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INTERSTITIAL PLASMIN ACTIVITY WITH EPSILON AMINOCAPROIC ACID: TEMPORAL AND REGIONAL HETEROGENEITY

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Abstract

Background—Epsilon aminocaproic acid (EACA) is used in cardiac surgery to modulate plasmin activity (PLact). The present study developed a fluorogenic-microdialysis system to measure *in-vivo* region specific temporal changes in PLact following EACA administration.

Methods—Pigs (25-35kg) received EACA (75mg/kg, n=7) or saline in which microdialysis probes were placed in the liver, myocardium, kidney and quadricep muscle. The microdialysate contained a plasmin specific fluorogenic peptide and fluorescence emission, which directly reflected PLact, determined at baseline, 30, 60, 90 and 120 minutes following EACA/vehicle infusion.

Results—EACA caused significant decreases in liver and quadricep PLact at 60, 90, 120 minutes and at 30, 60, 120 minutes respectively ($p<0.05$). In contrast, EACA induced significant biphasic changes in heart and kidney PLact profiles with initial increases followed by decreases at 90 and 120 minutes ($p<0.05$). The peak EACA interstitial concentrations for all compartments occurred at 30 minutes post infusion, and were 5-fold higher in the renal compartment and 4-fold higher in the myocardium, when compared to the liver or muscle ($p<0.05$).

Conclusions—Using a large animal model and *in-vivo* microdialysis measurements of plasmin activity, the unique findings from this study were 2-fold. First, EACA induced temporally distinct plasmin activity profiles within the plasma and interstitial compartments. Second, EACA caused region specific changes in plasmin activity profiles. These temporal and regional heterogeneous effects of EACA may have important therapeutic considerations when managing fibrinolysis in the perioperative period.

Introduction

Excessive perioperative bleeding is a major complication associated with cardiovascular surgery. The hemostatic goals associated with cardiovascular surgery, particularly in the context of cardiopulmonary bypass, requires a highly dynamic and sensitive balance of the coagulation and fibrinolytic states of the patient in the perioperative period. Antifibrinolytics,

which inhibit the fibrin degradation effects of plasmin, have been the pharmacological mainstay with proven efficacy in reducing blood loss and blood product transfusion requirements related to cardiovascular surgery. ^{1, 2, 3} However, such strategies to modulate fibrinolysis can be associated with significant adverse outcomes (thrombosis, acute renal injury and myopathies) ^{4,5,6,7,8,9,10,11,12} which may be due to differences in dosing regimens, off-target effects, dynamic coagulation/fibrinolytic states, temporal alterations in organ function and patient specific comorbidities. ^{13,14,15,16} Moreover, antifibrinolytics are often utilized in an empirical dosing fashion and have been associated with alterations in hepatic, renal and myocardial function. ^{5,17,18} However, no systematic study has been undertaken to determine the regional pharmacological effects following antifibrinolytic administration.

Common clinically utilized antifibrinolytics affect plasmin activity (PLact) primarily by inhibiting the enzymatic interaction of plasminogen and plasmin with fibrinogen and fibrin. Antifibrinolytics can be classified as either serine protease inhibitors or lysine analogues. ¹⁹ The serine protease inhibitor aprotinin significantly inhibits fibrinolysis, although this drug has been removed from clinical use. As a consequence, lysine analogues, such as epsilon aminocaproic acid (EACA), have now become the major class of pharmacological intervention in which antifibrinolytic therapy is indicated and has likely resulted in an increase use of EACA for this purpose. However, the basic regional and temporal PLact profiles following EACA administration remain unexplored. Such profiles may provide insight into dosing optimization to minimize adverse effects and maximize intended benefits. Consequently, the primary goal of this study was to characterize the effects of EACA on the regional and temporal PLact profiles in plasma and selected tissue compartments.

In order to explore the regional dynamics of PLact, a large animal model utilizing established microdialysis techniques was employed. ^{20,21} Such microdialysis techniques, utilizing a fluorogenic substrate, allowed the detection of interstitial enzymatic activity, such as plasmin. ²² Accordingly, the objectives of this study were two-fold. One, the validation and calibration of a fluorogenic peptide that could be used to assess PLact *in-vivo*. Second, the development of a porcine model to measure PLact in plasma and interstitial regions of clinical relevance utilizing this validated fluorogenic approach.

