Pharmacokinetics and pharmacodynamics of ε-aminocaproic acid in horses

Julie Ross, MA, VetMB; Barbara L. Dallap, VMD; Brett A. Dolente, VMD; Raymond W. Sweeney, VMD

Objective—To determine the pharmacokinetics and pharmacodynamics of ε-aminocaproic acid (EACA), including the effects of EACA on coagulation and fibrinolysis in healthy horses.

Animals—6 adult horses.

 Procedures—Each horse received 3.5 mg of EACA/kg/min for 20 minutes, IV. Plasma EACA concentration was measured before (time 0), during, and after infusion. Coagulation variables and plasma α1-antiplasmin activity were evaluated at time 0 and 4 hours after infusion; viscoelastic properties of clot formation were assessed at time 0 and 0.5, 1, and 4 hours after infusion. Plasma concentration versus time data were evaluated by use of a pharmacokinetic analysis computer program.

 Results—Drug disposition was best described by a 2-compartment model with a rapid distribution phase, an elimination half-life of 2.3 hours, and mean residence time of 2.5 ± 0.5 hours. Peak plasma EACA concentration was 462.9 ± 70.1 μg/mL; after the end of the infusion, EACA concentration remained greater than the proposed therapeutic concentration (130 μg/mL) for 1 hour. Compared with findings at 0 minutes, EACA administration resulted in no significant change in plasma α1-antiplasmin activity at 1 or 4 hours after infusion. Thirty minutes after infusion, platelet function was significantly different from that at time 0 and 1 and 4 hours after infusion. The continuous rate infusion that would maintain proposed therapeutic plasma concentrations of EACA was predicted (ie, 3.5 mg/kg/min for 15 minutes, then 0.25 mg/kg/min).

 Conclusions and Clinical Relevance—Results suggest that EACA has potential clinical use in horses for which improved clot maintenance is desired. (Am J Vet Res 2007;68:1016–1021)

E-aminocaproic acid is a synthetic derivative of the amino acid lysine. The antifibrinolytic effect of EACA was first described in 1957; since then, EACA has been used frequently in humans to decrease the need for blood product administration (eg, during cardiopulmonary bypass procedures, prior to surgical intervention to treat subarachnoid hemorrhage, following dental extraction in hemophilic patients, during extracorporeal membrane oxygenation in neonatal cardiac surgery, and during orthopedic and vertebral column surgery). In horses, it has been suggested that EACA may be useful only in those with active hyperfibrinolysis, although in human medicine, EACA is thought to be effective even when bleeding is not associated with clinicopathologic signs of excessive hyperfibrinolysis.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>EACA</td>
<td>ε-aminocaproic acid</td>
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<tr>
<td>α1-AP</td>
<td>α1-Antiplasmin</td>
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<tr>
<td>FDP</td>
<td>Fibrin degradation product</td>
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<tr>
<td>PTT</td>
<td>Partial thromboplastin time</td>
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<td>PT</td>
<td>Prothrombin time</td>
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<td>AT</td>
<td>Antithrombin</td>
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<td>V1</td>
<td>Volume of distribution of the central compartment</td>
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<td>MRT</td>
<td>Mean residence time</td>
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<tr>
<td>AUC</td>
<td>Area under the time-concentration curve (determined by the trapezoidal rule with extrapolation to infinity)</td>
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Inhibition of plasminogen activation by EACA results in altered clot maintenance without affecting clot formation. Kahn et al used modified whole blood clotting time as a measure of fibrinolytic activity and determined that EACA had an inhibitory effect on clot lysis in clinically normal humans. In an investigation of the mechanism of action of EACA by Thorsen, EACA decreased the adsorption of plasminogen to fibrin. Other researchers have investigated the effect of EACA on other factors involved in fibrinolysis, including α1-AP, FDPs, and D-dimers. However, the exact mechanism of action remains unclear.

