Laboratory measures of hemostasis and fibrinolysis after intravenous administration of \(\varepsilon\)-aminocaproic acid in clinically normal horses and ponies

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**Objective**—To determine whether \(\varepsilon\)-aminocaproic acid (EACA) administered IV affects hemostasis and fibrinolysis in clinically normal horses and ponies.

**Animals**—20 clinically normal adult horses and ponies.

**Procedures**—Blood samples were collected 24 hours before (baseline) and 1 and 5 hours after IV administration of a low dose (30 mg/kg) or high dose (100 mg/kg) of EACA. Platelet count, fibrinogen concentration, prothrombin time, partial thromboplastin time (PTT), D-dimer concentration, \(\alpha_2\)-antiplasmin activity, and thrombin-antithrombin complex concentration were measured. Values at 1 and 5 hours were compared with baseline values.

**Results**—1 hour after administration of a low dose of EACA, mean fibrinogen concentration was significantly lower than baseline concentration. Mean PTT was significantly shorter than the baseline value 5 hours after administration of a low dose of EACA. One hour after administration of 100 mg of EACA/kg, mean \(\alpha_2\)-antiplasmin activity was significantly higher than baseline activity. Mean fibrinogen concentration was significantly lower than baseline concentration 1 and 5 hours after administration of a high dose of EACA. Mean PTT was significantly shorter than the baseline value 6 hours after administration of a high dose of EACA.

**Conclusions and Clinical Relevance**—IV administration of 30 and 100 mg/kg of EACA to clinically normal horses significantly modified some laboratory measures of hemostasis, consistent with its known anti-fibrinolytic effects. Although enhanced clot maintenance and diminished bleeding were not directly ascertained, accessed, or repaired during surgery, \(\varepsilon\)-Aminocaproic acid has been given to horses with guttural pouch mycosis and partial compromise of the internal carotid artery and to horses with intra-abdominal bleeding in which the source of bleeding cannot be ascertained, accessed, or repaired during surgery.

Administration of EACA to veterinary species has been advocated because of its reported antifibrinolytic effect in humans. \(\varepsilon\)-Aminocaproic acid has been used empirically in animals with ongoing bleeding that cannot be controlled via compression, surgical ligation or cautery of blood vessels, or other mechanical means. \(\varepsilon\)-Aminocaproic acid has been used prior to surgical repair of congenital cyanotic heart disease, and humans undergoing major orthopedic surgery are reported. \(\varepsilon\)-Aminocaproic acid applied topically minimizes rebleeding rates in humans with traumatic hyphema and inhibits fibrinolysis postoperatively.

To the authors' knowledge, no studies have been performed in which the safety and efficacy of EACA have been determined in horses; effective doses in horses have also not been reported. The purpose of the study reported here was to determine whether doses of EACA that are commonly used in horses are associated with changes in laboratory measures of hemostasis and fibrinolysis and adverse effects in clinically normal horses. Variables selected were specifically those that are consistently affected after EACA administration in humans.

**Materials and Methods**

**Animals**—On the basis of physical examination, 20 clinically normal adult horses and ponies (including 10 from the Oregon State University Equestrian Center and 10 from
the Oregon State University College of Veterinary Medicine teaching herd) were selected for inclusion in the study. Breeds included 2 Appaloosas, 2 Arabians, 4 Thoroughbreds, 8 Quarter Horses, 1 Standardbred, 1 warmblood-Thoroughbred cross, and 2 Shetland ponies. Ages ranged from 4 to 24 years (mean, 13.2 ± 9.9 years), and there were 4 geldings and 16 mares. Results of hematologic and serum biochemical analyses in all equids were within respective reference ranges established by the Veterinary Diagnostic Laboratory at Oregon State University. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Oregon State University.

Procedures—Each equid was weighed (mean ± SD, 453.6 ± 97.7 kg [range, 207 to 568 kg]), and blood samples were collected via direct jugular venipuncture into standard sodium citrate tubes (baseline samples). A 14-gauge catheter was placed in a jugular vein and flushed once with 2 mL of heparinized saline solution (2 units of sodium heparin/mL of 0.9% NaCl) immediately after placement. Catheters were also flushed with 2 mL of heparinized saline solution 8 and 16 hours after catheter placement.

