

PHARMACOKINETIC STUDY OF ORAL ϵ -AMINOCAPROIC ACID IN THE NORTHERN ELEPHANT SEAL (*MIROUNGA ANGUSTIROSTRIS*)

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Abstract: ϵ -Aminocaproic acid (EACA) is a lysine analogue antifibrinolytic drug used to treat bleeding disorders in humans and domestic animals. Its use in zoological medicine is rare, and dosage is anecdotal. One possible application of EACA is to treat bleeding associated with prepatent *Otostrongylus* arteritis in Northern elephant seals (*Mirounga angustirostris*) presenting to wildlife rehabilitation centers. This study used an in vitro model of hyperfibrinolysis and a thromboelastograph-based assay to estimate the therapeutic plasma concentration of EACA in elephant seals (85 $\mu\text{g}/\text{ml}$, 95% confidence interval = 73.8–96.8 $\mu\text{g}/\text{ml}$). A concurrent pharmacokinetic study of orally administered, single-dose EACA found that doses of 75 and 100 mg/kg achieved therapeutic plasma concentrations (>85 $\mu\text{g}/\text{ml}$), but the drug was rapidly eliminated and remained in the therapeutic range for only 0.4 and 1.5 hr, respectively. Models of repeated oral dosing at 100 mg/kg every 6 hr predict that therapeutic plasma concentration will be maintained for 31.7% (7.6 hr) of a 24-hr period. More frequent dosing would be required to maintain continuous therapeutic concentrations but would be impractical in a wildlife rehabilitation setting. Further pharmacodynamic studies to evaluate the duration of action of EACA in elephant seals and a prospective, placebo-controlled study are needed to determine if EACA is effective in decreasing bleeding associated with prepatent *Otostrongylus* arteritis and other bleeding disorders in this species.

Key words: Coagulopathy, ϵ -aminocaproic acid, *Mirounga angustirostris*, Northern elephant seal, *Otostrongylus circumlitus*, thromboelastography.

INTRODUCTION

ϵ -aminocaproic acid (EACA) is a lysine analogue antifibrinolytic drug used widely in human medicine to treat a variety of hemorrhagic disorders, including perioperative bleeding¹⁸ and hemorrhage secondary to trauma-induced coagulopathy.²⁴ This drug class prevents clot lysis through the competitive binding of lysine residues on plasminogen, inhibiting plasmin formation and reducing fibrinolysis. In veterinary medicine, EACA has been demonstrated to decrease postoperative hemorrhage in greyhounds²⁰ and is used empirically for cases of uncontrolled bleeding in horses¹⁷ and dogs.¹⁰ ϵ -Aminocaproic acid represents a potentially useful therapy for managing hemorrhage in zoological species, especially in cases where fresh

frozen plasma or whole blood transfusions are not available. However, dose extrapolation from domestic species may not be appropriate, and further investigation is needed before these drugs can be effectively and safely used in nondomestic animals.

One potential application of EACA is the prevention of fatal hemorrhage in Northern elephant seals (NES; *Mirounga angustirostris*) presenting with prepatent *Otostrongylus* arteritis, a bleeding diathesis associated with aberrant larval migration of the lungworm *Otostrongylus circumlitus*. This syndrome was first described in 1996 in stranded, 3- to 4-mo-old NES undergoing rehabilitation at The Marine Mammal Center (TMMC) in Sausalito, California.¹⁶ The syndrome continues to occur annually from April to July in approximately 12% of stranded NES at TMMC.⁴ Presenting clinical signs are usually seen within 2 wk of stranding and often include lethargy, anorexia, regurgitation and vomiting, epistaxis, and hemoptysis. Signs can progress to dyspnea, uncontrollable hemorrhage from the nose and mouth, and death. The case fatality rate over the past 13 seasons is 89%, making up 26% of NES mortalities at TMMC (unpublished data).

