Effect of aminocaproic acid on clot strength and clot lysis of canine blood determined by use of an in vitro model of hyperfibrinolysis

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OBJECTIVE
To determine pharmacodynamic and pharmacokinetic profiles of aminocaproic acid (ACA) by use of a thromboelastography (TEG)-based in vitro model of hyperfibrinolysis and high-performance liquid chromatography–mass spectrometry.

ANIMALS
5 healthy adult dogs.

PROCEDURES
A single dose of injectable ACA (20, 50, or 100 mg/kg) or an ACA tablet (approximately 100 mg/kg) was administered orally. Blood samples were collected at 0, 15, 30, 45, 60, 90, 120, and 240 minutes after ACA administration for pharmacokinetic analysis. Samples were obtained at 0, 60, and 240 minutes for pharmacodynamic analysis by use of a TEG model of hyperfibrinolysis.

RESULTS
No adverse effects were detected. In the hyperfibrinolysis model, after all doses, a significantly higher TEG maximum amplitude (clot strength), compared with baseline, was detected at 60 and 240 minutes. Additionally, the percentage of fibrinolysis was reduced from the baseline value at 60 and 240 minutes, with the greatest reduction at 60 minutes. At 240 minutes, there was significantly less fibrinolysis for the 100 mg/kg dose than the 20 mg/kg dose. Maximum plasma ACA concentration was dose dependent. There was no significant difference in pharmacokinetic parameters between 100 mg/kg formulations.

CONCLUSIONS AND CLINICAL RELEVANCE
In an in vitro model of hyperfibrinolysis, ACA inhibited fibrinolysis at all doses tested. At 240 minutes after administration, the 100 mg/kg dose inhibited fibrinolysis more effectively than did the 20 mg/kg dose. Thus, ACA may be useful for in vivo prevention of fibrinolysis in dogs.

IMPACT FOR HUMAN MEDICINE
These data may improve research models of hyperfibrinolytic diseases. (*Am J Vet Res* 2016;77:1258–1265)

As a blood clot forms, fibrin monomers are cross-linked by coagulation factor XIIIa, which stabilizes the thrombus. Fibrinolysis typically occurs slowly over time as the clot is enzymatically dissolved by plasmin. In some disease states, there is accelerated clot lysis (ie, hyperfibrinolysis), and it can result in substantial morbidity and even fatality of individual patients as a result of hemorrhage.[1] Antifibrinolytic agents such as ACA and tranexamic acid can slow fibrinolysis. Authors of a meta-analysis[2] of human studies concluded that the use of antifibrinolytic agents during elective surgical procedures reduced blood loss by one-third. Acute traumatic coagulopathy, a syndrome of hyperfibrinolysis after trauma, has also been described in humans,[3] and authors of a prospective study[1] reported a significant survival benefit for trauma victims treated with tranexamic acid. Because of the results of that study[1] and others, tranexamic acid has become the standard of care for people at risk for hyperfibrinolysis following trauma and shock.

Fifteen percent of severely traumatized dogs have evidence of acute traumatic coagulopathy at the time of hospital admission.[4] Similarly, hyperfibrinolysis also occurs in dogs with severe, acute blood loss as a consequence of hemoperitoneum.[5] Antifibrinolytic agents may be indicated for use in both populations of dogs. A subset of Greyhounds predisposed to excessive fibrinolysis and bruising after surgery also appeared to benefit from perioperative ACA administration.[6-8] In addition, other canine patients at risk for

ABBREVIATIONS

ACA	Aminocaproic acid
AUC	Area under the thromboelastography tracing curve
Cmax	Maximum concentration of aminocaproic acid
G	Shear modulus strength
MA	Maximum amplitude
MRL	Maximum rate of clot lysis
MRTG	Maximum rate of thrombus generation
TEG	Thromboelastography
Tmax	Time until maximum concentration of aminocaproic acid
tPA	Tissue plasminogen activator

hemorrhage after routine or emergency surgery may benefit from medications that delay fibrinolysis.

Aminocaproic acid is a synthetic lysine derivative that reversibly binds to plasminogen, which prevents the association of fibrin with plasminogen.[7] Aminocaproic acid is supplied as 100 mg tablets and an injectable solution (250 mg/mL). Oral administration of the less expensive injectable formulation may be appropriate in veterinary medicine; however, no data are available regarding the efficacy for the injectable solution administered via the oral route of administration, compared with results for oral administration of the 100 mg tablets. Although the authors of an in vitro study[9] indicated the ideal plasma ACA concentrations for reversal of hyperfibrinolysis in dogs, the pharmacokinetics of specific doses of ACA in dogs has not been described.

Therefore, the objectives of the study reported here were to determine pharmacodynamic and pharmacokinetic profiles for ACA after oral administration to dogs by use of an in vitro model of hyperfibrinolysis and high-performance liquid chromatography–mass spectrometry, respectively. Our hypotheses were that administration of ACA would result in a dose-dependent reduction in clot lysis and that oral administration of the injectable solution of ACA to healthy dogs would yield pharmacokinetics similar to that after oral administration of the tablets.

Materials and Methods

Animals

Five purpose-bred spayed female Beagles (mean body weight, 10.7 kg; range, 7.8 to 13.3 kg) were included in the study. Dogs were eligible for inclusion if they were deemed healthy on the basis of results of physical examination, a CBC, serum biochemical analysis, and urinalysis and had results for TEG coagulation analysis within institutional reference ranges. The University of Georgia Institutional Animal Care and Use Committee approved all procedures.