Methods

The present study was conducted in two stages. First, *in-vitro* validation studies were performed to develop a PLact measurement system using a plasmin specific fluorogenic substrate. ²² This validated PLact measurement system was utilized to perform *in-vivo* PLact measurements, via microdialysis probes, within targeted regions. Then, EACA was infused intravenously and PLact was continuously monitored within these regions. Finally, plasma and interstitial EACA concentrations were measured.

In-vitro Validations

Several *in-vitro* validation studies were performed using a plasmin specific fluorogenic substrate ²² (Sigma-Aldrich, Cat. # A8171). In particular, this substrate contained a validated fluorogenic peptide which, when specifically cleaved by plasmin, yielded a coumarin fluorescent moiety with excitation/emission wavelengths of 365/440nm respectively. ²² The first *in-vitro* validation study determined the response of the fluorogenic substrate to increasing concentrations of plasmin in a solution of reference normal porcine plasma. Briefly, 6.0 μ M of plasmin substrate was injected into a 96-well polystyrene plate (Nalge Nunc) with increasing concentrations of plasmin (0-125 μ g/mL, Sigma-Aldrich, Cat #P1867) and diluted control porcine plasma (1:32). Following 5 minute incubation at 37°C, the plate was placed into a fluorescence microplate reader (FLUOstar Galaxy, BMG LABTECH Inc) and the fluorescence

emission was recorded. Fluorescence emission, reflective of PLact, increased with increasing concentrations of plasmin (Figure 1A).

Next, a series of *in-vitro* experiments were performed using a solution of reference normal porcine plasma which determined the EACA plasma concentration inhibition curve. Specifically, plasmin substrate (6 μ M), plasmin (62.5 μ g/mL) and diluted control porcine plasma (1:32) were incubated with increasing concentrations of EACA (0-62.5 mg/mL, Hospira, Inc, Lake Forest, IL) and subjected to the same fluorescence measurement procedure previously described. In Figure 1B, the fluorescence emission, reflective of PLact, decreased in response to increasing concentrations of EACA in a classic, logarithmic, concentration-dependent manner. A logarithmic equation was matched to this data using regression analysis.

In addition, a series of studies were performed to determine the comparative response of the plasmin specific fluorogenic substrate in dialysate solution to varying concentrations of plasmin and EACA. Specifically, a phosphate buffered dialysate solution (KCl 2.68mM, KH_2PO_4 1.47mM, NaCl 136.89 mM, Na_2HPO_4 8.10 mM, MP Biomedicals, Solon OH, Cat # 2810305) of plasmin substrate (6.0 μ M) and plasmin (0.98-7.81 μ g/mL) were incubated alone and with two concentrations of EACA (5 μ g/mL and 10 μ g/mL) respectively. These solutions were then subjected to the same fluorescence measurement procedure previously described. The fluorescence emission for this series of experiments is shown in Figure 1C. This series of *in-vitro* experiments demonstrated the specificity and sensitivity of the fluorogenic substrate in dialysate solution to plasmin, over a physiological range of concentrations, and the inhibiting effects by EACA.

Therefore, these *in-vitro* studies established the optimal substrate concentration, demonstrated specificity of the substrate for plasmin, determined the fluorescence emission inhibition curve for EACA in porcine plasma and established the response of the measurement system in a simulated interstitial dialysis environment. The development of this PLact measurement system was then translated to the *in-vivo* PLact studies described below.

Animal and Surgical Preparation

Yorkshire pigs (n= 15, castrated male, 25-35 kg, Hambone Farms, Reevesville, SC) were instrumented to measure plasma and interstitial PLact. All animals were treated and cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Institutes of Health, 1996). Approval of all animal care and use protocols was obtained from the Medical University of South Carolina Institutional Animal Care and Use Committee, AR# 2786.