The hemostatic process can be assessed by use of viscoelastic methods such as viscoelastic coagulation (sonoclot) analysis or thromboelastography.
Elastic coagulation analysis measures the impedance of movement of a small probe caused by clot development in a blood sample. This provides qualitative and quantitative information relating to the in vitro coagulation process, including fibrin formation, fibrin monomer polymerization, platelet interaction, clot retraction, and clot lysis. Results are displayed graphically, detailing clot signal over time. In humans, fibrinolysis occurs after the period of testing provided by viscoelastic coagulation analysis (ie, after 60 minutes). Thus, fibrinolysis is generally not detected by use of that analyzer. However, in hyperfibrinolytic patients, the trace obtained from the analyzer changes in a characteristic way as clot strength decreases, and the degree of hyperfibrinolysis and the effects of treatment can be monitored with repeated use of the analyzer. Viscoelastic coagulation analysis provides dynamic information about the coagulation pathway and has advantages over traditional coagulation testing in clinical settings. In human medicine, viscoelastic coagulation analysis has been used to monitor coagulation in patients undergoing a variety of procedures, including cardiac surgery and transplant surgery, and is a useful predictor of postoperative bleeding after cardiopulmonary bypass. Viscoelastic coagulation analysis has been used to evaluate neonatal foals and is thought to be useful for assessment of platelet function and detection of coagulation abnormalities in neonates with sepsis.

Anecdotally, EACA has been used by veterinarians to treat a variety of bleeding disorders in horses, including castration-associated hemorrhage, uterine artery hemorrhage, mycosis of the auditory tube diverticulum (guttural pouch), and intra-abdominal hemorrhage. At present, the efficacy of EACA has not been definitively determined, the therapeutic plasma drug concentration is unknown, and the pharmacokinetics of EACA in horses have not been elucidated. However, it is known that administration of EACA to horses results in alterations in PTT, plasma α2-AP activity, and plasma fibrinogen concentration. The purpose of the study reported here was to determine the pharmacokinetics and pharmacodynamics of EACA in healthy horses.

Materials and Methods

Animals—Six adult healthy mares (mean ± SD weight, 520.8 ± 48.9 kg) were selected for inclusion in the study. All horses were selected from the teaching herd at New Bolton Center, University of Pennsylvania. None of the mares in the study were pregnant or undergoing any form of treatment. Prior to the start of the investigation, the horses were determined to be healthy on the basis of physical examination findings. Coagulation variables were evaluated prior to the onset of the study. Among the 6 horses, 1 horse had a mildly high plasma concentration of FDPs, but otherwise all coagulation variables were within reference ranges (ie, PT and PTT, < 20% greater than the value obtained from a control horse; AT activity, 150% to 220%; plasma concentration of FDPs, < 10 μg/mL; plasma fibrinogen concentration, 150 to 375 mg/dL; and platelet count, > 100,000 platelets/μL). The control horse used to determine the reference range for PT and PTT was a healthy horse from our teaching herd. This sampling was conducted by the clinical laboratory and thus was beyond the control of the investigators. The horses involved in the study were housed in individual stalls and had access to hay and water ad libitum during the investigation. Horses were allowed a 24-hour acclimatization period before the start of the study and were monitored for adverse effects for 24 hours after completion of the study. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Drug administration—An IV catheter was aseptically placed in each jugular vein of each horse after the acclimatization period. ε-Aminocaproic acid was added to 1 L of saline (0.9% NaCl) solution under sterile conditions and administered through the catheter in the right jugular vein (3.5 mg of EACA/kg/min administered over a period of 20 minutes).