Twenty-four hours after catheter placement, each equid was given EACA. Ten randomly chosen equids (5 from each site of origin) were given 100 mg of EACA/kg (high dose) diluted in 1 L of lactated Ringer’s solution, IV. The other 10 were given 30 mg of EACA/kg (low dose) diluted in 1 L of lactated Ringer’s solution, IV. The total dose was administered over a minimum time of 30 minutes to a maximum time of 1 hour. The IV catheter was flushed with 2 mL of heparinized saline solution immediately after the infusion.

One hour after completion of EACA administration, blood samples were collected via direct jugular venipuncture into tubes containing potassium EDTA and 0.129M sodium citrate. Blood samples were immediately packed on ice for transport prior to processing. Citrated plasma was separated by centrifugation at 3,000 rpm for 3 minutes. Measurements were performed by a technician unaware of the treatment status of each equid. Abnormalities, including platelet clumping, were noted. Serum creatine kinase activity, platelet count, and hematologic and serum biochemical analyses in all equids were within respective reference ranges established by the Veterinary Diagnostic Laboratory at Oregon State University. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Oregon State University.

Sample analyses—A CBC was performed on blood samples collected 1 and 5 hours after administration of EACA; an automated hematology analyzer was used. Blood smears were examined by a veterinary clinical pathologist (SJT) unaware of the treatment status of each equid. Abnormalities, including platelet clumping, were noted. Serum creatine kinase activity in blood samples collected 5 hours after EACA administration was measured by use of an automated chemistry analyzer.

Plasma fibrinogen concentration was estimated by use of an automated hematology analyzer and refractometer; fibrinogen concentration was determined by calculating the difference between total plasma protein concentrations determined before and after heat precipitation of plasma at 36°C for 3 minutes. Measurements were performed by a technician unaware of the treatment status of each equid.

Secondary hemostasis was assessed via measurement of prothrombin time (PT) and partial thromboplastin time (PTT) in blood samples (citrated plasma) collected prior to (baseline) and 1 and 5 hours after EACA administration. Assays were performed by use of a photo-optical clot detection instrument; a thromboplastin reagent was used for the PT assay, and PTT and calcium chloride reagents were used for the PTT assay.

Assays of fibrinolytic potential were chosen on the basis of results of previous studies in humans and on the availability of assays validated for use in horses. Fibrinolysis was assessed via measurement of the concentration of the specific fibrin breakdown product fragment D (ie, D-dimer). A latex agglutination test kit in which D-dimer concentration in citrated plasma samples is measured was used. The kit assay is one of the most accurate D-dimer assays available and has been validated for use in horses. Fibrinolysis was also assessed via measurement of α2-antiplasmin activity in citrated plasma. The assay, which uses a chromogenic substrate method, was performed by use of a commercially available kit. The method has been validated for use with equine plasma and is expressed as a percentage of normal activity.

Activation of coagulation was assessed via measurement of concentration of thrombin-antithrombin (T-A) complexes in citrated plasma by use of an ELISA. The method has been validated for use in healthy and sick horses.

Statistical analyses—The study was designed as a hierarchical randomized crossover trial with self-paired controls. Measurement of D-dimer concentration resulted in an ordinal outcome (<250, 250 to 500, 500 to 1,000, and 1,000 to 2,000 ng/mL); therefore, for analysis of D-dimer concentrations, a Wilcoxon signed rank test for 1-sample analysis was used to compare each outcome after administration of low and high doses of EACA with baseline values. These ordinal values were converted to numerical terms (1, 2, 3, 4) for calculation of mean outcome.

All continuous outcome measures (PT, PTT, fibrinogen concentration, α2-antiplasmin activity, T-A complex concentration, creatine kinase activity, and platelet count) were analyzed by use of a 2-way ANOVA for repeated measures to determine whether values obtained after administration of EACA were significantly different than those at baseline and whether there were significant differences in values between high-dose and low-dose groups at corresponding 1- and 5-hour time points after administration of EACA. Values of P < 0.05 were considered significant. Statistical analyses were performed by use of a statistical software package.