After sedation with diazepam (100 mg po, Elkins-Sinn Inc (ESI), Cherry Hill, NJ), general inhalational anesthesia was induced using isoflurane (3%, Baxter Healthcare Corp., Deerfield, IL) mixed with oxygen and nitrous oxide (67:33%) and peripheral intravenous access obtained. A stable surgical plane of anesthesia was established and maintained throughout the protocol using sufentanil (2 μ g/kg IV, ESI), etomidate (0.1 mg/kg IV, ESI), vecuronium (10mg IV bolus, 0.5 mg/kg/hr IV infusion, Ben Venue Laboratories Inc, Bedford, OH), morphine sulfate (3 mg/kg/hr IV, ESI) and isoflurane (1%, Baxter Healthcare Corp.) Tracheal intubation was achieved via tracheostomy and mechanical ventilation established (Narkomed 2B, North American Drager, Telford, PA). Intravenous fluids (lactated Ringer's) were administered per established weight based protocols for maintenance fluids and estimated blood loss replacement. A single lumen catheter (8 Fr) was placed into the right external jugular vein for fluid and drug administration. An arterial line catheter (7 Fr) was placed into the right carotid artery to continuously monitor systemic blood pressures and obtain blood samples. Following a 60 minute baseline and stabilization period, each pig was randomly assigned to receive EACA

(Hospira, Inc, Lake Forest, IL) (75 mg/kg, diluted into normal saline, total volume = 50mL) or vehicle (50mL normal saline) over a 10 minute period.

Microdialysis Techniques

Microdialysis probes (CMA/Microdialysis, North Chelmsford, MA) with a molecular weight cutoff of 20 kDa and an outer diameter of 0.5 mm were surgically placed interstitially in the anterior myocardium of the left ventricle, right lobe of the liver, lower pole of the right kidney and left quadricep muscle compartments. Placement of the microdialysis probes required a median sternotomy, a subxyphoid intra-abdominal incision, a subcostal flank incision and a medial mid-thigh incision with associated tissue dissections respectively.

The microdialysis probes were connected to precision infusion pumps and controller system (BASi, West Lafayette, IN). A flow rate of 6.0 μ L/min was established and an iso-osmotic dialysis performed. Dialysate was infused for 30 minutes to allow for equilibration with each of the respective tissue compartments. The microdialysate infusion contained the validated fluorogenic peptide (Sigma A-8171, 10 μ M). Preliminary studies demonstrated this microdialysate concentration yielded a steady state fluorescence emission within 30 minutes of the initiation of dialysis, indicative of equilibration with the interstitial space of the target tissue. The fluorescence emission of the interstitial fluid collected from each of the microdialysis probes, which directly reflected PLact, was determined at steady-state baseline, 30, 60, 90 and 120 minutes following EACA/vehicle infusion, using fluorescence measurement techniques previously described.

Plasma Sampling

Arterial blood samples (50 mL) were collected at the beginning of two 30 minute baseline time intervals. The plasma from these blood samples was used to develop a reference normal porcine plasma solution for *in-vitro* validations previously described. At baselines and at 30 minute intervals throughout the protocol, coinciding with the microdialysis samples, arterial blood samples (10 mL) were collected. All blood samples were collected in heparinized tubes, centrifuged and the plasma decanted and frozen for subsequent measurement of PLact using the previously described fluorescence measurement system. In addition, EACA plasma concentrations were determined as described below.

Plasma and Interstitial EACA Concentration Measurements

An Acquity UPLC coupled to a Quattro Premier XE mass spectrometer (Waters, Milford, MA) was used to measure plasma and interstitial EACA concentrations. Chromatographic separation was performed on an Acquity UPLC HSS C18 2.1 $\times$ 100mm (1.8 μ m) column preceded by an Acquity UPLC HSS C18 (1.8 μ m) pre-column. Samples were acidified with 100 μ L sulfosalicylic acid (4.3 % v/v) and eluted isocratically over 5 minutes. The mobile phase consisted of 10% acetonitrile in 2mM ammonium acetate (pH 3.5) with a flow rate of 0.15 mL/min. The mass spectrometer was operated in positive ion mode with capillary voltage 3.1 kV, source temperature 120°C, desolvation temperature 400°C and nitrogen gas flow at 700 L/Hr. Data acquisition was performed using MassLynx 4.1 and quantification using QuanLynx 4.1 (Waters, Milford, MA). EACA plasma and interstitial dialysate concentrations were determined from precalibrated EACA standards (0.5-100 μ g/mL and 1.25-40 μ g/mL respectively) with Methyl-L-DOPA as internal standards.