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On gross necropsy, the syndrome is characterized by the presence of *O. circumlitus* larvae and adults in the right atrium, pulmonary artery, and inferior pulmonary vasculature, with multifocal hemorrhage and pulmonary thrombosis.¹⁵ Histologic findings include severe pulmonary arteritis, disseminated microthrombi, and evidence of septicemia.¹⁵ Parasite-induced arteritis with subsequent disseminated intravascular coagulation (DIC) was postulated to be the mechanism of hemorrhage.¹⁶ However, certain laboratory parameters are inconsistent with the most common findings in DIC in other species, including normal to elevated platelet counts, normal antithrombin concentrations, and low concentrations of fibrin degradation products.¹⁶ These results allow for alternative hypotheses on the mechanism of hemorrhage in these cases, including the possible contribution of hyperfibrinolysis similar to that seen in schistosomiasis⁷ and heartworm disease.¹⁴ However, we cannot definitively exclude the possibility that DIC in elephant seals leads to clinicopathologic abnormalities that are different than other species.

Previous studies investigating therapeutic plasma concentrations of EACA in domestic species have documented significant species variation, with nearly 100-fold difference found between horses and dogs.^{10,11,17} We hypothesized that NES would be hypofibrinolytic compared to people and dogs, with a lower minimum therapeutic plasma concentration of EACA compared to these species, and subsequently would require lower oral dosage to achieve therapeutic effects.

MATERIALS AND METHODS

Estimate of therapeutic plasma concentration of EACA

Sample collection: This study was approved by the Internal Animal Care and Use Committees of both TMMC and Cornell University. Blood was opportunistically collected immediately prior to release from 27 NES that stranded on the California coast in the spring of 2013 and were presented to TMMC for rehabilitation. Each individual was determined to be healthy at time of sampling; this assessment was based on physical exam, complete blood count, and biochemical profile performed in-house at TMMC. Antemortem testing for prepatent *Otostrongylus* infestation is not available, so subclinical parasite burden was unknown. Samples were collected from the epidural sinus with a Vacutainer (Becton, Dickenson and Company, Franklin Lakes, New

Jersey 07417, USA) directly into citrated tubes (0.3 ml of 3.2% sodium citrate) and centrifuged on-site at TMMC. Aliquots of citrated plasma were transferred into 1.5-ml plastic cryovials labeled with the individual NES number and date of collection. Samples were batched and shipped on dry ice to Cornell University (Ithaca, New York 14853, USA), where they were stored at -80°C until time of use.

Coagulation testing was performed for each sampled individual, including prothrombin time, activated partial thromboplastin time (APTT), D-dimer and fibrinogen concentrations, and anti-thrombin activity. Two outliers were detected using the Dixon statistic and were excluded;¹² 1.5 ml of plasma from each of the NES with normal coagulation parameters were pooled and refrozen at -80°C in 0.7-ml aliquots, generating a plasma pool for use in the study.

Thromboelastograph assays: Each TEG assay was performed in duplicate on the Thromboelastograph Analyzer 5000 (Haemonetics Corporation, Braintree, Massachusetts 02184, USA) using citrated pooled plasma. Assays were run for 60 min. Initial testing of TEG activators (kaolin, tissue factor [TF], and the RapidTEG activating reagent [Haemonetics Corporation]) showed that the RapidTEG activator yielded the most reproducible fibrinolysis parameters and was therefore used for all subsequent TEG assays.

Model of hyperfibrinolysis: Using a previously described technique to estimate therapeutic plasma concentrations of EACA in humans, dogs, and horses,^{10,11,22,25} tissue plasminogen activator (tPA) was added to the healthy pooled plasma to induce a hyperfibrinolytic state in vitro. TEG assays were run with the following concentrations of tPA: 1,600, 2,000, 3,000, 4,000, and 5,000 U/ml. A negative control was run using 20 μl HEPES + 2% bovine albumin in place of tPA. Estimated percent lysis (EPL) at each tPA concentration was derived by examining the percent of the clot strength remaining at 30 min after the maximum clot strength was reached. The minimum concentration of tPA required to consistently produce EPL >90% was selected as the final model of hyperfibrinolysis and was used in subsequent trials with EACA.

