)	MRTG (Δdyn/cm²)	TMRTG (min)	TG (dyn•cm²)	MRL (Δdyn/cm²)	TMRL (min)	L (dyn•cm²)
0	Canine	3.85 (0.055)	2.45 (0.38)	69.0 (0.034)	22.2 (0.038)	0 (0)	1,427.4 (0.050)	6.62 (0.19)	4.59 (0.051)	161.7 (0.015)	0 (0)	–	0 (0)
	Human	9.40 (0)	3.45 (0.020)	52.0 (0.021)	24.6 (0.011)	0 (0)	1,630.8 (0.018)	3.51 (0.068)	10.8 (0.022)	199.6 (0.024)	0 (0)	–	0 (0)
200	Canine	3.95 (0.054)	–	68.2 (0.012)	16.1 (0.013)	98.8 (0.0043)	956.0 (0.021)	5.92 (0.004)	4.54 (0.065)	94.1 (0.027)	0.47 (.47)	8.13 (.17)	94.1 (0.027)
	Human	9.20 (0)	2.6 (0)	50.8 (0.076)	21.0 (0.15)	99.4 (0.001)	1,330.8 (0.19)	3.94 (0.24)	10.63 (0.006)	132.0 (0.19)	1.28 (0.26)	20.42 (0.006)	132.0 (0.19)
400	Canine	4.10 (0.034)	–	56.1 (0.059)	8.2 (0.10)	98.5 (0.0014)	446.8 (0.10)	3.30 (0.15)	4.59 (0.026)	39.2 (0.087)	2.21 (0.22)	5.75 (0.042)	39.2 (0.087)
	Human	8.15 (0.009)	–	48.2 (0.063)	15.8 (0.062)	99.2 (0)	939.1 (0.077)	3.12 (0.16)	9.46 (0.019)	92.1 (0.076)	1.71 (0.12)	14 (0.008)	92.1 (0.076)
600	Canine	3.85 (0.018)	–	38.7 (0.057)	4.0 (0.070)	96.9 (0.002)	208.6 (0.082)	2.28 (0.031)	4.08 (0)	20.5 (0.027)	1.91 (0.067)	5.08 (0)	20.5 (0.027)
	Human	9.55 (0.096)	–	42.4 (0.12)	10.4 (0.089)	98.8 (0.001)	576.4 (0.095)	2.69 (0.11)	10.46 (0.096)	54.1 (0.083)	2.04 (0.035)	12.5 (0.066)	54.1 (0.083)
800	Canine	–	–	– (0.089)	1.9 (0)	100 (0.094)	– (0)	0.9 (0)	4.08 (0.10)	0.11 (0)	0.68	5.08	–
	Human	8.30 (0)	–	52.4 (0.11)	8.2 (0.10)	98.5 (0.001)	449.6 (0.11)	3.20 (0.17)	8.88 (0.007)	38.8 (0.12)	2.58 (0.13)	10.04 (0.006)	38.8 (0.12)
1,000	Canine	–	–	–	1.7 (0.33)	100 (0)	– (0.34)	0.77 (0.048)	3.71 (0.20)	0.14 (.42)	0.59 (.038)	4.63	–
	Human	8.95 (0.008)	–	19.2 (0.38)	2.9 (0.20)	95.6 (0.009)	149.6 (0.21)	0.81 (0.48)	9.17 (0.013)	13.6 (0.20)	0.66 (0.25)	10.42 (0.012)	13.6 (0.20)

– = Not determined

the pooled plasma, and it was determined that prothrombin time, activated partial thromboplastin time, antithrombin activity, as well as concentrations of fibrinogen, D-dimer, fibrin degradation product, alpha-2-antiplasmin, and plasminogen were within reference ranges. Commercial pooled human plasma,[a] confirmed to have normal coagulation function, was used for comparison. According to the National Institutes of Health guidelines, use of commercially available products for which individual donors cannot be identified does not require institutional approval. For each TEG trial, pooled plasma was thawed in a 37°C water bath and assays were initiated within 30 minutes of thawing.

TEG protocol—The TEG protocol was based on that described by Yurka et al[13], and Nielsen et al.[15] Kaolin-activated TEG was performed in duplicate with an analyzer[b] with standard disposable cups and pins. Commercial recombinant tPA[c] was reconstituted with sterile water to a final concentration of 250 µg/mL (145,000 U/mL). An initial tPA dose-response analysis was completed by adding serial dilutions of the tPA solution to 980 µL of pooled citrated plasma to yield final concentrations of tPA ranging from 0 U/mL to 1,000 U/mL in increments of 200 U/mL. Potassium phosphate buffer solution (pH, 7.4) was added (up to approx 13 µL) to achieve a final sample volume of 1 mL, which was added to a vial containing 1% kaolin.[b] The sample was mixed by inverting the kaolin vial 5 times per the manufacturer's recommendation. Subsequently, 340 µL of the sample was pipetted into a cup containing 20µL of 10% calcium chloride. All samples were analyzed in duplicate at 37°C. Tracings were stopped a minimum of 30 minutes after MA was achieved. On the basis of the results of this investigation, the 1,000 U/mL concentration of tPA was used for further assays.