Sample collection and handling—Blood samples were collected from the left jugular catheter immediately prior to the onset of the experiment (time 0), 10 minutes after the start of infusion, and at the end of infusion (20 minutes). Blood samples were then collected at 10, 20, 30, and 45 minutes and 1, 2, 3, 4, 6, 9, and 12 hours after completion of drug administration. At each time point, blood was collected into K3 EDTA blood collection tubes for determination of plasma EACA concentration; blood was similarly collected at 0 minutes and at 4 hours after infusion for determination of platelet count. Blood was collected into blood collection tubes containing buffered 3.2% sodium citrate solution at 0 minutes and at 1 and 4 hours after infusion for determination of plasma α2-AP activity; at 0 minutes and at 30 minutes and 1 and 4 hours after infusion for assessment of clot dynamics by use of a viscoelastic coagulation analyzer; and at 0 minutes and at 4 hours after infusion for determination of PT, PTT, AT activity, and plasma fibrinogen concentration. Blood was collected into tubes containing Bothrops atrox venom with soybean trypsin inhibitor for determination of plasma concentration of FDPs at time 0 and 4 hours after infusion. A 10-mL volume of blood was withdrawn from the catheter and discarded prior to each sample being collected. Heparin was not used in the IV catheters until after the fourth hour of the study because of the concern that heparin might affect the results of the clot dynamic tests. After that time, the sample collection catheter was flushed with 5 mL of saline solution containing heparin (10 U of heparin/mL) after each use. Blood collected for viscoelastic coagulation analysis and assessment of platelet count, PT, PTT, plasma concentrations of fibrinogen and FDPs, and AT activity was analyzed immediately after collection. Blood collected for assessment of plasma EACA concentration and α2-AP activity was immediately centrifuged (15 minutes at 2,500 × g), and plasma was stored at –70°C until analysis (< 3 months).

Sample analyses—Plasma EACA concentrations were measured by use of high-performance liquid chromatography, as described. Antiplasmin activity was assayed in plasma samples by use of a commercially available colorimetric assay that has been previ-
viously used in a study of coagulation in horses. Plasma fibrinogen concentration, activated PPT, PT, and AT activity were determined by use of an automated system. Activated PTT was assessed by use of rabbit brain phospholipids and calcium chloride to activate the intrinsic coagulation path. Prothrombin time and plasma fibrinogen concentration were assessed by use of lyophilized rabbit brain calcium thromboplastin to activate the extrinsic coagulation cascade. Plasma AT activity was determined with a chromogenic assay by incubating thawed plasma with bovine factor Xa in heparin and then quantifying the residual factor Xa activity. Plasma concentrations of FDPs were measured by use of a commercial latex agglutination kit. For case of statistical analysis, plasma concentration of FDPs was reported as 0 (< 10 µg/mL), 1 (≥ 10 but < 40 µg/mL), or 2 (≥ 40 µg/mL). This method has been used previously when assessing coagulation variables in horses.

Platelet count was determined by use of an automated method. Viscoelastic properties of clot formation were evaluated via viscoelastic coagulation analysis. The viscoelastic coagulation analyzer works by activating clot formation in whole or citrated blood samples and measuring resistance sensed by a disposable probe placed on a transducer oscillating at 200 Hz within the sample. The output data are processed and displayed in units of clot signal over time. The generated clot signal curve provides information regarding time to clot formation, rate of clot formation, plateau function, maximum clot signal (clot strength), and subjective quality of fibrinolysis. After collection, the blood sample was allowed to cool to the top of each sample. At each time point at which the viscoelastic coagulation analysis was performed, time to clot formation, rate of clot formation, and clot strength were recorded from the clot signal curve.

**Pharmacokinetics analysis—** Plasma EACA concentration versus time data were fit to 1- and 2-compartment models by use of a pharmacokinetic analysis program that employs nonlinear least square analysis. The pharmacokinetic rate constants and V1 that provided the best fit for the disposition curves during and following IV infusion of EACA were determined. The model of best fit was selected by use of Akaike’s information criterion. For the 2-compartment model, the hybrid parameters A, α, B, and β that describe the disposition curve following a bolus dose were calculated from V1 and the microconstants k12, k21, and ke1. Model independent variables included MRT, which was calculated as follows:

\[ MRT = \frac{AUMC}{AUC} - \frac{T}{2} \]

where AUMC is the area under the moment curve and T is the duration of the infusion (corrected for drug excretion during the infusion). Clearance was calculated as dose/AUC. On the basis of the mean rate constants determined in the study, the computer program was used to predict plasma EACA concentration following various infusion protocols. Therapeutic blood concentration for EACA has not been experimentally evaluated in horses, and the assumed target therapeutic blood concentration of EACA used (130 µg/mL) was derived from findings in humans.