EACA dose-response curve: Following previously published techniques,^{10,11,22,25} increasing concentrations of EACA (aminocaproic acid USP, 250 mg/ml, Hospira, Inc., Lake Forest, Illinois 60045, USA) were serially added to the hyperfibrinolytic plasma model. TEG assays were run at the following EACA concentrations: 0 (negative con-

trol), 20, 40, 60, 80, and 100 $\mu\text{g/ml}$. The relationship between EACA concentration and EPL was described, and regression analysis of the arcsin square transform of the EPL data was used to estimate the minimum concentration of EACA required to completely inhibit fibrinolysis for 30 min after the maximum clot strength was reached (EPL of 0%).

Pharmacokinetics of orally administered EACA

Study population: Twenty-four 3- to 4-mo-old NES that had stranded along the California coast in March–April 2014 and that were presented to TMMC for rehabilitation were included. All seals were assessed to be clinically healthy based on physical exam at the time of the study and had been determined by the TMMC veterinary staff to be ready for release.

Study protocol: The 24 NES recruited were randomly assigned to one of three dosage groups (50, 75, or 100 mg/kg EACA) with eight seals in each group. Sampling included baseline (0 hr) and 0.5, 1, 2, 4, 6, 8, 12, and 24 hr. A pooled pharmacokinetic approach was utilized in which each individual NES was scheduled for baseline blood collection and sampling at three additional time points. Ultimately, this yielded three data points ($n = 3$) per time point per dosage group. This pooled pharmacokinetic protocol allowed for sufficient replication while limiting handling and sampling episodes in each individual.

Prior to administration of EACA, a body weight was recorded for each seal, and blood was collected from the epidural sinus using a 20-ga 1.5-inch needle on a Vacutainer set. At baseline, three 4-ml lithium heparin tubes, two 4-ml citrate tubes, two 4-ml clot tubes, and one 2-ml EDTA tube were collected. These samples were used for biochemical profile, complete blood count, serum banking, baseline data for pharmacokinetic analysis, and future coagulation and fibrinolysis research. Following baseline blood collection, each seal was administered EACA at 50, 75, or 100 mg/kg. EACA was mixed with 100 ml of electrolyte solution and administered via oral gavage. At each subsequent time point, one lithium heparin tube for drug analysis and two citrate tubes for future coagulation analysis were collected.

Heparinized plasma, citrated plasma, and serum were obtained by centrifugation of whole blood at 2,500 g for 20 min. Heparinized and citrated plasma were stored in 1.5-ml cryovial aliquots at -80°C . Following completion of data

collection, heparinized plasma was shipped to the reference lab for EACA concentration analysis.

Drug quantification: EACA concentrations were evaluated in plasma samples at Iowa State University's Veterinary Diagnostic Laboratory using a liquid chromatography–mass spectrometry method on an LTQ XL ion trap mass spectrometer (Thermo Scientific, San Jose, CA 95134, USA). The method was validated with drug-free NES plasma collected at baseline.

Pharmacokinetic analysis: A noncompartmental analysis of pooled data from each dose group was performed using WinNonlin Professional (WinNonlin version 5.3, Pharsight Corp., Mountain View, California 94041, USA) to obtain initial pharmacokinetic (PK) parameter estimates, including terminal elimination rate (k), half-life ($t_{1/2}$), apparent volume of distribution/bioavailability (V/F), total systemic clearance/bioavailability (CL/F), area under the plasma drug concentration–time curve (AUC), maximum plasma concentration (C_{max}), and time to maximum plasma concentration (T_{max}). These parameters were used as initial estimates to support development of a computational model to describe the drug concentration time profile for each dose. The model was fit using both naïve-pooled data and naïve-averaged data approaches.¹³ 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 to select the best-fit model using WinNonlin.^{2,13} A two-compartmental model was found to best capture the observed data. The parameter estimates were then fixed, and simulations were performed evaluating different doses and frequencies of administration, assuming linear and stationary kinetics.¹ The goal was to identify a treatment schedule that would achieve the estimated therapeutic concentration of 85 $\mu\text{g/ml}$.