To estimate the effective plasma concentrations of TEA and EACA, a standard hyperfibrinolytic assay was used, consisting of 6.89 µL (1,000 units) of the tPA dilution added to 10 µL of either EACA[d] or TEA[e] of various concentrations and 980 µL of pooled citrated plasma. A volume of 3.11 µL of PBS solution (pH, 7.4) was added to this final solution to yield a final sample volume of 1 mL in a vial containing 1% kaolin.

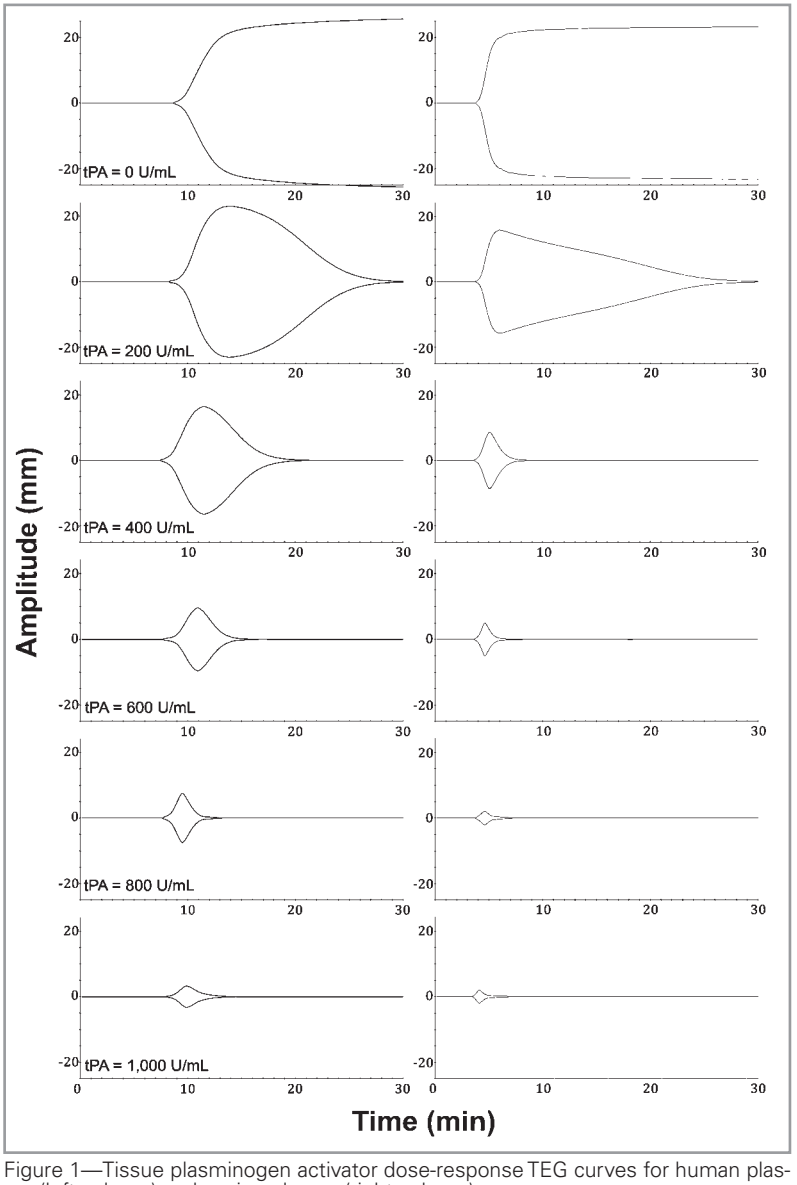

Figure 1—Tissue plasminogen activator dose-response TEG curves for human plasma (left column) and canine plasma (right column).

For human and canine plasma, concentrations of either TEA or EACA were sequentially increased until complete inhibition of fibrinolysis was observed for 30 minutes after the tracing reached MA. Complete inhibition was defined as an EPL of 0% at 30 minutes after MA on the basis of results of previous studies of human plasma concentrations of EACA and TEA performed with a TEG assay.[13,15] The EPL was calculated with the equation 100 × (MA – A30)/MA, where A30 is the amplitude of the TEG tracing 30 minutes after MA is achieved. The EPL represents the percentage decrease in clot strength 30 minutes after the MA. The EACA and TEA were diluted with sterile water so that the volume added to the final 1-mL sample was always 10 µL. The EACA concentrations evaluated in canine plasma were 0, 80, 130, 250, 350, 400, 450 and 500 µg/mL. The EACA concentrations evaluated in human plasma were 0, 30, 60, 80, 100, and 130 µg/mL. The TEA concentrations evaluated in canine plasma were 0, 12.5, 30, 60, 80, 100, 120 and 140 µg/mL. The TEA concentrations evaluated in human plasma were 0, 2.5, 5, 7.5, 10, 12.5 and 15 µg/mL.

The primary outcome variable examined was the TEG EPL. This allowed comparisons with previous studies investigating adult and neonatal human fibrinolysis. Dose response curves for EACA and TEA were generated, and the EPL of fibrinolysis-induced human and canine plasma were compared. In addition, the

standard TEG coagulation variables (R time, K time, α angle, MA, and G) as well as the TEG clot formation and lysis velocity variables (MRTG, TMRTG, TG, MRL, TMRL, and L) were recorded.