Experimental design

By use of a crossover Latin square design, dogs were randomly[a] assigned to receive a single dose of injectable (liquid) ACA solution[b] (20, 50, or 100 mg/kg, PO) or a commercially available ACA tablet[c] (approximately 100 mg/kg, PO), with a washout period ≥ 5 days between subsequent doses. Food was withheld for 12 to 18 hours prior to administration of ACA. Investigators were not aware of the dose or formulation administered to each dog. The tablets were placed in a small amount of canned food for administration; thus, a small bolus of canned food was administered to each dog after each drug administration to ensure drug formulation was not known by the investigators. Dogs were observed for clinical signs of toxicoses after drug administration.

Collection of blood samples

The day before a drug was administered, dogs were sedated with dexmedetomidine[d] (5 µg/kg, IV) to facilitate placement of an indwelling 20-gauge, 12-inch catheter[e] into a jugular vein. Catheters were placed by use of a modified Seldinger technique. Catheters were flushed with sterile saline (0.9% NaCl) solution[b] and subsequently flushed with 0.5 mL of a 50% dextrose solution[b] to maintain catheter patency and obviate the use of an anticoagulant flush. The following day (day of drug administration), catheters were flushed with 3 mL of sterile saline solution, and 1 mL of blood was withdrawn and discardedprior to each collected sample. A total of 22.4 mL of blood was collected on each test day. After collection of a sample, the catheter was flushed with 3 mL of sterile saline solution. If the period between sample collections was to be > 30 minutes, an additional 0.5 mL of 50% dextrose solution was used as a flush. Catheters were removed after acquisition of the final blood sample (4 hours after drug administration) on each test day.

Pharmacodynamics

For each dose of ACA, blood samples (1.8 mL/sample) were collected via the indwelling catheter before (0 minutes [baseline]) and 60 and 240 hours after drug administration. Blood was transferred into vacuum tubes containing 3.2% sodium citrate[f] (final blood-to-citrate ratio, 9:1). These samples were allowed to sit undisturbed at room temperature (22° to 24°C) for 30 minutes; samples then were subjected to TEG analysis by a single researcher (BMB), who was unaware of treatment dose for each sample.

A modified TEG assay[g] was performed as described elsewhere.[9] Briefly, assays were performed at 37°C by use of tissue factor[h] (final dilution, 1:3,400) as an activator in the reaction cup. Blood for analysis was recalcified with 20 µL of 0.2M calcium chloride (total reaction volume, 340 µL). Two analyses were performed for each blood sample: one with only tissue factor activation (TF group) and the other with hyperfibrinolysis (HF group). For the HF group, 10 µL of tPA[i] diluted in HEPES[j] and 2% bovine serum albumin was added to the reaction mixture (final concentration, 100 U/mL). The TF group had 10 µL of HEPES[j] with 2% bovine serum albumin[j] added. All assays were continued for 60 minutes after each clot achieved MA, which represented maximum clot strength. Standard TEG values (R time, α angle, and MA)[10] were recorded. In addition, fibrinolysis variables that represented the amount of fibrinolysis 30 and 60 minutes after MA was achieved were determined **(Figure 1)**. These fibrinolysis variables represented the percentage of each clot lysed at a given time point (ie, 30 and 60 minutes after MA was achieved) and were calculated as a ratio of the AUC at MA to the AUC at a given time point during fibrinolysis. Additional TEG variables measured and calculated by the TEG

software[k] included clot kinetic time (K), G (which was calculated as [5,000 × MA]/[100 – MA]), thrombodynamic potential index (which was calculated as [{100 × MA}/{100 – MA}]/K, where K is the clot kinetic time), tracing amplitude at 30 and 60 minutes after MA, clot lysis time, MRTG, and MRL.[11] The time to achieve MRTG and MRL were also recorded.

Pharmacokinetics

Blood samples (2 mL) for pharmacokinetic analysis were collected into heparinized blood tubes[f] before (0 minutes [baseline]) and 15, 30, 45, 60, 90, 120, and 240 minutes after oral administration of each dose of ACA. At time points when it was required that 2 samples be collected, samples were collected sequentially into separate syringes, with the sample for TEG analysis collected first. Heparinized samples were held on ice until centrifugation (1,500 × g for 10 minutes at room temperature), which was performed within 30 minutes after collection. Plasma supernatant was removed and stored at –80°C for a maximum of 4 months before high-performance liquid chromatography–mass spectrometry analysis at a commercial laboratory.[l]

Statistical analysis

Data were tested for normality by use of the Shapiro-Wilk test. The influence of ACA dose (20, 50, and 100 mg/kg [both formulations]) or time in relation to administration of dose (baseline, 60 minutes, or 240 minutes) on TEG variables was evaluated with a 2-way repeated-measures ANOVA. Correction for multiple comparisons was performed with the Holm-Sidak method, when indicated. Comparison of values between the TF group and HF group was performed with a paired t test. Significance was set at values of P < 0.05. Data were reported as mean ± SEM or median and range, as appropriate for the data distribution. All statistical analyses were performed by use of commercial software.[m,n]