Data Analysis

Comparisons for net change in fluorescence for all time points post infusion within each region were made using an analysis of variance (ANOVA) followed by pairwise tests of individual time points means using Bonferroni bounds. Two-sample t-tests were performed on the

computed net change in fluorescence compared to baseline for all time points within each region. Comparisons for area under the curve (AUC) and peak interstitial EACA concentrations post infusion within each region were made using ANOVA. All statistical procedures were performed using STATA statistical software (Intercooled STATA 8.0). Results are presented as mean $\pm$ SEM with p values <0.05 considered to be statistically significant.

Results

Using a validated fluorogenic plasmin substrate with established microdialysis techniques, the fluorescence emission of the interstitial fluid collected from each tissue compartment, was determined at steady-state baseline, 30, 60, 90 and 120 minutes following EACA/vehicle infusion. The fluorescence emission of the plasma was determined at the same time points. The relative fluorescence emission is reflective of change in PLact induced by the administration of EACA. Therefore, in order to directly examine the effects of EACA on PLact, the absolute fluorescence emission values were transformed to yield a net change in mean fluorescence emission with respect to time matched mean vehicle values for each of the selected compartments at the specified times intervals (Figure 2). Thus, the values plotted in Figure 2 are the differences between the mean EACA and mean vehicle fluorescence emission of the respective tissue compartments at the specified time points. With respect to vehicle values, EACA did not significantly alter plasma PLact at any of the specified time intervals. However, in the interstitial compartments, temporal and regional differences in PLact were observed following EACA administration. Specifically, there was a significant decrease in liver PLact at 60, 90 and 120 minutes. The nadir in liver PLact at 90 minutes occurred 30 minutes after the maximal decline in plasma PLact. In contrast, EACA induced biphasic changes in the heart and kidney PLact profiles with significant increases at 60 and 30 followed by significant decreases at 90 and 120 minutes respectively. There was also an overall progressive reduction in quadricep PLact with significant decreases at 30, 60 and 120 minutes.

The EACA interstitial concentrations for each tissue compartment at baseline, 30, 60, 90 and 120 minutes post EACA infusion are shown in Figure 3. The peak EACA interstitial concentrations (peak-EACA) for all tissue compartments occurred at 30 minutes post EACA infusion with a general decline in concentration in a time dependent manner. Moreover, the peak-EACA interstitial values for the kidney and heart were significantly higher when compared to the liver or quadricep. There were also significant differences in the area under the EACA-time concentration curves (AUC-EACA) for the heart and kidney with respect to liver or quadricep compartments.

The EACA plasma concentrations for time intervals 30, 60, 90 and 120 minutes post EACA infusion are shown in Figure 4. The peak EACA plasma concentration occurred at 30 minutes post EACA infusion and subsequently decreased in a negative logarithmic time dependent manner consistent with first order elimination pharmacokinetics.

Discussion

Perioperative hemorrhage constitutes an important risk factor for morbidity and mortality in most major surgical procedures; notably cardiovascular surgery.^{4,23,24,25} Accordingly, blood product transfusions, coagulation factor delivery and pharmacological strategies targeted at the coagulation/fibrinolytic mechanisms are important clinical maneuvers to reduce blood loss in the perioperative setting.^{1, 2, 3, 23} However such interventional modalities can be associated with untoward outcomes which may be due to diverse dosing regimens, dynamic coagulation/fibrinolytic states, off-target effects, temporal alterations in organ function and genetic polymorphisms.^{4,5,6,7,8,9,10,11,12,13,14,15,16} One commonly utilized antifibrinolytic is epsilon aminocaproic acid (EACA), which can modulate the fibrinolytic pathway by inhibiting

