Interstitial Plasmin Activity With Epsilon Aminocaproic Acid: Temporal and Regional Heterogeneity

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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 after EACA administration.**

Methods. **Pigs (25 to 35 kg) received EACA (75 mg/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 after EACA/vehicle infusion.**

Results. **Epsilon aminocaproic acid caused significant decreases in liver and quadricep PLact at 60, 90, 120 minutes, and at 30, 60, and 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 after infusion, and were fivefold higher in the renal compartment and fourfold higher in the myocardium, when compared with 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 twofold. 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 heterogeneic effects of EACA may have important therapeutic considerations when managing fibrinolysis in the perioperative period.**

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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 pharmacologic mainstay with proven efficacy in reducing blood loss and blood product transfusion requirements related to cardiovascular surgery [1–3]. However, such strategies to modulate fibrinolysis can be associated with significant adverse outcomes (thrombosis, acute renal injury and myopathies) [4–12], 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 [13–16]. 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 pharmacologic effects after 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 pharmacologic intervention in which antifibrinolytic therapy is indicated and has likely resulted in an increased use of EACA for this purpose. However, the basic regional and temporal PLact profiles after 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.

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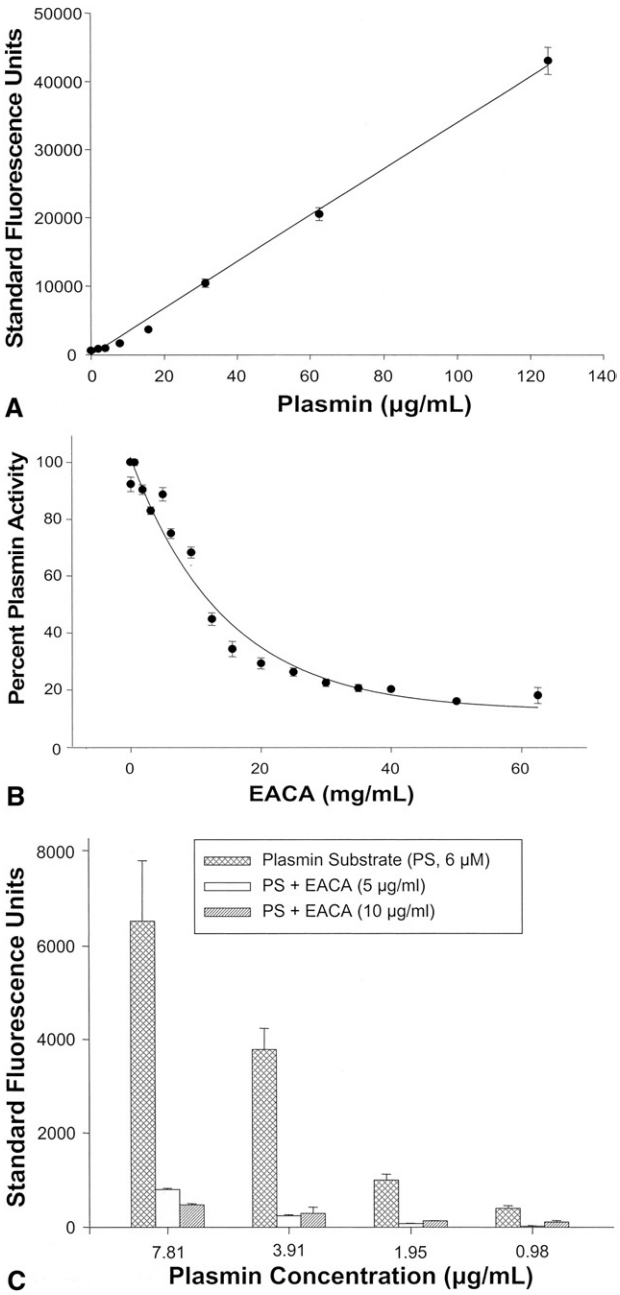
To explore the regional dynamics of PLact, a large-animal model utilizing established microdialysis techniques was employed [20, 21]. Such microdialysis techniques, utilizing a fluorogenic substrate, allowed the detection of interstitial enzymatic activity, such as plasmin [22]. Accordingly, the objectives of this study were twofold: first, 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.