Pharmacokinetic analysis

A noncompartmental analysis was performed with commercial software[n] to obtain initial estimates of pharmacokinetic parameters, including terminal elimination rate, half-life, apparent volume of distribution/bioavailability, total systemic clearance/bioavailability, AUC, Cmax, and Tmax. These parameters were used as initial estimates to support development of a computational pharmacokinetic model to describe the drug concentration–time profile for each dose. The model was fit by use of both naïve-pooled data and naïve-average data approaches.[12] Residuals, accuracy, precision, confidence intervals, correlation between parameters, condition number, and objective criteria (Akaike information criteria, Schwarz criteria, sum of squares, and estimator criterion value) were analyzed to evaluate goodness of fit as well as select the model of best fit.[12,13] A 1-compartment model best fit the observed data. Parameter estimates were then fixed, and simulations were performed to evaluate various doses and the frequency of administration, assuming linear and stationary kinetics.[14] The goal was to identify a treatment schedule that would achieve an a priori minimum effective concentration of 25 µg/mL over the longest period (based on the mean ± SD plasma concentration at 240 minutes for the 100 mg/kg doses, which was 26.29 ± 14 µg/mL). For the simulations, it was assumed that pharmacokinetic parameters were linear and stationary for the conditions examined.
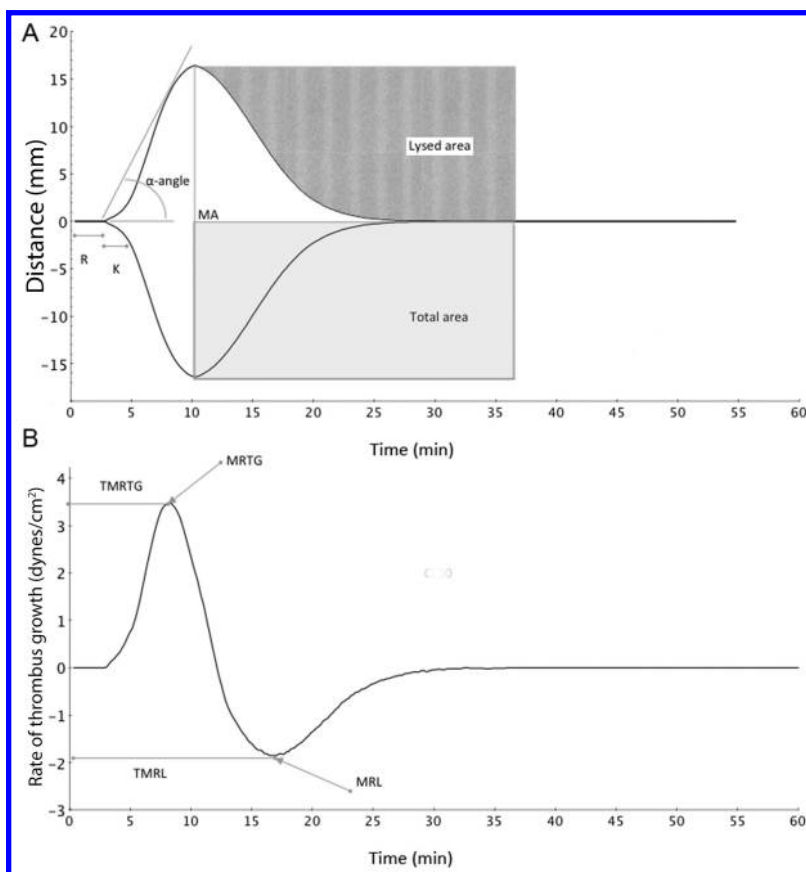

Figure I—A representative TEG tracing (A) and first derivative of the TEG tracing (B) for a blood sample obtained from a healthy dog and analyzed in a hyperfibrinolysis assay. In panel A, notice the reaction time (R), clot kinetic time (K; defined as the time to reach a specified clot strength [amplitude, 20 mm]), and MA and values for the percentage of lysis at 30 minutes after MA was achieved (calculated as a ratio of the AUC at MA to the AUC at 30 minutes after MA). In panel B, MRTG, time to MRTG (TMRTG), MRL, and the time to MRL (TMRL) are indicated.

Table 1—Mean ± SD values for TEG in samples collected from 5 healthy dogs before (0 minutes) and 60 and 240 minutes after oral administration of ACA and evaluated by use of an in vitro model of tPA-induced hyperfibrinolysis.

| Variable | Reference interval | Time (min) | Injectable solution (mg/kg) | | | Tablet (mg/kg) |
			20	50	100	100
MA (mm)	54.4 ± 8.8	0	28.5 ± 22.3	19.7 ± 12.4	31.0 ± 16.3	20.3 ± 19.9
	—	60	50.7 ± 27.1*	58.2 ± 8.1*	51.3 ± 6.3*	58.3 ± 10.8*
	—	240	54.1 ± 13.4*	53.6 ± 13.6*	60.3 ± 4.8*	53.6 ± 11.7*
Lysis 30 (%)	2.9 ± 6.0	0	85.4 ± 7.5	86.4 ± 7.7	84.4 ± 8.8	86.8 ± 8.8
	—	60	36.6 ± 24.4†‡	25.3 ± 18.3†‡	20.2 ± 21.5†‡	20.1 ± 21.1†‡
	—	240	59.3 ± 10.3†	45.6 ± 38.0†	27.9 ± 23.5†§	33.7 ± 27.9†§

Reference interval represents an institutional reference interval. There was a washout period of ≥ 5 days between subsequent doses.

,†Within a row, value differs significantly ($P < 0.001$; †$P < 0.05$) from the value at 0 minutes. ‡Within a column within a variable, value differs significantly ($P < 0.05$) from the values at 0 and 240 minutes. §Within a row, value differs significantly ($P = 0.01$) from the value for 20 and 50 mg/kg.

— = Not applicable. Lysis 30 = Percentage of lysis at 30 minutes after MA was achieved (calculated as a ratio of the AUC at MA to the AUC at 30 minutes after MA).