Elimination half-life (the time required for plasma concentration of drug to be reduced by 50%), MRT (mean amount of time that the drug remains in the body), and clearance (proportionality constant between amount of drug eliminated per unit time and the concentration of drug in plasma) are all convenient expressions of how rapidly drug is eliminated and are useful for determining dosing regimens and comparing drug elimination between species.

**Statistical analysis—** Analysis was performed by use of a commercially available software program. A Student t test was used to compare platelet count, PT, PTT, and plasma fibrinogen concentration before and 4 hours after administration of EACA was completed. The Wilcoxon matched-pairs signed rank test was used to compare FDP values before and 4 hours after treatment was completed. Repeated-measures ANOVA with a Bonferroni multiple comparisons test were used to compare plasma α2-AP activity before administration of EACA and 1 and 4 hours after EACA infusion. The viscoelastic coagulation analysis data were tested by use of a repeated-measures ANOVA. The null hypothesis was rejected at a value of P < 0.05. Post hoc testing was performed by use of the Tukey-Kramer multiple comparisons test.

**Results**

Following the 20-minute infusion, mean ± SD peak plasma concentration of EACA was 462.9 ± 70.1 µg/mL (Figure 1). The drug disposition was best described by
the 2-compartment model with a rapid distribution phase, an elimination phase half-life of 2.3 hours, and an MRT of 2.5 ± 0.5 hours (Table 1). The apparent V1 is a mathematical proportionality constant that permits prediction of the initial drug concentration immediately following administration of an IV bolus dose. For many drugs, it is conceptually similar to the intravascular blood volume, and in the present study, V1 (0.11 L/kg) was similar to the blood volume of horses. Similarly, without representing a specific fluid space or tissue, the apparent volume of distribution at steady state represents the ratio of the concentration of drug in plasma and the total amount of drug in the body when a steady state has been reached. In the present study, the apparent volume of distribution at steady state (0.26 L/kg) was similar to the extracellular fluid volume of the horse, suggesting that extensive distribution of the drug to tissues did not occur.

Drug concentrations remained greater than the proposed therapeutic concentration of 130 µg/mL for 1 hour after the end of the IV infusion. No detrimental effects of EACA administration were detected during the infusion or during the observation period after drug administration. On the basis of computer-simulated infusions, we determined that a loading dose of 3.5 mg of EACA/kg/min for 15 minutes followed by 0.25 mg/kg/min would be an effective regimen with which plasma concentrations of EACA > 130 µg/mL could be maintained (Figure 2).

Coagulation variables were within reference limits in all 6 horses at time 0, except for 1 horse in which plasma concentration of FDPs was scored as 1 (≥ 10 but < 40 µg/mL). Administration of EACA did not result in a significant change in PT, PTT, plasma AT activity, or plasma concentration of FDPs or fibrinogen at 4 hours after completion of the infusion. There was a significant (P = 0.045) decrease in platelet count at 4 hours after EACA administration (112,300 ± 15,820 platelets/µL), compared with the value at time 0 (121,500 ± 20,280 platelets/µL). Plasma α,-AP activity was not significantly different at 1 or 4 hours after EACA administration (77.6 ± 4.930% and 79.8 ± 4.604%, respectively), compared with the value at time 0 (79.4 ± 4.722%); however, the difference in activity at 1 and 4 hours after EACA administration was significant (P = 0.029). The differences in mean time to clot formation, rate of clot formation, and clot strength among the various time points were not significant (Table 2). At 30 minutes after completion of EACA treatment, PF was significantly (P < 0.05) different from PF at time 0 and at 1 and 4 hours after infusion.

**Discussion**

In horses, administration of EACA at 3.5 mg/kg/min for 20 minutes resulted in a mean plasma drug concentration > 130 µg/mL (the proposed therapeutic concentration) during the infusion and for 1 hour after the end of drug administration. The elimination half-life of EACA in horses was 2.3 hours (138 minutes), whereas in healthy humans, the elimination half-life is 77 to 294 minutes. In the present study, mean ± SD clearance of EACA in horses was 98 ± 17 mL/kg/h and mean volume of distribution at steady state was calculated as 0.26 ± 0.04 L/kg. In healthy humans, clearance and volume of distribution were calculated as 157 mL/kg/h and 0.39 L/kg, respectively.