Statistical analysis—Values of the standard TEG and velocity variables for the duplicate assays are presented as mean and coefficient of variation, and were compared qualitatively between the human and canine plasma tPA dose-response curves. These same values were compared qualitatively, for plasma samples from each species with 1,000 U/mL of tPA added, between 4 representative concentrations of EACA and TEA for each species, representing complete lysis, minimal lysis, and intermediate lysis at 30 minutes after MA.

Effective plasma concentrations of EACA and TEA were estimated by use of linear regression modeling of the data used to generate the EPL dose-response curves. Each individual data point from the paired TEG analyses was included in the regression model. Because the EPL is a ratio and the curve is a sigmoidal shape, an arcsin of the square root transform was applied to linearize the data.[19] At the lowest concentrations of EACA and TEA, repeated EPL values of 100% were obtained because of complete lysis of the clot, leading to redundancy in the data. At the highest concentrations, flooring was present because EPL decreased to 0% with complete inhibition of fibrinolysis. Redundancy and flooring of data at the lowest and highest EACA and TEA concentrations were avoided by selecting limited data points in the mid-range of concentrations tested that resulted in unique EPL values that were < 100% and > 0%. Linear regression analysis was then used to estimate the relationship between EACA or TEA concentration and EPL, and the intercept from the regression analysis (along with the 95% confidence intervals) was calculated to estimate the minimum EACA and TEA concentrations required to achieve an EPL of zero (ie, complete inhibition of fibrinolysis at 30 minutes after MA).[19]

Results

Values for TEG coagulation and lysis variables and velocity variables for clot formation and clot lysis were determined for control samples containing graded concentrations of tPA (from 0 to 1,000 U/mL) but without antifibrinolytic agents for canine and human plasma (**Table 1**; **Figures 1 and 2**). In the absence of either antifibrinolytic agent (concentrations of 0 µg/mL), clots lysed more rapidly and MA decreased as tPA concentration increased. Qualitatively, canine plasma clotted more quickly (shorter R times), but the clots were weaker (slightly smaller MA and G) at all tPA concentrations. No clot lysis occurred within 30 minutes in the absence of tPA (EPL = 0%; L = 0 dyn/cm^2) in either the canine or human plasma, but the clots were effectively fully lysed within 30 minutes in both species at all concentrations of tPA from 200 to 1,000 U/mL (EPL > 95.6%). At 1,000 U/mL, although MA appeared smaller in the canine plasma (1.7 mm) than in the human plasma (2.9 mm), the TEG tracings were similar between the 2 species.

The addition of EACA or TEA inhibited fibrinolysis in a concentration-dependent fashion in both human and canine plasma exposed to 1,000 U/mL of tPA (**Tables 2–4**; **Figure 3**). Minimum plasma concentrations of EACA and TEA necessary for complete inhibition of fibrinolysis at 30 minutes (EPL = 0%) obtained from the inverse prediction analysis based on the linear regression results were determined Higher concentrations of both drugs were required to inhibit fibrinolysis in canine plasma, compared with in human plasma.