Table 2—Mean ± SD values for TEG variables in samples collected from 5 healthy dogs before (0 minutes) and 60 and 240 minutes after oral administration of ACA and evaluated by use of an in vitro model of tPA-induced hyperfibrinolysis.

| Variable | Reference interval | Time (min) | Injectable solution (mg/kg) | | | Tablet (mg/kg) |
			20	50	100	100
R (min)	2.0 ± 0.4	0	2.1 ± 0.9	2.2 ± 0.5	2.0 ± 0.6	2.2 ± 0.7
	—	60	2.0 ± 0.6	2.0 ± 2.0	1.9 ± 0.6	2.0 ± 1.2
	—	240	1.4 ± 0.9	1.1 ± 0.5	1.7 ± 1.2	1.5 ± 0.5
K (min)	1.4 ± 0.8	0	1.3 ± 0.3	1.9 ± 1.6	1.1 ± 0.3	1.1 ± 0.4
	—	60	1.1 ± 0.3	1.5 ± 0.6	1.4 ± 0.4	1.5 ± 0.8
	—	240	1.0 ± 0.3	1.1 ± 0.6	1.1 ± 0.2	2.2 ± 2.2
α angle (°)	70.8 ± 7.8	0	69.8 ± 32.0	59.7 ± 28.1	71.7 ± 35.2	58.2 ± 59.0
	—	60	72.6 ± 49.1	68.0 ± 16.8	72.7 ± 9.4	70.9 ± 28.3
	—	240	78.2 ± 18.3	79.1 ± 12.6	75.0 ± 6.9	70.5 ± 27.4
G (dynes/s)	6,320 ± 2,038	0	2,772 ± 3,240	1,341 ± 1,028	2,592 ± 1,852	1,636 ± 1,821
	—	60	7,025 ± 4,488	7,307 ± 2,388	5,420 ± 1,407	7,617 ± 3,031
	—	240	6,659 ± 3,365	6,491 ± 3,730	7,715 ± 1,476	6,279 ± 2,675
TPI (/s)	57.22 ± 32.53	0	44.77 ± 47.57	18.75 ± 19.73	31.70 ± 3.81	33.30 ± 7.21
	—	60	86.68 ± 47.19	61.84 ± 42.24	43.32 ± 15.33	68.24 ± 49.03
	—	240	70.84 ± 51.52	66.33 ± 53.89	75.70 ± 24.93	59.55 ± 50.03
A30 (min)	51.4 ± 12.4	0	0.12 ± 0.10	0.13 ± 0.10	0.14 ± 0.10	0.12 ± 0.00
	—	60	19.28 ± 19.80	33.70 ± 21.50	27.20 ± 21.90	34.60 ± 23.00
	—	240	3.10 ± 2.30	21.00 ± 31.90	26.30 ± 26.40	22.10 ± 23.40
A60 (min)	47.4 ± 14.5	0	0.12 ± 0.05	0.13 ± 0.05	0.14 ± 0.06	0.12 ± 0.04
	—	60	3.16 ± 3.68	33.72 ± 21.50	7.58 ± 7.81	11.12 ± 16.12
	—	240	0.14 ± 0.06	12.10 ± 20.27	13.15 ± 16.56	3.18 ± 4.01
Lysis 60 (%)	6.9 ± 11.5	0	93.9 ± 7.5	93.35 ± 7.0	92.7 ± 8.9	93.3 ± 8.6
	—	60	63.7 ± 39.6	46.7 ± 72.7	39.8 ± 54.4	46.9 ± 61.7
	—	240	79.4 ± 11.8	77.5 ± 71.4	52.8 ± 56.8	60.9 ± 54.9
CLT (min)	—	0	9.8 ± 5.8	8.3 ± 5.3	10.7 ± 6.3	7.5 ± 7.7
	—	60	27.3 ± 18.0	ND	37.0 ± 0.2	ND
	—	240	33.8 ± 10.4	24.8 ± 12.1	34.3 ± 5.5	35.9 ± 8.3
MRTG (mm/min)	17.57 ± 7.11	0	19.24 ± 11.49	14.41 ± 9.69	14.12 ± 7.63	20.51 ± 8.97
	—	60	21.59 ± 10.14	17.63 ± 9.84	15.65 ± 4.40	17.49 ± 9.52
	—	240	21.06 ± 9.88	19.57 ± 7.78	20.28 ± 6.41	18.79 ± 12.31
MRL (mm/min)	—	0	5.50 ± 3.10	3.53 ± 0.41	4.90 ± 1.67	5.07 ± 1.12
	—	60	6.20 ± 3.04	5.53 ± 4.83	4.72 ± 1.82	5.34 ± 2.09
	—	240	5.68 ± 1.53	5.22 ± 3.84	5.38 ± 1.36	5.41 ± 2.59
TMRTG (min)	2.92 ± 0.50	0	2.58 ± 0.39	2.86 ± 0.46	2.78 ± 0.22	2.79 ± 0.29
	—	60	2.94 ± 0.35	2.67 ± 0.84	2.81 ± 0.27	2.93 ± 0.62
	—	240	2.13 ± 0.39	1.92 ± 0.38	2.49 ± 0.63	2.29 ± 0.34
TMRL (min)	—	0	12.00 ± 5.53	10.83 ± 5.89	13.75 ± 7.88	14.50 ± 3.29
	—	60	37.50 ± 5.81	36.03 ± 7.92	39.62 ± 11.82	40.46 ± 7.65
	—	240	24.58 ± 3.01	23.79 ± 8.08	33.75 ± 3.18	39.89 ± 18.27

Reference interval represents an institutional reference interval.