Results

PT—No significant difference between mean baseline PT and PT 1 hour after administration of a low dose of EACA was found (Table 1). Mean PT 5 hours after administration of a low dose of EACA was significantly (P < 0.02) longer than mean baseline PT; however, the total mean increase was <0.2 seconds, a 1.8% change from the baseline value. No significant differences between mean baseline PT and PT 1 and 5 hours after administration of a high dose of EACA were found. No significant differences between mean PT after administration of high and low doses of EACA at corresponding time points were found.

PTT—Mean PTT 5 hours after administration of EACA at both doses was significantly shorter, compared with baseline values (Table 1). A total mean decrease of 2.9 seconds (8.3%) from the baseline value 5 hours after administration of a low dose of EACA (P = 0.049) and a total mean decrease of 3.8 seconds (10.9%) from the baseline value 5 hours after administration of a high dose of EACA (P < 0.016) were found. No significant differences in mean PTT 1 hour after administration of a low or high dose of EACA, com-
comparing with baseline values, were found. No significant differences between PTT after administration of high and low doses of EACA at corresponding time points were found.

**Fibrinogen concentration**—Mean fibrinogen concentration was significantly (P = 0.005) lower 1 hour after administration of a low dose of EACA, compared with the baseline value, but was not significantly different than the baseline value 5 hours after administration of a low dose of EACA (Table 1). Mean fibrinogen concentrations were lower than baseline values 1 hour (P = 0.001) and 5 hours (P = 0.017) after administration of a high dose of EACA. No significant difference between mean fibrinogen concentration 1 hour after administration of a low dose of EACA and 1 hour after administration of a high dose of EACA was found; however, mean fibrinogen concentration was significantly (P = 0.034) lower 5 hours after administration of a high dose of EACA than 5 hours after administration of a low dose of EACA.

**α₂-Antiplasmin activity**—Mean α₂-antiplasmin activities 1 and 5 hours after administration of a low dose of EACA were not significantly different than baseline activity (Table 1). Mean α₂-antiplasmin activity was significantly (P = 0.024) higher 1 hour after administration of a high dose of EACA, compared with baseline activity; activity increased from a mean of 113% to 119%. Mean α₂-antiplasmin activity increased from a baseline activity of 113% to 118% 5 hours after administration of a high dose of EACA; however, this increase was not significant (P = 0.065). No significant differences between α₂-antiplasmin activities after administration of low and high doses of EACA at corresponding time points were found.

**D-dimer and T-AT complex concentrations, creatine kinase activity, and platelet count**—No significant differences in mean D-dimer and T-AT complex concentrations, creatine kinase activity, and platelet count 1 and 5 hours after administration of a high and low dose of EACA, compared with baseline values, were found (Table 1). No significant differences between values of corresponding parameters after administration of EACA at high and low doses at corresponding time points were found. Platelet clumping was detected on some blood smears; however, there was no significant association of platelet clumping with any group. The baseline and 1- and 5-hour platelet count values in both groups included some measurements below the lower limit of the Oregon State University Diagnostic Laboratory hematology reference ranges. Platelet clumping likely caused these findings. No other hematologic abnormalities were identified.

Two adverse effects were noted during the study. One gelding had clinical signs of colic 18 hours after administration of a low dose of EACA. Approximately 5 weeks after EACA administration, fetal death was detected in a pony mare in the ninth month of gestation. It was not known whether these findings were related to the administration of EACA.