RESULTS

Plasma pool

Of the 27 recruited NES, two were excluded from the plasma pool due to abnormalities on routine coagulation testing, including an elevated APTT (33.7 sec; median [95% confidence interval (CI)] = 24.5 [24–25.8]) and elevated D-dimer (313 ng/ml; median [95% CI] = 93 ng/ml [61–107.5]), respectively; 1.5 ml of plasma from each of the 25 remaining NES were pooled for a total sample

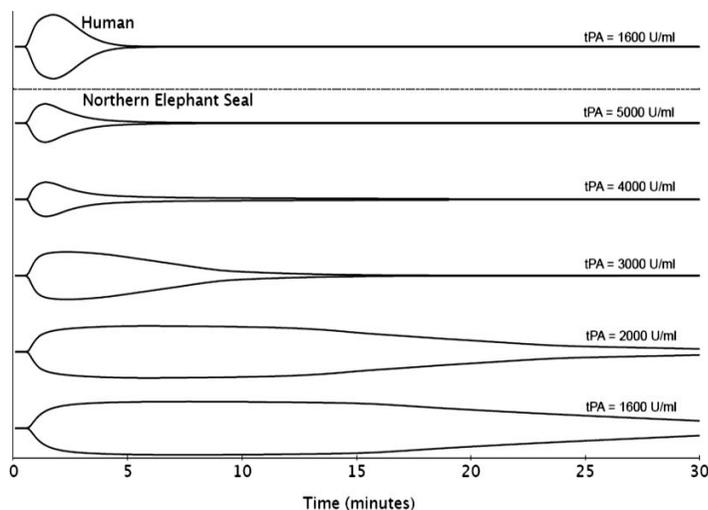


Figure 1. Thromboelastograph tracings of pooled plasma from healthy Northern elephant seals, modified with increasing concentrations of tPA. Healthy pooled human plasma with 1,600 U/ml tPA added is provided for comparison.

volume of 37.5 ml, and the plasma was refrozen at -80°C until the time of in vitro analysis.

Estimate of therapeutic plasma concentration of EACA

Model of hyperfibrinolysis: The degree of fibrinolysis at 30 min post maximum amplitude (EPL) increased with increasing concentrations of tPA (Fig. 1). Consistent EPL $> 90\%$ was achieved with a tPA concentration of 5,000 U/ml. This concentration of tPA was added to pooled plasma in subsequent trials with EACA.

EACA dose-response curve: TEG tracings of several concentrations of EACA are shown in Figure 2. Increasing concentrations of EACA resulted in decreasing EPL. The resulting dose-response curve is sigmoidal because the EPL is a ratio. Regression analysis was used to estimate the lowest plasma concentration of EACA at which EPL would be 0%. The regression analysis was done on the arcsine square transform of the EPL curve to linearize the sigmoidal data (Fig. 3)²¹ and yielded an estimated therapeutic plasma concentration for EACA of 85 $\mu\text{g}/\text{ml}$ (estimate = 85.3, 95% CI = 73.8–96.8 $\mu\text{g}/\text{ml}$).

Pharmacokinetics of orally administered EACA

Two of the seals initially recruited into the study were excluded, one due to a marked leukocytosis and the second due to a dosing error. Two additional seals (for a total of 26) were recruited to complete the study so that the final

sample size was 24 seals with eight seals per dosage group. No adverse effects of restraint, sample collection, or drug administration were noted. Pharmacokinetic parameters of orally administered, single-dose EACA at 50, 75, and 100 mg/kg are presented in Table 1.

The drug showed rapid oral absorption ($t_{\text{max}} \sim 1$ hr) at all doses tested. At 50 mg/kg, therapeutic concentrations were not achieved at any time point ($C_{\text{max}} = 61.9 \mu\text{g}/\text{ml}$). Single-dose administration of 75 and 100 mg/kg achieved therapeutic concentrations ($C_{\text{max}} > 85 \mu\text{g}/\text{ml}$), but the drug was eliminated rapidly and remained in the therapeutic range for only 0.4 and 1.5 hr, respectively (Fig. 4).