A30 = Clot strength (amplitude) 30 minutes after MA was achieved. A60 = Clot strength (amplitude) 60 minutes after MA was achieved. CLT = Clot lysis time (defined as the time from MA to complete clot lysis). K = Clot kinetic time (defined as the time to reach a specified clot strength [amplitude, 20 mm]). Lysis 60 = Percentage of lysis at 60 minutes after MA was achieved (calculated as a ratio of the AUC at MA to the AUC at 60 minutes after MA). ND = No data. R = Reaction time. TMRL = Time to MRL. TMRTG = Time to MRTG. TPI = Thrombodynamic potential index.

See Table 1 for remainder of key.

Table 3—Mean ± SD values for noncompartmental analysis of pharmacokinetic parameters in samples collected from 5 healthy dogs 45 minutes after oral administration of ACA.

Parameter	Injectable solution (mg/kg)			Tablet (mg/kg)
	20	50	100	100
Tmax (min)	63.0 ± 26.8	42.0 ± 28.9	54.0 ± 37.6	63.0 ± 24.6
Cmax (μg/mL)	29.6 ± 15.5	68.7 ± 14.5	166.0 ± 46.7*	161.0 ± 47.6*
β (/min)	0.0107 ± 0.0027	0.0101 ± 0.0011	0.0097 ± 0.0024	0.0093 ± 0.0025
$t_{1/2\beta}$ (min)	70.0 ± 25.5	69.0 ± 7.1	76.9 ± 26.1	81.6 ± 33.6
AUC_{0-4h} (min•μg/kg•mL)	3,160 ± 1,140	7,470 ± 598	16,900 ± 3,660	15,600 ± 2,490
$AUC_{0-\infty}$ (min•μg/kg•mL)	3,640 ± 1,190	8,480 ± 930	19,800 ± 3,570	19,700 ± 5,730
Vd/F (mL)	594 ± 224	589 ± 52	575 ± 204	597 ± 119
CL/F (mL/min)	6.06 ± 2.27	5.96 ± 0.71	5.23 ± 1.21	5.42 ± 1.48
AUC_{inf}/D (min/mL)	0.182 ± 0.0594	0.170 ± 0.0188	0.198 ± 0.0357	0.197 ± 0.0573
Vd/F/D (kg•mL/min)	0.0297 ± 0.0112	0.0118 ± 0.0010	0.0058 ± 0.0020	0.0060 ± 0.0012
CL/F/D (kg•mL/min•mg)	$3.06 \times 10^{-4} \pm 1.14 \times 10^{-4}$	$1.19 \times 10^{-4} \pm 1.41 \times 10^{-5}$	$5.23 \times 10^{-5} \pm 1.21 \times 10^{-5}$	$5.42 \times 10^{-5} \pm 1.48 \times 10^{-5}$

*Value differs significantly ($P < 0.001$) from the value for 20 mg/kg.

AUC_{0-4h} = The AUC from time of administration until 4 hours after administration. $AUC_{0-\infty}$ = The AUC from time of administration extrapolated until infinity. AUC_{inf}/D = Dose-normalized area under the plasma concentration–time profile. β = Terminal phase of plasma drug clearance. CL/F = Total body clearance/bioavailability. CL/F/D = Dose-normalized total body clearance/bioavailability. $t_{1/2\beta}$ = Terminal half-life. Vd/F = Volume of distribution/bioavailability. Vd/F/D = Dose-normalized volume of distribution/bioavailability.

Results

Animals

The ACA administration was tolerated well by all dogs. No abnormal clinical signs indicative of adverse drug effects were observed in any dog for any dose of ACA.

Pharmacodynamics

All TEG values for the TF group were within institutional reference intervals for all ACA doses and time points (data not shown). For the HF group, a significant ($P < 0.001$) increase in MA and α-angle from baseline values was identified at 60 and 240 minutes for all ACA doses **(Table 1)**. The percentage of lysis at 30 and 60 minutes after MA was achieved was significantly decreased (ie, fibrinolysis was decreased) for all ACA doses in samples collected 60 and 240 minutes after drug administration, compared with baseline values. The percentage of lysis at 30 minutes after MA was achieved measured in the sample collected 60 minutes after drug administration was significantly less than that measured in the sample collected 240 minutes after drug administration for all doses. The amount of decrease in the percentage of lysis at 30 minutes after MA was achieved in samples collected 60 and 240 minutes after ACA administration for dogs receiving both 100 mg/kg doses was significantly ($P = 0.01$) greater than that for dogs when they received the 20 mg/kg dose of ACA. The MRTG was significantly ($P = 0.025$) greater than baseline values at 60 and 240 minutes after ACA administration at all doses, and the time to MRTG was less for all doses in samples collected at 240 minutes, compared with values for samples collected at baseline and 60 minutes. Despite these findings, there was no significant ($P = 0.821$) difference in MRTG between doses. For samples collected at 60 and 240 minutes, the time to MRL was significantly ($P = 0.025$) greater for all doses than that measured in samples collected at baseline. In addition to these parameters, the R time measured in samples collected at 240 minutes was significantly less than that measured in samples collected at baseline ($P = 0.017$) or 60 minutes ($P = 0.025$) for all doses. Comparison of MA values for the TF group and HF group revealed significant differences only at baseline for each dose, which was similar to results described in another study.[9] The MA values at 60 and 240 minutes for the HF group were not significantly different from those for the TF group. All parameters for the HF group were summarized **(Table 2)**.