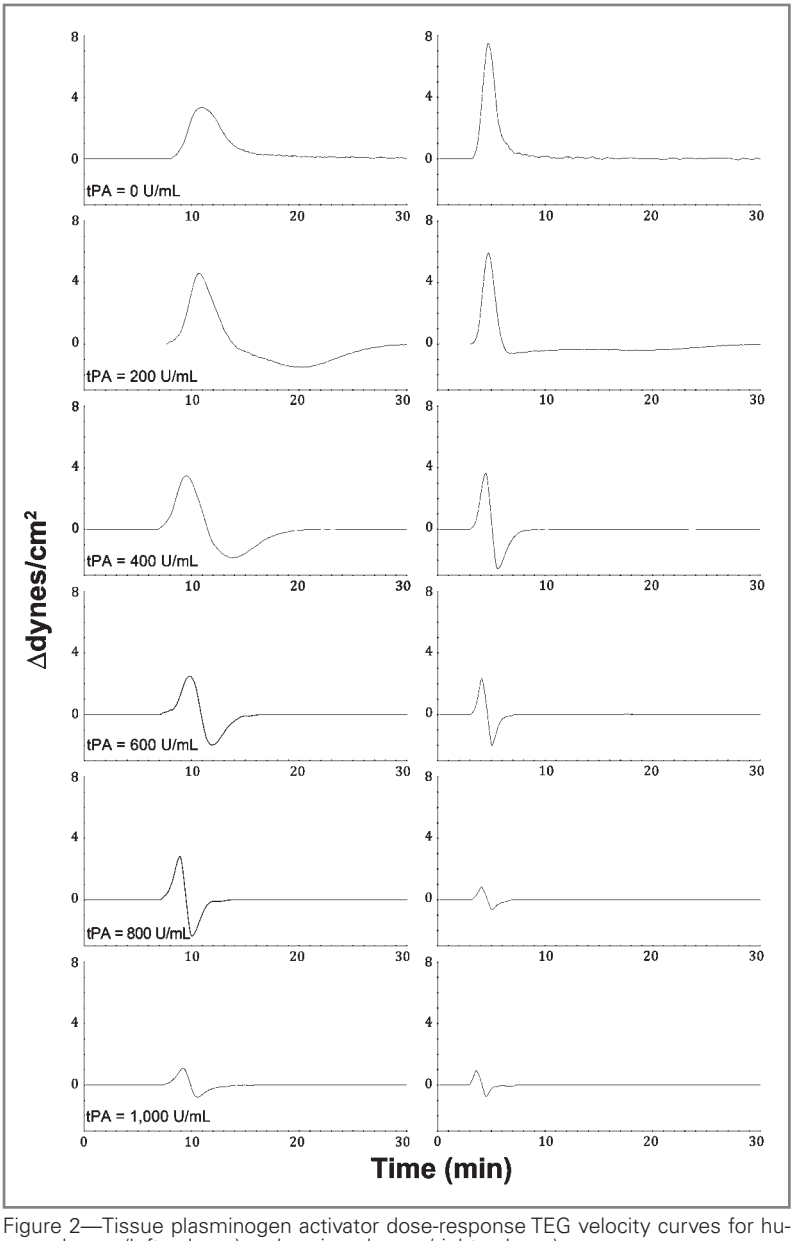

Figure 2—Tissue plasminogen activator dose-response TEG velocity curves for human plasma (left column) and canine plasma (right column).

Table 2—Mean (coefficient of variation) values for variables of clot strength, lysis, and velocity in human and canine plasma treated with 4 concentrations of EACA and 1,000 U of tPA/mL.

	EACA concentration in human plasma				EACA concentration in canine plasma			
Variable	**30 μg/mL**	**80 μg/mL**	**100 μg/mL**	**130 μg/mL**	**80 μg/mL**	**250 μg/mL**	**450 μg/mL**	**500 μg/mL**
G (dyn/cm²)	865.2 (0.032)	1,117.7 (0.051)	1,711.2 (0.027)	2,061.9 (0.056)	580.9 (0.29)	1,081.1 (0.059)	1,144.9 (0.21)	1,208.9 (0.12)
EPL (%)	99.3 (0.001)	93.6 (0.005)	8.87 (0.001)	0 (0)	99.0 (0.003)	94.1 (0.016)	56.7 (0.77)	0 (0)
MRTG (Δdyn/cm²)	3.14 (0.18)	3.87 (0.15)	3.69 (0.10)	4.58 (0.21)	3.71 (0.29)	3.66 (0.16)	4.85 (0.013)	4.15 (0.055)
TMRTG (min)	11.0 (0.042)	9.46 (0.006)	9.71 (0.018)	9.46 (0.019)	4.75 (0)	4.38 (0.015)	4.09 (0.029)	4.08 (0)
TG (dyn•cm²)	84.1 (0.038)	111.0 (0.052)	131.1 (0.048)	145.7 (0.12)	56.1 (0.29)	88.1 (0.093)	131.4 (0.003)	124.8 (0.005)
MRL (Δdyn/cm²)	2.18 (0.062)	1.44 (0.12)	0.12 (0)	0 (0)	1.46 (0.36)	1.09 (0.15)	1.33 (0.19)	0 (0)
TMRL (min)	14.25 (0.008)	17.30 (0.031)	26.0 (0.034)	–7.96 (0.038)	15 (0.024)	33.9 (0.050)	–	
L (dyn•cm²)	84.1 (0.038)	103.8 (0.13)	15.7 (0.090)	–56.1 (0.29)	86.9 (0.099)	121.3 (0.010)	0 (0)	

– = Not determined

Table 3—Mean (coefficient of variation) values for variables of clot strength, lysis, and velocity in human and canine plasma treated with 4 concentrations of TEA and 1,000 U of tPA/mL.

	TEA concentration in human plasma				TEA concentration in canine plasma			
Variable	**5 μg/mL**	**7.5 μg/mL**	**12.5 μg/mL**	**15 μg/mL**	**30 μg/mL**	**80 μg/mL**	**120 μg/mL**	**140 μg/mL**
G (dyn/cm²)	1,070.5 (0.034)	1,225.5 (0.033)	1,252.1 (0.043)	1,297.1 (0.015)	1,016.5 (0.025)	1,819.5 (0.017)	1,802.2 (0.026)	1,850.1 (0.009)
EPL (%)	99.2 (0.004)	65.5 (0.048)	16.0 (0.079)	0 (0)	99.4 (0.001)	94.0 (0.028)	10.0 (0.008)	0 (0)
MRTG (Δdyn/cm²)	3.84 (0.057)	4.89 (0.023)	3.58 (0.13)	–	6.79 (0.028)	8.36 (0.12)	6.84 (0.068)	6.86 (0.071)
TMRTG (min)	9.71 (0.031)	9.75 (0.011)	9.75 (0.011)	–	3.59 (0.034)	3.5 (0)	3.46 (0.12)	3.96 (0.014)
TG (dyn•cm²)	106 (0.035)	121.9 (0.033)	126.8 (0.047)	–	98.3 (0.027)	181.9 (0.12)	154.3 (0.20)	184.1 (0.014)
MRL (Δdyn/cm²)	1.49 (0.019)	0.57 (0.15)	0.18 (0.040)	–	2.67 (0.024)	1.2 (0.64)	0.4 (0.11)	0 (0)
TMRL (min)	16.04 (0.041)	24.29 (0.056)	33.80 (0.005)	–	5.08 (0)	32.5 (0.079)	49.6 (0.26)	-
L (dyn•cm²)	106 (0.035)	88.6 (0.074)	26.8 (0.024)	–	98.3 (0.027)	165.8 (0.13)	62.2 (0.39)	0 (0)