**Discussion**

Despite the poor sensitivity of the heat precipitation method for detection of decreases in plasma fibrinogen concentration, the results of our study revealed significant decreases in plasma fibrinogen concentrations, compared with baseline values, after IV administration of EACA. We also detected significantly lower fibrinogen concentration 5 hours after administration of a high dose of EACA, compared with fibrinogen concentration 3 hours after administration of a low dose of EACA, suggesting the possibility that decreases in fibrinogen concentration after administration of EACA are dose related. e−Aminocaproic acid prevents fibrinolysis by inhibiting plasmin and enhancing antiplasmin activity and promotes clot stabilization. Increased fibrinogen and fibrin degradation products are independently associated with an increased risk of abnormally increased tendency toward coagulation, as in ischemic heart disease in humans.16
Previous studies of fibrinogen concentration after administration of IV EACA have reported conflicting results. In 1 study, human infants treated surgically for cyanotic heart disease and given EACA had higher fibrinogen concentrations than infants not treated with EACA. Plasma fibrinogen concentrations decreased from baseline values in swine treated with a fibrin bandage impregnated with EACA and did not change in nontreated swine. In our study, hemodilution as a result of IV administration of 1 L of a crystalloid solution may have contributed to decreased plasma fibrinogen concentrations; however, the changes in fibrinogen concentrations exceeded the potential effect of IV administration of 1 L of fluids, even in the smallest equid (207 kg). Studies utilizing a more sensitive method of quantifying changes in fibrinogen concentration from horses receiving EACA are warranted to investigate the possibility of clinically important decreases in fibrinogen concentration that may lead to development of transient hypocoagulability. Results of a previous study in humans receiving high doses of EACA when undergoing neurosurgical procedures revealed a period of transient hypocoagulability, although alterations in fibrinogen concentration were not reported.

The results of our study revealed that administration of EACA at a dose of 100 mg/kg resulted in higher α2-antiplasmin activity within 1 hour of administration, compared with baseline values; activity remained higher than baseline activity 5 hours after administration of 100 mg of EACA/kg, although the difference was not significant. Renal excretion, the primary route of elimination of EACA after IV or PO administration, is rapid, and the effects of α2-antiplasmin are understood to be time dependent. In horses, all measurable quantities of EACA are excreted within 6 hours of IV administration. Our findings support the expectation that the maximum effect of EACA on inhibition of fibrinolysis in horses occurs within the first 1 to 2 hours after administration of a single bolus.

The lack of significantly higher α2-antiplasmin activity, compared with baseline activity, at any time point 1 and 5 hours after administration of EACA at a dose of 30 mg/kg suggests that the empiric dosage (30 mg of EACA/kg, IV, 2 to 4 times daily) typically used in horses may not be sufficient to affect indicators of fibrinolysis. However, administration of a high-dose bolus of EACA may predispose to transient hypocoagulability. This effect was reported in a study in which humans were given 1 daily dose > 400 mg of EACA/kg. Because of the short half-life of EACA in humans, a loading dose of 50 to 150 mg of EACA/kg/h for a minimum of 8 hours or until bleeding is controlled is recommended. Plasma EACA concentrations of 0.13 mg/mL are suggested to achieve successful inhibition of fibrinolysis in humans. Continuous-rate infusions have not been evaluated in horses but could prove to be more effective than bolus administration for maximizing antifibrinolytic potential.

The natural inhibitors of fibrin clot maintenance (plasmin, antithrombin) are inhibited by EACA. This occurs only after a clot has formed as a result of activation of the coagulation cascade; therefore, no changes in the extrinsic or intrinsic coagulation activation pathways as measured via PT and PTT, respectively, would be expected. The prolonged PT detected 5 hours after administration of a low dose of EACA was < 0.2 seconds longer than the baseline value, a 1.8% change. The clinical importance of this small change in PT is debatable; the decreases in PTT 5 hours after administration of EACA at both doses suggest that enhanced secondary hemostasis may occur as a result of feedback or another unidentified mechanism, indirectly related to the known antifibrinolytic effects of EACA. Alterations in PTT following administration of EACA have not been documented in human patients.

In our study, no significant changes from baseline values were detected in D-dimer or T-AT complex concentrations after administration of low and high doses of EACA. Hypercoagulability is characterized by increased thrombin formation and has been evaluated in horses via measurement of T-AT complexes, which are found in direct proportion to thrombin. Results of studies in humans reveal that T-AT complex concentrations are unchanged after EACA administration. Concentrations of D-dimer decreased from baseline values in humans treated with EACA, consistent with inhibition of fibrinolysis. The kit used to measure D-dimer concentration is capable of detecting increases in D-dimer concentration in horses with colic, but it is not known whether the test is sufficiently sensitive to detect minor changes in D-dimer concentration in clinically normal horses. Further studies in which D-dimer and T-AT complex concentrations are measured during episodes of traumatic and surgically induced blood loss in horses treated and not treated with EACA are warranted.