Oral dosage simulations: The PK parameters obtained from noncompartmental analysis were used as initial estimates for compartmental modeling. The data were best described using a two-compartment pharmacokinetic model (Fig. 3). Parameter estimates were fixed using the two-compartment model, and simulations were performed at 10, 50, 75, 100, and 200 mg/kg assuming linear and stationary kinetics (Fig. 4). At the maximum dose simulated (200 mg/kg), the majority of drug was cleared rapidly and did not maintain plasma concentrations over 85 $\mu\text{g}/\text{ml}$ for more than 3 hr (Table 2).

Simulations of repeated oral dosing at 6- and 8-hr intervals were performed for 75-, 100-, and 200-mg/kg doses (Table 2). The plasma concentration reached steady state within 24 hr. When administered at 100 mg/kg every 6–8 hr, the

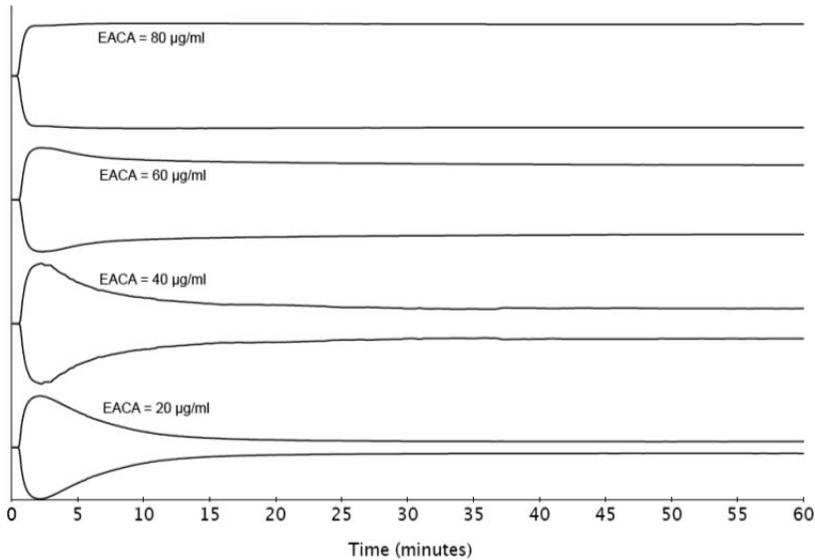


Figure 2. Thromboelastograph tracings of pooled plasma from Northern elephant seals, modified with 5,000 U/ml tPA and with increasing concentrations of ϵ -aminocaproic acid.

predicted plasma EACA concentration would be within the therapeutic range for only 22.5–31.7% of a 24-hr period (5.4–7.6 hr; Table 2). A dose of 200 mg/kg given q. 6–8 hr was predicted to have greater exposure (AUC) above the target 85 μ g/ml but would still result in therapeutic concentrations for only 50% of a 24-hr treatment period.

DISCUSSION

ϵ -Aminocaproic acid is a potentially useful therapy for controlling hemorrhage in NES affected by prepatent *Otostrongylus* arteritis. It is not a holistic therapy, and further symptomatic and specific treatments are required to support seals with this disorder. Treatment of the underlying worm burden with fenbendazole or ivermectin has anecdotally resulted in peracute death, hypothesized to be secondary to massive release of worm antigen, as reported in cases of *Dirofilaria* in dogs and cats.³ Because of these early cases, treatment at TMMC currently focuses on managing the arteritis syndrome with gavage feeding, subcutaneous fluids, and treating secondary bacterial infections rather than addressing the worm burden. Ultimately, a reassessment of the use of antiparasitic agents may be warranted.