– = Not determined

Table 4—Estimated concentrations and 95% confidence intervals of EACA and TEA required for antifibrinolysis in human and canine plasma.

Drug	**Estimate (μg/mL)**	**95% CI (μg/mL)**
EACA		
Human	122.0	106.2–137.8
Canine	511.7	433.2–590.3
TEA		
Human	14.7	13.7–15.6
Canine	144.7	125.2–164.2

Significant ($P < 0.001$) linear associations between the arcsin square root transformed EPL data and EACA and TEA concentrations were identified. The minimum effective plasma concentrations of EACA and TEA were greater for canine plasma than for human plasma, with nonoverlapping 95% confidence intervals. Concentration-effect curves for EACA and TEA were generated (**Figure 4**).

Discussion

In the absence of additional tPA or antifibrinolytic agents, TEG analysis revealed that canine plasma was relatively hypercoagulable, compared with human plasma, and had shorter R and K times, steeper α angle, higher MRTG, and shorter TMRTG with comparable MA and G. This was consistent with results of previous studies that indicated that compared with humans, dogs have shorter clotting times and increased coagulation factor activity.[8,20] Although the literature also suggests that dogs are hyperfibrinolytic, compared with humans, in the absence of additional tPA neither the human nor canine plasma had any fibrinolysis within 30 minutes of MA (EPL = 0%). Therefore, the accuracy of TEG for diagnosis of clinical hyperfibrinolysis is questionable, and a previous study failed to detect substantial lysis by use of TEG in dogs that responded favorably to treatment with anti-fibrinolytic drugs.[12]

More recently, modified TEG assays using additional tPA have revealed measurable differences in TEG fibrinolysis variables between human and canine patients with disorders of fibrinolysis and healthy subjects.[14,21] By use of a similar approach, our qualitative tPA dose-response analysis of canine and human plasma revealed that higher tPA concentrations resulted in more profound decreases in MA, TG, and MRTG in canine plasma than in human plasma, but the TEG tracings had similar shapes. This suggests that canine plasma may be more sensitive to tPA than human plasma. The MRLG and L variables had less consistent patterns, which was likely attributable to the overall decrease in clot formation in both canine and human plasma at higher tPA doses, affecting the velocity curves and the ability to measure the magnitude of fibrinolysis. With the addition of 1,000 U of tPA/mL, the TEG tracings from the canine plasma were slightly smaller than those from the human plasma (MA, 1.7 vs 2.9 mm), but the overall shapes of the TEG curves were similar. We chose to estimate the plasma concentrations of EACA and TEA required to inhibit fibrinolysis by use of 1,000 U of tPA/mL because the TEG curves were similar in both species at this concentration and allowed direct comparison with the findings from the literature.[13,15]

The concentrations of EACA and TEA required to inhibit fibrinolysis in canine plasma in this in vitro study were 511.7 μg/mL and 144.7 μg/mL, respectively. The required concentration of EACA in human plasma determined in this study, 122.0 μg/mL, was similar to