Pharmacokinetics

Pharmacokinetic parameters were determined after oral administration of a single dose of ACA at 20, 50, and 100 mg/kg **(Table 3)**. The drug was rapidly absorbed after oral administration (Tmax = 1 hour) for all doses tested. At 20 mg/kg, the minimum effective concentration (25 μg/mL) was barely reached (Cmax = 29.6 μg/mL). Doses of 50 and 100 mg/kg achieved the minimum effective concentration, but the drug was eliminated rapidly and remained at a concentration > 25 μg/mL for only 2 and 3 hours, respectively **(Figure 2)**. Maintenance of an effective concentration would likely require a continuous rate infusion administered IV. However, when administered orally at 100 mg/kg every 6 hours, an effective plasma concentration would be maintained for 51.7% of a 24-hour period, and oral administration of 100 mg/kg every 8 hours would maintain an effective concentration for 38.8% of a 24-hour period **(Figure 3)**. We were unable to detect a significant difference in pharmacokinetics between the injectable solution and tablet formulations administered at a dose of 100 mg/kg **(Figure 4)**.
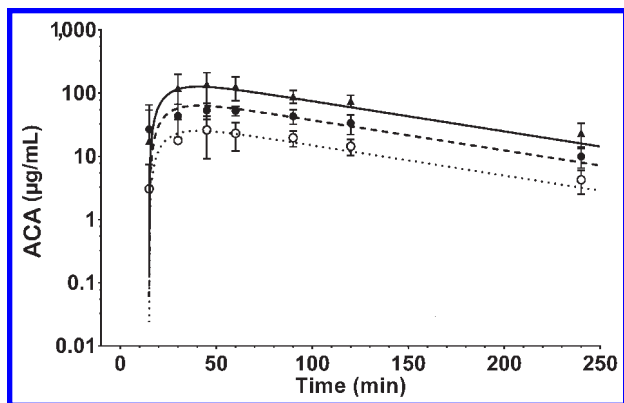
Figure 2—Predicted (lines) and measured (mean ± SD; symbols) ACA plasma concentrations over time after oral administration of an injectable solution of ACA at doses of 20 mg/kg (dotted line and white circles), 50 mg/kg (dashed line and black circles), and 100 mg/kg (solid line and black triangles) to 5 healthy dogs in a randomized crossover design. There was a washout period of ≥ 5 days between subsequent doses.
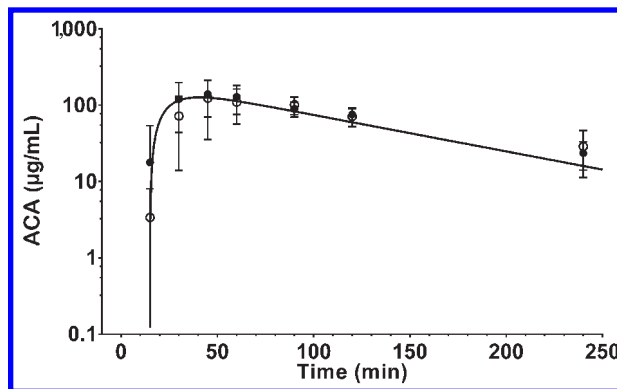

Figure 4—The predicted (line) and measured (mean ± SD; symbols) ACA plasma concentration over time after oral administration of a dose of 100 mg/kg as a tablet formulation (white circles) or an injectable solution (black circles) to 5 healthy dogs. The predicted value represents results for both formulations at a dose of 100 mg/kg.
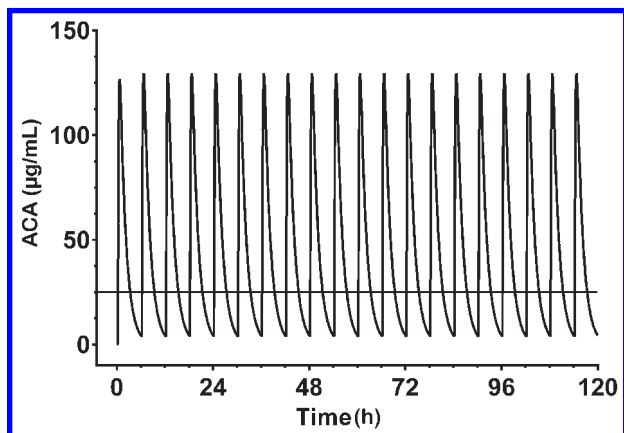

Figure 3—Results for a predictive model to estimate the ACA plasma concentration over time for ACA administered orally at a dosage of 100 mg/kg every 6 hours for 5 days to healthy dogs. The horizontal line indicates the a priori target plasma concentration of ACA (25 µg/mL).

Pharmacokinetic parameters obtained with non-compartmental analysis were used as initial estimates for compartmental modeling. The data were best described by use of a 1-compartment pharmacokinetic model (Figure 3). Model parameters were fixed, and the 1-compartment model was used to simulate pharmacokinetic profiles for various doses (20, 50, 100, and 200 mg/kg) and the frequency of administration (intervals of 6, 8, 12, and 24 hours); the simulations assumed linear and stationary kinetics. Plasma concentrations would reach steady state within 24 hours after oral administration. The model for the 200 mg/kg dose predicted an increase in drug exposure (ie, amount of time AUC > 25 µg/mL). However, even at the 200 mg/kg dose, the drug was predicted to be cleared rapidly from the systemic circulation. To our knowledge, studies have not been performed to de-

termine toxic effects of ACA at higher doses (such as 200 mg/kg), and such effects should be evaluated before these doses can be recommended for clinical use.