ϵ -Aminocaproic acid may not be an appropriate lone therapy in all cases of *Otostrongylus* arteritis or other causes of uncontrolled hemorrhage in seals. Antifibrinolytics remain contraindicated in seals without frank hemorrhage that are suspected of nonovert or chronic DIC and should be

reserved for seals with clinical signs of bleeding or evidence of hypocoagulability. Additionally, EACA would be contraindicated in seals suspected of the acute, hypercoagulable phase of DIC. The use of antifibrinolytics in people with early, acute DIC is generally not recommended due to a hypercoagulable state and inappropriate fibrin deposition.¹⁹ However, as DIC progresses to the hypocoagulable state with clinical bleeding, use of antifibrinolytics may have a role. A recent survey of members of the International Society on Thrombosis and Haemostasis showed that 22% of respondents consider antifibrinolytic therapy in patients in the hypocoagulable stage of DIC who are considered at risk of bleeding.⁶ This option may be especially relevant in seals with clinical signs of hemorrhage for which more traditional therapies, such as fresh frozen plasma or whole blood transfusions, are not possible. This limitation may be common in zoo or wildlife rehabilitation settings due to difficulties maintaining an indwelling intravenous catheter, lack of eligible donors, or financial and personnel constraints.

Using an in vitro model of hyperfibrinolysis in NES, this study found that an EACA plasma concentration of 85 μ g/ml was required to effectively inhibit fibrinolysis. This concentration is markedly below the therapeutic concentration for humans (130 μ g/ml)^{22,25} and dogs (512 μ g/ml)¹⁰ and markedly greater than that estimated for horses (5.82 μ g/ml).¹¹ In addition, while only 1,000 U/ml of tPA were required to induce near

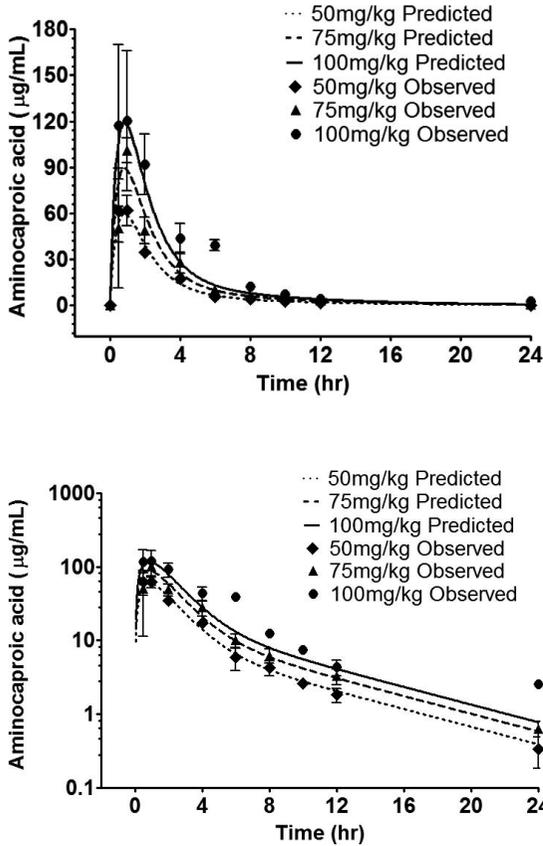


Figure 3. Two compartmental pharmacokinetic modeling of orally administered ε-aminocaproic acid in Northern elephant seals. Data are presented as mean plasma concentration ± SD; solid lines indicate model fits. Top panel is represented on a linear axis, and bottom panel is represented on a semilogarithmic axis.

Table 1. Pharmacokinetic parameters following single-dose, orally administered ε-aminocaproic acid (50, 75, or 100 mg/kg) in Northern elephant seals.^a

Parameters (unit)	Dose (mg/kg)		
	50	75	100
t _{max} (hr)	1.00	1.00	1.00
C _{max} (µg/ml)	61.9	101	120
k (hr ⁻¹)	0.164	0.152	0.125
t _{1/2} (hr)	4.22	4.55	5.53
AUCinf (hr·µg/kg/ml)	205	303	557
Vd/F (ml)	1,480	1,630	1,440
CL/F (ml/hr)	244	248	181

^a t_{max} indicates time to maximum plasma concentration; C_{max}, maximum plasma concentration; k, elimination rate; t_{1/2}, half-life; AUCinf, area under the curve; Vd/F; CL/F, plasma clearance.