local plasmin activity (PLact).²⁶ However, current EACA dosing schedules are largely empirically derived and, as such, no consensus exists as to appropriate dosing to provide optimal perioperative control of fibrinolysis.^{27,28,29} This lack of established clinical dosing parameters suggests that the modulation of fibrinolysis by EACA may be enhanced by regional and temporal measurements of PLact. Specifically, temporal and regional disparate differences in the effects of EACA on PLact may have a critical role in minimizing adverse effects (end organ injury and dysfunction) and more optimally controlling fibrinolysis. Accordingly, the central hypothesis of this study was that temporal and regional PLact profiles following EACA administration are quantifiable. The present study addressed this issue through the use of a validated fluorogenic-microdialysis approach in a large animal model, in order to provide direct serial measurements of interstitial PLact on a regional basis, following a common standardized dose of EACA.²⁹ The unique finding from this study was that interstitial and plasma PLact is differentially affected following EACA infusion in both a region and time dependent manner. For example, EACA induced temporally distinct PLact profiles within the plasma and selected interstitial compartments such as the heart, the kidney and the liver. These temporal and regional differences in the effects of EACA on PLact may have important therapeutic considerations when managing fibrinolysis in the perioperative period. Particularly, continuous PLact measurements may provide a means to specifically titrate antifibrinolytics, such as EACA, so as to minimize off-target effects and optimally modulate fibrinolysis.

The perioperative hemostatic management of cardiovascular surgery patients, particularly in the context of cardiopulmonary bypass, requires a temporally acute and sensitive balance of their coagulation and fibrinolytic states. Furthermore, the activation of both coagulation and fibrinolysis during cardiopulmonary bypass induces simultaneous and temporal changes in multiple key hemostatic factors some of which are influenced by individual genetic variations.^{13,14} In addition, the prophylactic use of lysine analogue antifibrinolytics during cardiac surgery has the potential to induce a hypercoagulable prethrombotic state.¹⁵ As such, thrombosis (DVT, pulmonary artery, renal pelvic and artery, bladder and cerebral vascular) and myopathies with respective concomitant organ injury and dysfunction have been associated with the use of antifibrinolytics such as EACA.^{4,7,8,9,10,11,12} The primary mechanism of elimination of EACA is via renal excretion. As such, acute temporal alterations in renal function associated with cardiac surgery further compound the complexity of maintaining a safe hemostatic state in clinical scenarios in which EACA is indicated.¹⁶ Thus there are several temporal and regional parameters that must be considered when attempting to balance the extensively dynamic and sensitive coagulation/fibrinolytic state(s) of cardiac surgical patients in the perioperative period. The continuous monitoring PLact may facilitate optimization of antifibrinolytic therapy in such challenging clinical scenarios.

While the pharmacology of EACA has been rigorously described previously with respect to mechanisms of action,²⁶ there have been no studies which have precisely quantified the effects of EACA on interstitial PLact *in-vivo* - the clinically relevant target for EACA with respect to modulating fibrinolysis. Furthermore, while past basic and clinical studies have described the utility of EACA in the context of cardiovascular surgery, such as that associated with cardiopulmonary bypass, optimal dosing strategies remain a subject of debate.^{27,28,29} Tissue plasminogen activator is synthesized and secreted by endothelial cells intravascularly and extravascularly, into the interstitial space, where it catalyzes the conversion of plasminogen to plasmin and thus facilitating fibrinolysis.³⁰ The present study is the first to develop an approach to continuously and directly measure *in-vivo* PLact, the major biological response variable relevant to EACA administration, within the interstitium of critical target tissues as well as the plasma. This study utilized a microdialysis approach to interrogate the interstitial compartment, an approach that has been well described previously in both animal and clinical studies.^{20,21} This microdialysis method was coupled with a fluorogenic substrate specific for plasmin, and therefore provided a means to quantify PLact within the interstitial space. This methodology

may provide a useful analytical approach to assess PLact with varying EACA dosing regimens, and thereby provide a basis for optimal EACA administration.