Discussion

In the study reported here, a model of hyperfibrinolysis was used to determine that orally administered ACA decreased fibrinolysis. Inhibition of fibrinolysis after a single dose was both time and dose dependent. Inhibition of fibrinolysis (ie, decreased percentage of lysis at 30 minutes after MA was achieved) was detected for all doses of ACA administered. Administration of ACA also resulted in an MA value for the HF group that was not significantly different from that for the TF group. These data indicated that treatment with ACA can enhance clot strength in patients that have diminished clot strength attributable to hyperfibrinolysis. Both the injectable and tablet formulations for the 100 mg/kg dose had a significantly greater decrease in fibrinolysis (ie, smaller percentage of lysis at 30 minutes after MA was achieved) for samples collected 240 minutes after administration, compared with results for the 20 mg/kg dose. This indicated persistence of an antifibrinolytic effect at higher doses of ACA. Assays performed on samples collected 6 and 8 hours after ACA administration would be helpful in defining the total duration of this effect.

In a previous study[9] conducted by evaluating canine plasma with a similar in vitro hyperfibrinolytic model, but with a much higher concentration of tPA (1,000 U/mL), an ACA plasma concentration of 511.7 µg/mL was required to completely inhibit fibrinolysis. Even at a plasma ACA concentration of 450 µg/mL, the percentage of lysis was 56.7%. None of the doses investigated in the study reported here achieved a Cmax that approached the effective plasma concentration derived in that previous study.[9] However, fibrinolysis was not completely inhibited in any samples in the present study. Even at the highest ACA doses,

which achieved a mean ± SD plasma Cmax of 140 ± 70 µg/mL, the mean percentage of lysis at 30 minutes after MA was achieved was 20 ± 22% of clot lysis. One reason for the discrepancy may have been the time of sample acquisition: Cmax was at approximately 45 minutes after drug administration, whereas the TEG was not conducted until 1 hour after drug administration, a time when plasma drug concentrations were already decreasing. Another interesting observation was that for the HF group, 3 of 5 dogs had a mean ± SD value for percentage of lysis at 30 minutes after MA was achieved of 5 ± 2%, whereas the other 2 dogs had a mean of 43 ± 9% after oral administration of the highest dose of ACA. These latter 2 dogs also had lower mean ACA concentrations at this time point (78 ± 12 µg/mL vs 163 ± 34 µg/mL for the other 3 dogs). This may have represented individual variability in the response to tPA or ACA (or both) or the speed of metabolism of ACA.

Despite the persistence of some clot lysis, improvement in clot strength (ie, MA) was not dose dependent. The MA was significantly higher for all doses and all time points, compared with baseline values, for the HF group. The MA after ACA administration for the HF group was not significantly different from MA for the TF group. From the perspective of a patient with hemorrhage, the relative benefit of clot strength restoration versus a decrease in fibrinolysis is unclear, and both aspects would likely contribute to the effectiveness of ACA.

A reason that reducing fibrinolysis may lead to a stronger clot is the continued formation of thrombin and activation of both factor XIII and thrombin-activatable fibrinolysis inhibitor, which downregulates fibrinolysis by removal of c-terminal lysine plasminogen binding sites from fibrin. Thrombin-activatable fibrinolysis inhibitor requires relatively high amounts of thrombin for activation, and the ability for the inhibitor to reach threshold limits to completely inhibit fibrinolysis is, in part, dependent on the presence of plasmin inhibitors.[15] Therefore, ACA inhibits the initiation of fibrinolysis, which subsequently enhances the threshold-dependent mechanism for thrombin-activatable fibrinolysis inhibitor during initial phases of clot formation.

Pharmacokinetic parameters did not differ significantly between the 100 mg/kg dose administered orally as a tablet or as the injectable solution. This provides a basis for oral administration of the injectable solution because it is generally much less expensive than the tablets in the United States. Absorption of drug from the tablets was slightly slower, but the lack of a significant difference in Cmax and AUC indicated that this was a viable route of administration. The slightly faster absorption of the injectable solution was likely a result of the rate of disintegration and dissolution of the tablet, but it may also have been attributable, in part, to differences in oral mucosal absorption. All of the drugs were administered to dogs from which food was withheld, although a small food bolus was used for administration, and the effect of a meal on absorption of the tablet or injectable solution was not evaluated.

Although clot strength remained the same, the decrease in inhibition of fibrinolysis at 240 minutes when dogs received ACA at a dose of 20 mg/kg, compared with results when dogs received ACA at a dose of 100 mg/kg, indicated that the 20 mg/kg dose may not be adequate unless administered at relatively frequent intervals. The mean ± SD ACA plasma concentration at 240 minutes when dogs received ACA at a dose of 20 mg/kg was 4.28 ± 2 µg/mL, compared with 26.3 ± 14 µg/mL when dogs received ACA at a dose of 100 mg/kg, which indicated a concentration at which the effects of ACA were evident in this model. Extrapolation to other dogs would target a plasma concentration ≥ 25 µg/mL to maintain an expected therapeutic drug effect. Modeling of the pharmacokinetic data indicated that a dose of 100 mg/kg administered orally as a tablet or liquid every 6 hours would achieve this plasma concentration and can be recommended for patients with hyperfibrinolysis. This recommended dosage and target plasma concentration are based on the assumption that decreasing fibrinolysis is more important than maintaining clot strength because MA was not significantly different between doses, which may not be the case in vivo. It also does not address whether lower doses may be used in a prophylactic scenario (ie, patients at risk for hyperfibrinolysis but not yet hemorrhaging), which remains to be investigated.