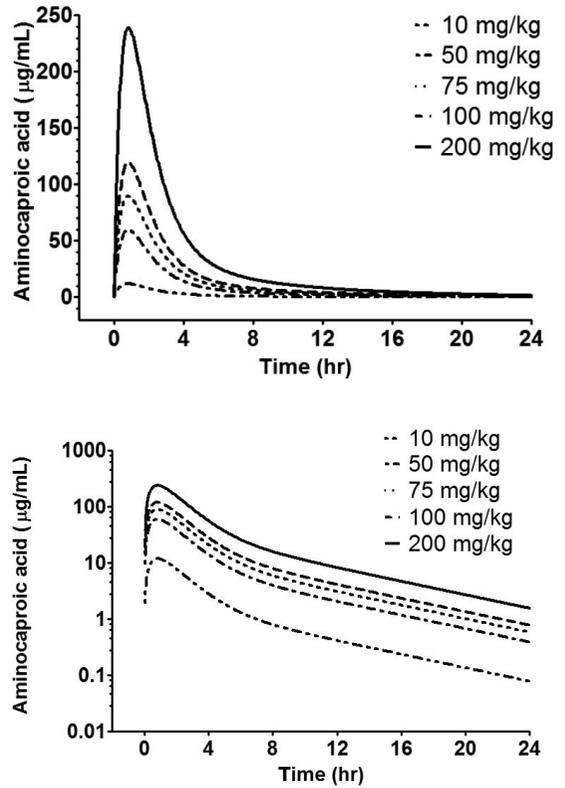


Figure 4. Single-dose pharmacokinetic simulation (10, 50, 75, 100, and 200 mg/kg) of orally administered ε-aminocaproic acid in Northern elephant seals.

complete clot lysis in human pooled plasma within 30 min, five times that concentration was required to achieve the same degree of fibrinolysis in pooled NES plasma. Although the endogenous activity of pro- and antifibrinolytic mediators in NES is unknown, from these data we hypothesize a tendency toward relative hypofibrinolysis compared to humans and dogs and relative hyperfibrinolysis compared to horses.

The NES plasma failed to reliably clot in the absence of standard TEG activators but did clot reliably using the RapidTEG activator, which contains a combination of TF, kaolin, and phospholipids. This suggests that NES likely have a tendency toward hypocoagulability compared to other species. We also found an unacceptable degree of intra-assay variation with TF-activated TEG, leading to the use of the more potent RapidTEG activator. Nonactivated TEGs are reported to have higher intra-assay variation than activated TEG,⁵ suggesting that inadequate activation of the NES samples by TF alone may have contributed to intra-assay variation seen during

Table 2. Pharmacokinetic simulation parameters for multiple-dose orally administered ϵ -aminocaproic acid in Northern elephant seals.

	Dose (mg/kg)											
	75				100				200			
	Time above 85 per dose (hr)	Time above 85 per day (hr)	AUCs ^a above 85 per day (hr· μ g/kg/ml)	Time above 85 per dose (hr)	Time above 85 per day (hr)	AUCs above 85 per day (hr· μ g/kg/ml)	Time above 85 per dose (hr)	Time above 85 per day (hr)	AUCs above 85 per day (hr· μ g/kg/ml)	Time above 85 per dose (hr)	Time above 85 per day (hr)	AUCs above 85 per day (hr· μ g/kg/ml)
q. 6 hr	1.1	4.4	33.6	1.9	7.6	188	3.7	14.8	1,344	3.7	14.8	1,344
q. 8 hr	0.9	2.7	14.1	1.8	5.4	111	3.5	10.5	873	3.5	10.5	873
q. 12 hr	0.8	1.6	4.66	1.6	3.2	59.4	3.2	6.4	506	3.2	6.4	506
q. 24 hr	0.4	0.4	0.772	1.5	1.5	25.0	3.0	3.0	232	3.0	3.0	232

^a AUC_{ss} indicates area under the curve at steady state.

the pilot trials. RapidTEG appears to be a more suitable activator for NES plasma.