In human medicine, controversy persists regarding the most beneficial dosing regimen for EACA to achieve sustained therapeutic plasma concentrations; some authors propose the use of continuous infusion, and others propose the use of intermittent bolus dosing. Because of the rapid elimination of EACA in horses, administration via continuous infusion may be the most

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**Table 1—Pharmacokinetic variables for EACA following IV administration (3.5 mg/kg/min; over a period of 20 minutes) to 6 healthy horses. Values are reported as mean ± SD except for half-life values, which are reported as the harmonic mean.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>k12 (h⁻¹)</td>
<td>1.16 ± 0.32</td>
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<tr>
<td>k21 (h⁻¹)</td>
<td>0.85 ± 0.12</td>
</tr>
<tr>
<td>kel (h⁻¹)</td>
<td>0.91 ± 0.12</td>
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<tr>
<td>A (µg/mL)</td>
<td>477.3 ± 59.6</td>
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<tr>
<td>α (h⁻¹)</td>
<td>2.63 ± 0.45</td>
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<tr>
<td>t₁/₂</td>
<td>0.26</td>
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<tr>
<td>B (µg/mL)</td>
<td>151.0 ± 33.9</td>
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<tr>
<td>β (h⁻¹)</td>
<td>0.29 ± 0.04</td>
</tr>
<tr>
<td>V₁ (L/kg)</td>
<td>0.11</td>
</tr>
<tr>
<td>Vss (L/kg)</td>
<td>0.26 ± 0.04</td>
</tr>
<tr>
<td>AUC (µg⋅h/mL)</td>
<td>734.2 ± 138.1</td>
</tr>
<tr>
<td>Clearance</td>
<td>0.098 ± 0.017</td>
</tr>
<tr>
<td>MRT* (h)</td>
<td>2.5 ± 0.5</td>
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*Corrected for drug elimination during infusion. k12 = Rate constant from V1 to peripheral compartment. k21 = Rate constant peripheral to V1. kel = Rate constant elimination from central compartment. A = Preexponential term for distribution (rapid) phase following bolus dose. α = Exponential term for distribution (rapid) phase. t₁/₂ = Distribution phase half-life. B = Preexponential term for elimination (slow) phase following bolus dose. β = Exponential term for elimination (slow) phase. t β = Elimination phase half-life. Vss = Volume of distribution at steady state.

**Figure 2—Results of a computer simulation of infusion of EACA (3.5 mg/kg/min) over a period of 10 (pink line) or 15 minutes (blue line) followed by continuous infusion of EACA (0.25 mg/kg/min) for 6 hours in horses. Plasma concentrations > 130 µg/mL are maintained for the duration of the continuous infusion following the initial 15-minute high-dose infusion.**
practical means of maintaining therapeutic plasma concentrations in horses. On the basis of computer-simulated infusions, we believe that a loading dose of 3.5 mg of EACA/kg/min for 15 minutes followed by 0.25 mg/kg/min would be an effective regimen to maintain blood concentrations of EACA > 130 µg/mL. Further research is needed to evaluate the safety and efficacy of this regimen in vivo. In human medicine, the duration of infusions are often limited to 4 to 6 hours.5

Given the paucity of safety studies of the use of EACA in humans and horses with regard to possible thrombotic complications, the safety of prolonged administration of EACA to horses is unknown at this time. Results of an investigation4 in humans have indicated that the elimination half-life is significantly increased in patients with renal disease, compared with persons with normal renal function. It is likely that this is also true in horses; thus, administration of EACA to horses with renal disease must be done with caution because reduced elimination of the drug might result in higher than desired plasma concentrations during infusion.