The study reported here had several limitations. This in vitro model did not account for the complex influence of endothelium on fibrinolysis. Local concentrations of tPA at the site of vascular damage[16] may be higher or lower than the concentrations used to simulate hyperfibrinolysis in the present study. Also, healthy dogs were used, so application of the derived pharmacodynamic or pharmacokinetic data to patients with altered perfusion or volume of distribution may not yield similar effectiveness. Additionally, only 5 healthy dogs were used in the study, which potentially limited the statistical power to document alterations in all of the TEG parameters evaluated. This may not necessarily affect assessment of the effectiveness and clinical outcome for ACA in animals with naturally occurring hyperfibrinolytic conditions.

In the study reported here, the antifibrinolytic effects of ACA were detected for all test doses. The therapeutic effect of the drug appeared to remain despite decreasing plasma drug concentrations, although higher doses (100 mg/kg) maintained inhibition of fibrinolysis for a longer period than did the lowest dose evaluated (20 mg/kg). Deleterious effects of ACA orally administered to dogs as the injectable solution or tablet formulation were not seen in this study. A dose of 100 mg/kg administered orally every 6 hours would be expected to achieve plasma ACA concentrations > 25 µg/mL for 3 hours after administration (ie, 12 hours of a 24-hour period).

Acknowledgments

Footnotes

a. Random number generator. Available at: www.randomizer.com. Accessed Jan 3, 2014.
b. Hospira, Lake Forest, Ill.
c. Amicar tablets, Xanodyne, Newport, Ky.
d. Pfizer, New York, NY.
e. Arrow Teleflex, Telford, Pa.
f. BD, Franklin Lakes, NJ.
g. TEG 5000, Haemonetics Corp, Braintree, Mass.
h. Dade Innovin in 2% albumin, Siemens Healthcare Diagnostics Inc, Newark, Del.
i. Genentech, South San Francisco, Calif.
j. Haemonetics, Braintree, Mass.
k. TEG software, version 4.2, Hemonetics, Braintree, Mass.
l. Veterinary Diagnostic Pharmacologic Analytical Laboratory, Iowa State University, Ames, Iowa.
m. Sigma Stat, Systat Inc, San Jose, Calif.
n. WinNonlin version 5.3, Pharsight Corp, Mountain View, Calif.

References

1. 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 randomized, placebo-controlled trial. *Lancet* 2010;10:1–10.
2. Ker K, Prieto-Merino D, Roberts I. Systemic review, meta-analysis and meta-regression of the effect of tranexamic acid on surgical blood loss. *Br J Surg* 2013;100:1271–1279.
3. Brohi K, Cohen MJ, Ganter MT, et al. Acute coagulopathy of trauma: hypoperfusion induces systemic anticoagulation and hyperfibrinolysis. *J Trauma* 2008;64:1211–1217.
4. Holowaychuk MK, Hanel RM, Wood RD, et al. Prospective multicenter evaluation of coagulation abnormalities in dogs following severe acute trauma. *J Vet Emerg Crit Care (San Antonio)* 2014;24:93–104.
5. Fletcher DJ, Rozanski EA, Brainard BM, et al. Assessment of the relationships among coagulopathy, hyperfibrinolysis, plasma lactate, and protein C in dogs with spontaneous hemoperitoneum. *J Vet Emerg Crit Care* 2015; 26:41–51.
6. Vilar P, Couto CG, Westendorf N, et al. Thromboelastographic tracings in retired racing Greyhounds and in non-Greyhound dogs. *J Vet Intern Med* 2008;22:374–379.
7. Marin 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* 2012;22:332–340.
8. Marin 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.
9. Fletcher DJ, Blackstock KJ, Epstein K, et al. Evaluation of tranexamic acid and ε-aminocaproic acid concentrations required to inhibit fibrinolysis in plasma of dogs and humans. *Am J Vet Res* 2014;75:731–738.
10. Spodsberg EH, Wiinberg B, Jessen LR, et al. Endogenous fibrinolytic potential in tissue-plasminogen activator-modified thromboelastography analysis is significantly decreased in dogs suffering diseases predisposing to thrombosis. *Vet Clin Pathol* 2013;42:281–290.
11. Hanel RM, Chan DL, Conner B, et al. Systematic evaluation of evidence on veterinary viscoelastic testing part 4: definitions and data reporting. *J Vet Emerg Crit Care* 2014;24:47–56.
12. Gabrielsson J, Weiner D. Pharmacodynamic models. In: Gabrielsson J, Weiner D, eds. *Pharmacokinetic pharmacodynamic data analysis: concepts and applications.* 4th ed. Stockholm: Swedish Pharmaceutical Press, 2007;438–463.
13. Arnold RD, Mager DE, Slack JE, et al. Effect of repetitive administration of doxorubicin-containing liposomes on plasma pharmacokinetics and drug biodistribution in a rat brain tumor model. *Clin Cancer Res* 2005;11:8856–8865.
14. Aljuffali IA, Brainard BM, Moore JN, et al. Pharmacokinetic assessment of ketanserin in the horse. *J Vet Pharmacol Ther* 2012;35:472–477.
15. Bouma BN, Mosnier LO. Thrombin activatable fibrinolysis inhibitor (TAFI)—how does thrombin regulate fibrinolysis? *Ann Med* 2006;38:378–388.
16. Noel P, Cashen S, Patel B. Trauma-induced coagulopathy: from biology to therapy. *Semin Hematol* 2013;50:259–269.