No significant changes in creatine kinase activity or platelet count from baseline values were detected after administration of EACA. Monitoring of creatine kinase activity and platelet count in experimental horses or sick horses after administration of EACA is warranted because long-term IV administration of EACA in human patients has been associated with skeletal muscle weakness with necrosis of muscle fibers. E-Aminocaproic acid appeared to be well tolerated by equids in our study; however, 2 equids developed different conditions during and after the study. One gelding had clinical signs of colic 18 hours after administration of a low dose of EACA. This horse had a history of episodes of gas colic; a change in feed and increased duration of confinement preceding the study may have predisposed this horse to development of colic. The horse was removed from the study and recovered fully without treatment other than reduction of feed for 18 hours. It is unlikely that EACA administration was related to the episode of colic. Approximately 5 weeks after EACA administration, fetal death was detected in a pony mare in the ninth month of gestation. Fetal intestines had herniated through the inguinal ring. Because of the long period of time that had elapsed between EACA administration and fetal death and the presence of a congenital defect in the fetus that likely predated EACA administration, it is unlikely that fetal death was related to the admin
istration of EACA; however, the possibility of abnormal clot formation in the uterine, placental, or fetal vasculature resulting in ischemia cannot be definitively excluded.

Overall, our results support the conclusion that IV administration of EACA is well tolerated by clinically normal equids. Administration of a high dose of EACA resulted in an increase in α2-antiplasmin activity over baseline values, suggesting the possibility of enhanced clot maintenance as previously reported in humans with similar increases in α2-antiplasmin activity. Because α2-antiplasmin is the primary inhibitor of plasmin-mediated fibrinolysis, assay of its activity is a more sensitive measure of overall coagulability, especially compared with the relatively insensitive heat precipitation method. α2-Antiplasmin is a potent endogenous antifibrinolytic enzyme that exerts its effect via inhibition of plasminogen activation and formation of a 1:1 complex with plasmin, preventing plasmin from binding with fibrinogen and therefore preventing fibrinolysis. When subjectively assessed, horses were more likely to have clots in their IV catheters after administration of EACA than before administration of EACA.

Direct measurement of coagulation is important to further justify the use of EACA in horses. Future studies may include measurement of duration of bleeding and volume of blood loss from a standard incision and assessment of in vitro phases of clot formation via measurement of activation of coagulation, platelet activity, and fibrin cross-linking by use of thromboelastography.

It has been suggested that EACA be used only in horses with active hyperfibrinolysis (ie, horses with disseminated intravascular coagulation, endotoxemia, or both) Many veterinary clinicians also suggest that treatment will have no benefit in clinically normal horses bleeding as a result of trauma. Our results indicate that certain parameters are modified after IV administration of EACA to clinically normal equids. This is an important finding because it suggests that EACA may benefit horses without coagulopathies (ie, horses with a primary traumatic injury or horses undergoing surgery).

Anecdotal evidence suggests that, in horses, EACA may be effective in attenuating the volume of blood loss or duration of bleeding in a variety of conditions (ie, uterine artery rupture and exercise-induced pulmonary hemorrhage [EIPH]). Use of EACA prior to athletic events has been suggested as a method to safely limit bleeding in horses predisposed to EIPH, possibly by modifying alterations in hemostasis. It has been hypothesized that EIPH in horses may be, in part, the result of inhibition of coagulation that occurs during intense exercise; therefore, controlled studies of the use of EACA as a preventative treatment for EIPH are warranted, especially because EACA is often administered empirically to horses with EIPH.

The potential effects of EACA on clot stability would not be expected to independently control high-volume blood loss from large vessels in horses with blood pressure within the reference range. Blood loss from small vessels could be minimized when EACA administration is combined with direct mechanical control of bleeding (ie, direct pressure, electrocautery, or ligation of blood vessels); EACA administration may also be useful when bleeding is restricted to small vessels or when severe hypotension is present.

References