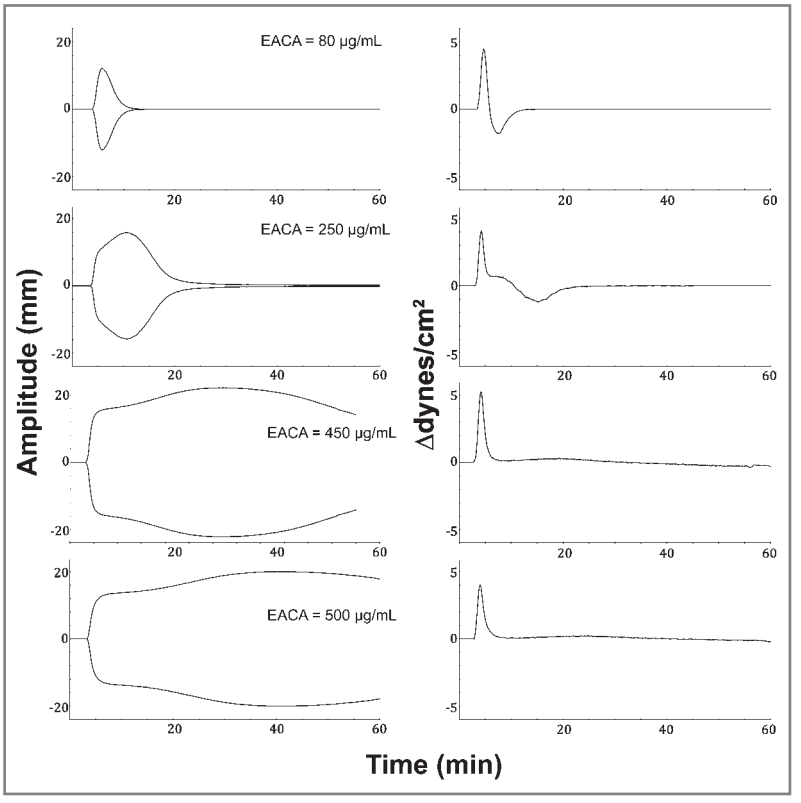

Figure 3—Thromboelastograph (left) and TEG velocity curves (right) for canine plasma treated with 1,000 U of tPA/mL and graded concentrations of EACA.

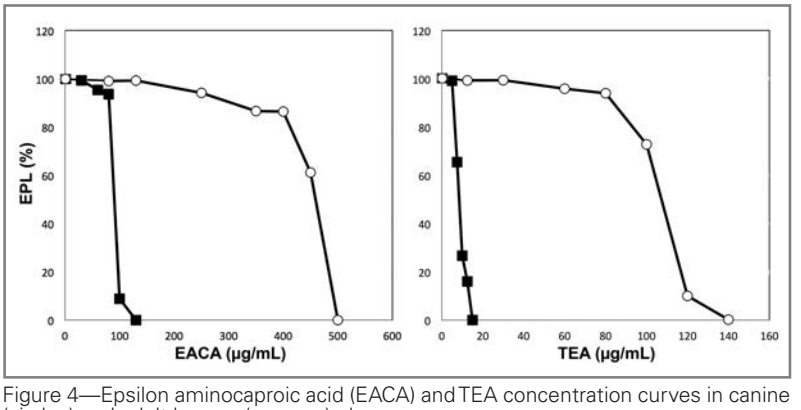

Figure 4—Epsilon aminocaproic acid (EACA) and TEA concentration curves in canine (circles) and adult human (squares) plasma.

the previously reported value (130 µg/mL). However, the required concentration of TEA in human plasma was higher than anticipated at 14.7 µg/mL. As hypothesized, concentrations of EACA and TEA necessary to completely inhibit clot lysis in canine plasma were significantly greater than those required in human plasma. Early dosage recommendations for EACA were based on in vitro studies investigating inhibition of fibrinolysis with the whole blood clot lysis assay, fibrin plate assay, or plasma euglobulin clot lysis assay.[17,22,23] McNicol et al[23] first reported the required human plasma concentration of EACA as 130 µg/mL in a study that used competitive inhibition of plasminogen activation by streptokinase, urokinase, and tissue factor. Data provided here as well as that determined by Nielsen et al[13] and Yurka[15] et al with a similar TEG assay in adult human plasma confirmed this concentration.

Early pharmacokinetic studies comparing TEA with EACA found TEA to be 6 to 10 times more potent than EACA via inhibition of streptokinase-induced plasminogen activation,[22,24] a whole blood clot lysis assay, and tPA activity inhibition.[17] By use of this comparative data, antifibrinolytic TEA plasma concentrations of 13 to 21.7 µg/mL would be expected, although the therapeutic concentration of TEA in humans is generally accepted to be approximately 10 µg/mL[18] Subsequent clinical trials with 10 µg of TEA/mL as a plasma concentration target for dose recommendations have found this dose to be therapeutic in several disease processes in humans.[5,25] To our knowledge, TEG has not been used to investigate concentrations of TEA in human or dog plasma required to inhibit fibrinolysis in vitro. Our in vitro data suggested a possibly therapeutic concentration of 14.7 µg/mL, within the range predicted by the reported relevant potency data, suggesting that higher plasma concentration targets for TEA may be considered in humans.

There are limited reports of the use of antifibrinolytic drugs in dogs.[7,26] However, recently the ability of EACA to reduce clinical postoperative bleeding in Greyhounds has been determined in a retrospective analysis of limb amputations and a prospective, randomized clinical trial of dogs undergoing elective ovariohysterectomy or orchiectomy.[11,12] Given the lack of pharmacokinetic and pharmacodynamic data in dogs, doses were extrapolated from human data.[27] The data presented here suggest that this may result in suboptimal plasma concentrations.

One explanation for the difference in therapeutic concentrations for these drugs among species is evidence that dogs may have an enhanced fibinolytic response, compared with humans. Although our data did not reveal differences between canine and human plasma EPL in the absence of tPA, the qualitative analysis of the tPA dose-response data revealed a greater decrease in MA, G, and MRTG with increasing tPA concentrations in dog plasma, compared with the decrease in human plasma. Several studies have examined the differences in the fibrinolytic response between dogs and humans, the earliest of which documented rapid pulmonary embolus resolution (within 24 to 48 hours) in dogs, as opposed to humans (weeks to months).[6,7,28] Increased in vitro fibrinolytic activity in dogs, up to 6 times that of humans, has also been reported.[29,30] Multiple mechanisms for this hyperfibrinolysis have been suggested. Plasminogen activator activity in canine plasma is approximately 25% higher than in human plasma.[10] In addition to increased plasma PA activity, Lang et al[9] found

significantly increased PA activity in canine platelets, with canine platelet membranes inducing a time- and dose-dependent fibrinolysis 50 times that reported for isolated human platelet membranes. Because our in vitro plasma study excluded the effect of platelets, the in vivo therapeutic concentrations of these drugs (ie, in the presence of platelets) may be even higher in dogs. Alternative explanations for the differences in therapeutic concentrations of these drugs could be species differences in receptor binding or affinity for the antifibrinolytic drugs as well as interaction with other fibrinolytic or coagulation factors. Regardless, the species differences in response to antifibrinolytic drugs determined in the present study were substantial and relevant for clinical application of these drugs in veterinary medicine.