The present study provided a means by which to measure temporal and regional PLact as a basis to move forward studies aimed at EACA dosing optimization. Moreover, the present study identified differences in PLact following EACA administration in critical target organs such as the liver and kidney, which may hold relevance in the clinical context of hepatic or renal dysfunction.^{4,5,6} For example, following a single bolus dose of EACA transient effects on PLact were observed in the heart and kidney, whereas there were persistent effects in the liver. While this acute study could not address this issue directly, the disparate effects on PLact may in turn affect hepatic and renal function, which the latter has been identified as a potential consequence of antifibrinolytics such as aprotinin.^{4,5,6} Moreover, any significant and unanticipated interstitial accumulation of EACA within the myocardium, kidney or liver may have undesired consequences. The continuous PLact profiling described in the current study may provide a means by which to address these issues and further optimize current and future antifibrinolytic therapies.³¹

The present study utilized EACA to investigate the effects of a commonly used antifibrinolytic agent on plasma and interstitial PLact profiles. The rationale for focusing this study on EACA with respect to PLact profiles was two-fold. First, the objective of this study was to demonstrate the proof of concept that regional and temporal heterogeneity exists with respect to one computed dose of an antifibrinolytic, and EACA was chosen as a prototypical example. Second, the serine protease inhibitor, aprotinin, while historically considered the first line agent for modulating PLact, has been withdrawn from clinical use, thus leaving lysine analogues such as EACA as the pharmacological mainstay for antifibrinolysis. Lysine analogues such as EACA affect PLact primarily by inhibiting the enzymatic interaction of plasminogen and plasmin with fibrinogen and fibrin which is pivotal to the enzymatic induction of fibrinolysis.²⁶ Thus, EACA served as a reasonable first step, with respect to clinical relevance, in determining the fundamental mechanistic underpinnings of the regional and temporal effects of lysine analogues on PLact profiles. It is likely that the regional and temporal PLact heterogeneity observed in the present study can be extrapolated, to some degree, to other fibrinolytic inhibitors (tranexamic acid and CU-2010).³¹

The peak EACA plasma concentrations obtained in the present study are consistent with those reported in prior clinical investigations.^{27,28,29} As such, the EACA dosing regimen used in the present study represents a clinically relevant dosing approach. The EACA plasma elimination profile obtained is congruent with previously reported EACA pharmacokinetics in humans,^{32,33} indicating that the large animal model used in the present study holds pharmacological relevance. Moreover, the occurrence for the peak plasma PLact inhibition following the peak EACA plasma concentration at 30 minutes demonstrates the pharmacological efficacy of the EACA within the vascular compartment. Furthermore, the time of the peak interstitial EACA concentrations for all tissue compartments at 30 minutes post EACA infusion coincided with the occurrence of the peak EACA plasma concentration. Accordingly, the large animal preparation and EACA dosing paradigm utilized in the present study is likely to be a clinically relevant simulation.

Unique to this study were the *in-vivo* serial measurements of regional EACA concentrations following a single computed EACA dose (Figure 3). No previous studies have directly measured serial interstitial EACA concentrations in a clinically relevant large animal model. The EACA interstitial concentrations obtained in the present study demonstrate the penetration of EACA within each of the critical tissue compartments. When compared to the respective PLact profiles, both regional and temporal heterogeneity exists between the pharmacokinetics and pharmacodynamic effects of EACA (Figures 2 and 3). For example, among all of the

interstitial compartments, the kidney had the highest EACA concentration at 30 minutes post infusion which paradoxically corresponded to the greatest interstitial PLact level for that time interval. In contrast, the lowest interstitial EACA concentration occurred in the quadricep compartment at 120 minutes post infusion which corresponded with the maximum inhibition of PLact within that compartment. These regional and temporal disparate differences in the pharmacokinetics and pharmacodynamic effects of EACA underscore the significance of direct *in-vivo* measurements of PLact when attempting to optimally modulate fibrinolysis.

Study Limitations

The primary potential limitations of the current study are two. First, the EACA regimen implemented involved a loading dose only without a subsequent continuous infusion of EACA. Second, the *in-vivo* investigations did not include the context of cardiopulmonary bypass or other heightened fibrinolytic states that are typical clinical conditions under which EACA is utilized. The primary objective of the current study was to quantify the regional and temporal effects of EACA on critical tissue compartment PLact profiles. Accordingly, the EACA regimen involved a loading dose only in order to exam the compartment specific temporal dynamics of EACA on PLact profiles which would have been potentially obscured by the subsequent administration of a continuous infusion of EACA. This initial study was not performed in the context of cardiopulmonary bypass in order to limit the complexity of hemostatic interactions which are significantly altered by requisite system heparinization and cardiopulmonary bypass.¹³ The extension of the current study findings will provide a basis for the pursuit of similar PLact investigations involving a clinically relevant cardiopulmonary bypass model.