The TEG assay used in this study was run using frozen plasma and does not account for the role of platelets, blood cells, and the vascular endothelium in clot formation and stability. However, we hypothesize that studies of whole blood TEG in NES will also result in a relatively hypocoagulable and hypofibrinolytic profile compared to humans and dogs, as previous research of platelets of NES is consistent with our hypothesis of global hypocoagulability. NES platelets in citrated platelet-rich plasma were less sensitive to activation by collagen, ADP, epinephrine, and thrombin as well as more resistant to activation due to atmospheric pressure decompression compared to human platelets.^{8,9}

Pharmacokinetic analysis of oral single-dose EACA (50, 75, and 100 mg/kg) showed a high terminal elimination rate, large volume of distribution, and rapid clearance, resulting in limited duration of plasma concentrations above the drug concentration found efficacious in vitro. In horses, intravenous continuous-rate infusions of EACA have been proposed as the most effective delivery method to maintain therapeutic concentrations.²³ While intravenous administration was not investigated in this study, our data suggest that a continuous-rate infusion may also be the most effective way to deliver EACA to NES. Two alternatives to improve drug exposure would be increasing oral dose quantity or decreasing the dosing interval.

Simulations using two-compartment modeling were used to investigate the effect of alternative oral dose quantities and intervals. Models of repeated oral dosing showed that even at steady-state concentrations, a dose of 100 mg/kg administered every 6 hr would remain in the therapeutic range for only 1.9 hr after administration (Table 2), with resulting therapeutic effects expected for only ~30% of a daily treatment period. Modeling of more frequent dosing, such as q. 2 or q. 4 hr, was not performed, as these dosing schedules were considered impractical in a clinical setting. Alternatively, models of higher dose quantity (200 mg/kg) did show improved exposure at steady state, suggesting that higher oral dosage (200 mg/kg or greater) may be an option for sustained therapeutic effects of oral EACA. However, the safety of EACA in NES at these higher doses has not been investigated, and no seal was administered 200 mg/kg or higher as part of this study. Because EACA binds to a lysine-binding site on plasminogen, preventing conversion to plasmin, it

is likely that the effect of the drug persists for some time after plasma concentrations have dropped, until EACA is displaced from the binding site. Further research is required to fully describe the pharmacodynamics of EACA in NES, including the duration of antifibrinolytic activity following drug administration. TEG analysis of serial plasma samples following EACA administration would likely answer this question and determine if less frequent dosing might still be sufficient to have an antifibrinolytic effect. Further research is also required to assess the clinical effects of the drug in seals affected with *Otostrongylus* arteritis and other bleeding disorders. A prospective, placebo-controlled clinical trial is needed to determine if the dosing regimens investigated in this study would be effective in decreasing bleeding in NES.

CONCLUSIONS

Antifibrinolytic drugs such as EACA are widely used in human medicine to control bleeding in a variety of clinical situations. Safe use in veterinary species requires knowledge of appropriate dosage and recognition of appropriate clinical situations. Antifibrinolytics are contraindicated in most cases of DIC, and early, hypercoagulable DIC should be ruled out prior to use. Importantly, the great variation in therapeutic plasma concentrations established for humans, dogs, and horses illustrates the need for species-specific dosing strategies for EACA in veterinary patients. The TEG-based assay described in this article provides a noninvasive method of estimating required therapeutic plasma concentrations in novel species. In NES plasma, inhibition of severe fibrinolysis *in vitro* is achieved at an EACA concentration of 85 $\mu\text{g}/\text{ml}$. Pharmacokinetic modeling of orally administered, single-dose EACA and simulations of repeated-dose EACA show that doses of 75 and 100 mg/kg achieve therapeutic concentration but for limited duration. Further studies are needed to determine the *in vivo* duration of action of EACA and to investigate the safety of higher oral doses that may provide improved systemic exposure. Until such studies are available, a dose of 100 mg/kg given every 6–8 hr can be expected to result in plasma concentrations of EACA at or above therapeutic concentrations for 22.5–31.7% of a 24-hr treatment period. A clinical trial is warranted to determine if this dosing regimen has the potential to limit bleeding in NES with bleeding disorders, and further *in vitro* investigation of the

mechanism of hemorrhage in cases of *Otostrongylus* arteritis would be valuable.

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