This in vitro study of hyperfibrinolysis used a supraphysiologic concentration of tPA (1,000 U/mL). The reference range for tPA in dogs and humans is 2.5 ± 0.9 U/mL and 0.5 ± 0.03 U/mL, respectively.[9] In humans, increased concentrations of tPA have been detected following surgery (particularly surgery involving cardiopulmonary bypass), with secretion rates from endothelial cells increasing as much as 6-fold.[2] Hypoperfusion also leads to increases in plasma tPA concentrations in humans, up to 26 U/mL.[31] Although TEG tracings obtained with lower concentrations of tPA were investigated, insufficient lysis was observed at 30 minutes after MA on the TEG tracings in this in vitro study, necessitating higher concentrations of tPA. Yurka et al[13] observed the same limitations with use of 400 U/mL. Because published studies of a similar methodology in humans also used 1,000 U of tPA/mL to induce hyperfibrinolysis, this higher tPA concentration was chosen to facilitate comparison. Additionally, although the reported systemic concentrations are lower than those used in this study, local concentrations of tPA are likely much higher because of enhanced production by endothelial cells in response to clot formation.[16]

There were several limitations to this study. It used an in vitro method of coagulation in plasma from healthy dogs, ignoring the effects of endothelial cells on fibrinolysis. In addition, because the TEGs were performed with plasma, the influence of platelets, leukocytes, and erythrocytes on fibrinolysis could not be evaluated. Because plasma from healthy dogs was used, the effects of concurrent drug administration and hemostatic variation caused by underlying disease were not examined, and extrapolation of these concentrations to sick dogs should be done cautiously. Platelets appear to enhance fibrinolysis in dogs, suggesting that even higher doses might be needed in dogs than indicated by these data. Despite the omission of platelets, significant differences between human plasma and canine plasma were still observed. Although the interplay of all of the aforementioned cellular components and disease states influence coagulation, it should be noted that current human dosages of both TEA and EACA have been based on in vitro data, including those from which this study was modeled. Because the canine plasma had some degree of increased sensitivity to tPA concentrations, compared with human plasma, as indicated by decreased MA and G, additional in vitro studies repeating this procedure with varying tPA concentrations would be useful.

Results of the study reported here suggest that concentrations of EACA and TEA of 511.7 µg/mL and 144.7 µg/mL, respectively, may inhibit fibrinolysis in healthy dogs. Dogs are hyper-fibrinolytic, compared with humans. Because this study did not establish dosage ranges, more extensive pharmacokinetic studies in dogs are needed. Given the data reported here, increased doses of EACA and TEA should be considered in dogs.

a. FACT, George King Biomedical, Overland Park, Kan.
b. TEG 5000, Haemoscope, Skokie, Ill.
c. Alteplase, Genentech, South San Francisco, Calif.
d. Aminocaproic acid for injection, Hospira Inc, Lake Forest, Ill.
e. Sigma-Aldrich Corporation, St Louis, Mo.