In the present study, administration of EACA had no significant effect on PT, PTT, plasma AT activity, or plasma concentrations of FDPs or fibrinogen in horses. Administration of EACA to healthy horses does not significantly alter the plasma concentration of FDPs;6 however, in clinical studies in humans,4 EACA use has been reported to decrease plasma concentrations of D-dimers and FDPs. In the horses in our study, the platelet count was significantly decreased 4 hours after administration of EACA, compared with the value before treatment. To our knowledge, this finding has not been identified in previous EACA studies. The clinical significance of this finding is questionable because the platelet count decreased to less than the reference limit (91,000 platelets/µL) in only 1 horse and because the changes in platelet counts in individual horses were small. Platelet counts performed on blood samples containing sodium citrate would have been useful to minimize any artifact associated with platelet activation. Coagulation variables were assayed before (time 0) and at 4 hours after EACA administration in our study. In retrospect, it would have been informative to have performed these measurements during the first hour after administration of EACA while plasma drug concentrations were within the proposed therapeutic concentration range. The minimal changes in coagulation profile identified at 4 hours after completion of EACA admin-

istration allow some assessment of the safety of this drug with regard to the coagulation system in horses. However, we cannot rule out the possibility of a delayed or more immediate effect of EACA on coagulation variables after administration that remained undetected with the study protocol. There is concern in human medicine that EACA may increase the risk of thrombotic complications in patients because of EACA's effect of inhibiting fibrinolysis without suppressing thrombin formation.13 To our knowledge, no large-scale clinical trial has yet identified an increased risk of thrombotic complications with EACA use, although case reports26,27 in which complications such as renal complications and formation of pulmonary microthrombi are described have been published. a-Aminocaproic acid use is contraindicated in humans with disseminated intravascular coagulopathy.27

Plasma α2-AP activity was not significantly changed at 1 or 4 hours after the end of EACA infusion, compared with that at time 0, but α2-AP activity at 1 and 4 hours after drug administration did differ significantly. These changes are difficult to interpret and do not reflect changes detected by other investigators. Heidmann et al.20 detected a significant increase in plasma α2-AP activity 1 hour after administration of EACA (100 mg/kg) to healthy horses. Increased de novo release and synthesis of α2-AP is one of the suggested mechanisms of action of EACA;13 administration of EACA to cardiopulmonary bypass patients prevents cardiopulmonary bypass-related decreases in plasma α2-AP activity and results in increased α2-AP activity 1 hour after cardiopulmonary bypass. It is possible that a more profound effect on plasma α2-AP activity would have been detected in the horses in the present study if activity had been monitored more frequently during the first hour after completion of EACA administration when plasma concentrations were within the proposed therapeutic concentration range.

In the present study, evaluation of the viscoelastic properties of the hemostatic process indicated that platelet function was increased at 30 minutes after EACA infusion, compared with findings at all other time points. It is not known what the platelet count was at 30 minutes after EACA infusion because that variable was not assessed at this time point. The importance of this finding is unclear and requires further investigation. In human medicine, there is debate over whether EACA has an effect on platelet function and whether this effect is part of the improvement in hemostasis associated with EACA use.4,28 Platelet function has not been previously evaluated in horses, to our knowledge. The viscoelastic coagulation analyzer4 assesses coagulation and platelet function in a sample for a period of 45 to 60 minutes, but in clinically normal humans, fibrinolysis occurs after that interval. Additional information regarding the effect of EACA on fibrinolysis may be obtained if humans or other animals with hyperfibrinolytic are monitored by use of the viscoelastic coagulation analyzer4 during EACA administration.

Additional research is needed to further assess the potential clinical benefits of EACA in horses in hyperfibrinolytic states and determine an effective therapeutic plasma concentration. The treatment regimen for
EACA in horses that we have suggested was based on an established therapeutic concentration calculated for humans, and that concentration may be different from the concentration needed to be effective in horses. Nevertheless, the pharmacokinetics and pharmacodynamics of EACA in horses were elucidated and results of the present study have indicated a dosage regimen that will maintain plasma EACA concentrations > 130 µg/mL. Novel methods that provide information regarding clot strength and platelet function that is not easily obtained via more traditional coagulation assessments are likely to be useful in evaluations of the effects of EACA on the coagulation system in horses and other species.

References