Conclusions

The present study provided a proof of concept approach for continuous and regional measurement of PLact which is a fundamental pharmacological target for antifibrinolytic therapy. The present study also demonstrated in a clinically relevant large animal model that regional and temporal heterogeneity in PLact exists following a single computed dose of EACA, a prototypical antifibrinolytic. Although commonly utilized, EACA and similar antifibrinolytics are not FDA approved for routine use to reduce blood loss and blood component transfusions in patients. In addition, the use of antifibrinolytics such as lysine analogues are currently administered using empirical dosing regimens and as a consequence may result in adverse effects in local tissue compartments such as the kidney, heart or liver. Thus, at the current time, antifibrinolytic therapies remain a significant clinical need and the best approach to implementation continues to be controversial.³⁴ The results of the present study set the stage for future investigations whereby this continuous approach to measure interstitial PLact may provide a means to optimize and individualize antifibrinolytic therapy.

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Figure 1.

(A) Fluorescence emission of the plasmin specific substrate (6 µM), reflective of PLact, increased with increasing concentrations of plasmin (0-125 µg/mL, Sigma-Aldrich, Cat #P1867) in diluted control porcine plasma (1:32) in a linear concentration dependent manner (n=3, plotted values are mean±SEM; linear regression, $y(x) = 339.06 \cdot x$, $r^2 = 0.998$, $p < 0.0001$).

(B) Fluorescence emission of the plasmin specific substrate (6 µM), reflective of PLact, in the presence of plasmin (62.5 µg/mL, Sigma-Aldrich, Cat #P1867) and diluted control porcine plasma (1:32) decreased in response to increasing concentrations of EACA (0-62.5 mg/mL) in a classic logarithmic concentration dependent manner (n=3, plotted values are mean±SEM; linear regression, $y(x) = 12.78 + 88.93 \cdot e^{-0.096 \cdot x}$, $r^2 = 0.970$, $p < 0.0001$).

(C) The comparative fluorescence emission response of the plasmin specific substrate (6 µM) in dialysate solution to decreasing concentrations of plasmin (7.81-0.976 µg/mL, Sigma-Aldrich, Cat #P1867) and two fixed concentrations of EACA (5 µg/mL and 10 µg/mL) demonstrates the specificity and sensitivity of the substrate in dialysate solution to plasmin, over a physiological range of concentrations, and the inhibiting effects by EACA (n=3, plotted values are mean± SEM).

Figure 2.

The computed net change in mean fluorescence emission, reflective of changes in PLact, with respect to time matched vehicle values following EACA infusion for selected compartments demonstrates the unique temporal and regional differences in the affects of EACA on PLact (plotted values are mean±SEM, * $p < 0.05$ versus baseline). Specifically, EACA significantly decreased liver PLact at 60, 90 and 120 minutes. Similarly, there was a temporally progressive reduction in quadricep PLact with significant decreases at 30, 60 and 120 minutes. In contrast, EACA induced biphasic changes in the heart and kidney PLact profiles with significant increases at 60 and 30 followed by significant decreases at 90 and 120 minutes respectively. With respect to vehicle values, EACA did not significantly alter plasma PLact at any of the specified time intervals.

Figure 3.

EACA interstitial concentrations determined by high performance liquid chromatography / mass spectrometry techniques obtained at base line (time 0), 30, 60, 90 and 120 minutes post EACA infusion. Peak-EACA interstitial concentrations for all tissue compartments occurred at 30 minutes post EACA infusion with a general decline in values in a time dependent manner. Peak-EACA and AUC-EACA values for the kidney and heart were significantly higher when compared to the liver or quadriceps compartments (plotted values are mean±SEM, $p < 0.05$).

Figure 4.

EACA plasma concentrations determined by high performance liquid chromatography / mass spectrometry techniques obtained at time intervals 30, 60, 90 and 120 minutes post EACA infusion decreased in a negative logarithmic time dependent manner consistent with first order elimination pharmacokinetics (plotted values are mean $\pm$ SEM, regression, $y(x) = 146.90 * e^{-0.067 * x}$, $r^2 = 0.995$, $p = 0.0024$).