References

1. Theusinger OM, Wanner GA, Emmert MY, et al. Hyperfibrinolysis diagnosed by rotational thromboelastometry (ROTEM) is associated with higher mortality in patients with severe trauma. *Anesth Analg* 2011;113:1003–1012.
2. Chandler WL, Velan T. Secretion of tissue plasminogen activator and plasminogen activator inhibitor 1 during cardiopulmonary bypass. *Thromb Res* 2003;112:185–192.
3. Mangano DT, Miao Y, Vuylsteke A. Mortality associated with aprotinin during 5 years following bypass graft surgery. *JAMA* 2007;297:471–479.
4. Henry DA, Moxey AJ, Carless PA, et al. Anti-fibrinolytic use for minimising perioperative allogeneic blood transfusion. *Cochrane Database Syst Rev* 2011;CD001886.
5. Shakur H, Roberts I, Bautista R, et al. Effects of tranexamic acid on death, vascular occlusive events, and blood transfusion in trauma patients with significant haemorrhage (CRASH-2): a randomised, placebo-controlled trial. *Lancet* 2010;376:23–32.
6. Moser KM, Guisan M, Bartimmo EE, et al. In vivo and post mortem dissolution rates of pulmonary emboli and venous thrombi in the dog. *Circulation* 1973;48:170–178.
7. Moser KM, Cantor J, Olman M, et al. Chronic pulmonary thromboembolism in dogs treated with tranexamic acid. *Circulation* 1991;83:1371–1379.
8. Cade JF, Robinson TF. Coagulation and fibrinolysis in the dog. *Can J Comp Med* 1975;39:296–298.
9. Lang IM, Marsh JJ, Konopka RG, et al. Factors contributing to increased vascular fibrinolytic activity in mongrel dogs. *Circulation* 1993;87:1990–2000.
10. Lanevschi A, Kramer JW, Greene SA, et al. Fibrinolytic activity in dogs after surgically induced trauma. *Am J Vet Res* 1996;57:1137–1140.
11. Marín LM, Iazbik MC, Zaldivar-Lopez S, et al. Retrospective evaluation of the effectiveness of epsilon aminocaproic acid for the prevention of postamputation bleeding in retired racing Greyhounds with appendicular bone tumors: 46 cases (2003–2008). *J Vet Emerg Crit Care San Antonio* 2012;22:332–340.
12. Marín LM, Iazbik MC, Zaldivar-Lopez S, et al. Epsilon aminocaproic acid for the prevention of delayed postoperative bleeding in retired racing Greyhounds undergoing gonadectomy. *Vet Surg* 2012;41:594–603.
13. Yurka HG, Wissler RN, Zanghi CN, et al. The effective concentration of epsilon-aminocaproic acid for inhibition of fibrinolysis in neonatal plasma in vitro. *Anesth Analg* 2010;111:180–184.
14. Kupesiz OA, Chitlur MB, Hollon W, et al. Fibrinolytic parameters in children with noncatheter thrombosis: a pilot study. *Blood Coagul Fibrinolysis* 2010;21:313–319.
15. Nielsen VG, Cankovic L, Steenwyk BL. Epsilon-aminocaproic acid inhibition of fibrinolysis in vitro: should the "therapeutic" concentration be reconsidered? *Blood Coagul Fibrinolysis* 2007;18:35–39.
16. Hrafnkelsdottir T, Gudnason T, Wall U, et al. Regulation of local availability of active tissue-type plasminogen activator in vivo in man. *J Thromb Haemost* 2004;2:1960–1968.

17. Andersson L, Nilsoon IM, Colleen S, et al. Role of urokinase and tissue activator in sustaining bleeding and the management thereof with EACA and AMCA. *Ann N Y Acad Sci* 1968;146:642–658.
18. Verstraete M. Clinical application of inhibitors of fibrinolysis. *Drugs* 1985;29:236–261.
19. Neter J, Kutner M, Nachtsheim C, et al. Inverse predictions. In: Neter J, Kutner M, Nachtsheim C, et al, eds. *Applied linear regression models*. 3rd ed. Chicago: McGraw-Hill/Irwin, 1996;167–169.
20. Karges HE, Funk KA, Ronneberger H. Activity of coagulation and fibrinolysis parameters in animals. *Arzneimittelforschung* 1994;44:793–797.
21. Spodsberg EH, Wiinberg B, Jessen LR, et al. Endogenous fibrinolytic potential in tissue-plasminogen activator-modified thromboelastography analysis is significantly decreased in dogs suffering from diseases predisposing to thrombosis. *Vet Clin Pathol* 2013;42:281–290.
22. Melander B, Gliniecki G, Granstrand B, et al. Biochemistry and toxicology of amikapron, the antifibrinolytically active isomer of AMCHA. (A comparative study with epsilon-aminocaproic acid). *Acta Pharmacol Toxicol (Copenh)* 1965;22:340–352.
23. McNicol GP, Douglas A. 223. Aminocaproic acid and other inhibitors of fibrinolysis. *Practitioner* 1966;197:102–111.
24. Andersson L, Nilsson IM, Niléhn JE, et al. Experimental and clinical studies on AMCA, the antifibrinolytically active isomer of p-aminomethyl cyclohexane carboxylic acid. *Scand J Haematol* 1965;2:230–247.
25. Fiechtner BK, Nuttall GA, Johnson ME, et al. Plasma tranexamic acid concentrations during cardiopulmonary bypass. *Anesth Analg* 2001;92:1131–1136.
26. Marsh JJ, Konopka RG, Lang IM, et al. Suppression of thrombolysis in a canine model of pulmonary embolism. *Circulation* 1994;90:3091–3097.
27. Nilsson IM. Clinical pharmacology of aminocaproic and tranexamic acids. *J Clin Pathol Suppl (R Coll Pathol)* 1980;14:41–47.
28. Dalen JE, Mathur VS, Evans H, et al. Pulmonary angiography in experimental pulmonary embolism. *Am Heart J* 1966;72:509–520.
29. Hedlin AM, Monkhouse F, Milojevic S. A comparative study of fibrinolytic activity in human, rat, rabbit, and dog blood. *Can J Physiol Pharmacol* 1972;50:11–16.
30. Tentoni J, Polini N, Casanave E. Comparative vertebrate fibrinolysis. *Comp Clin Pathol* 2010;19:225–234.
31. Brohi K, Cohen MJ, Ganter MT, et al. Acute coagulopathy of trauma: hypoperfusion induces systemic anticoagulation and hyperfibrinolysis. *J Trauma* 2008;64:1211–1217.