Material and 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 (Cat. # A8171; Sigma-Aldrich, St. Louis, MO) [22]. 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 365/440 nm, 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 μM plasmin substrate was injected into a 96-well polystyrene plate (Nalge Nunc; Thermo Fisher Scientific, Rochester, NY) with increasing concentrations of plasmin (0 to 125 μg/mL [Cat #P1867; Sigma-Aldrich) and diluted control porcine plasma (1:32). After a 5-minute incubation at 37°C, the plate was placed into a fluorescence microplate reader (FLUOstar Galaxy; BMG LABTECH, Offenburg, Germany) and the fluorescence emission was recorded. Fluorescence emission, reflective of PLact, increased with increasing concentrations of plasmin (Fig 1A).

Next, a series of in vitro experiments were performed using a solution of reference normal porcine plasma, which determined the EACA plasma concentration inhi-

Fig 1. (A) Fluorescence emission of the plasmin specific substrate (6 μM), reflective of PLact, increased with increasing concentrations of plasmin (0 to 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, $y(x) = 339.06*x$, $r^2 = 0.998$, $p < 0.0001$). (B) Fluorescence emission of the plasmin specific substrate (6 μM), reflective of PLact, in the presence of plasmin (62.5 μg/mL) and diluted control porcine plasma (1:32) decreased in response to increasing concentrations of epsilon aminocaproic acid (EACA [0 to 62.5 mg/mL]) in a classic logarithmic concentration-dependent manner (n = 3; plotted values are mean ± SEM; linear regression, $y(x) = 12.78 + 88.93*e^{-0.096*x}$, $r^2 = 0.970$, $p < 0.0001$). (C) The comparative fluorescence emission response of the plasmin specific substrate (6 μM [coarse-hatched bars]) in dialysate solution to decreasing concentrations of plasmin (7.81 μg/mL to 0.976 μg/mL) and two fixed concentrations of EACA (5 μg/mL [gray bars] and 10 μg/mL [fine-hatched bars]) demonstrates the specificity and sensitivity of the substrate in dialysate solution to plasmin, over a physiologic range of concentrations, and the inhibiting effects by EACA (n = 3; plotted values are mean ± SEM).

bition curve. Specifically, plasmin substrate (6 μM), plasmin (62.5 μg/mL), and diluted control porcine plasma (1:32) were incubated with increasing concentrations of EACA (0 to 62.5 mg/mL [Hospira, 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 was 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 2.68 mM, KH_2PO_4 1.47 mM, NaCl 136.89 mM, Na_2HPO_4 8.10 mM [Cat # 2810305; MP Biomedicals, Solon, OH]) of plasmin substrate (6.0 μM) and plasmin (0.98 to 7.81 μg/mL) were incubated alone and with two concentrations of EACA (5 μg/mL and 10 μg/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 physiologic 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 males, 25 to 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 orally [Elkins-Sinn Inc (ESI), Cherry Hill, NJ]), general inhalational anesthesia was induced using isoflurane (3% [Baxter Healthcare, 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 μg/kg intravenously (IV [ESI]); etomidate, 0.1 mg/kg IV (ESI); vecuronium, 10 mg IV bolus, 0.5 mg/kg/hr IV infusion (Ben Venue Laboratories, Bedford, OH); morphine sulfate, 3 $mg \cdot kg^{-1} \cdot h^{-1}$ IV (ESI); and isoflurane (1% [Baxter Healthcare]) Tracheal intubation was achieved through tracheostomy, and mechanical ventilation established (Narkomed 2B; North American Drager, Telford, PA). Intravenous fluids (lactated Ringer's) were administered according to established weight-based protocols for maintenance fluids and estimated blood loss replacement. A single-lumen catheter (8F) was placed into the right external jugular vein for fluid and drug administration. An arterial line catheter (7F) was placed into the right carotid artery to continuously monitor systemic blood pressures and obtain blood samples. After a 60-minute baseline and stabilization period, each pig was randomly assigned to receive EACA (Hospira), 75 mg/kg, diluted into normal saline (total volume = 50 mL) or vehicle (50 mL 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 midthigh 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 μL/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 μM. 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 after 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 × 100 mm (1.8 μm) column preceded by an Acquity UPLC HSS C18 (1.8 μm) precolumn. Samples were acidified with 100 μL sulfosalicylic acid (4.3% v/v) and eluted isocratically over 5 minutes. The mobile phase consisted of 10% acetonitrile in 2 mM 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/h. Data acquisition was performed using MassLynx 4.1 and quantification using QuanLynx 4.1 (Waters). The EACA plasma and interstitial dialysate concentrations were determined from precalibrated EACA standards (0.5 to 100 μg/mL and 1.25 to 40 μg/mL, respectively) with methyl-L-dopa as internal standards.

Data Analysis

Comparisons for net change in fluorescence for all time points after infusion within each region were made using an analysis of variance (ANOVA) followed by pair-wise tests of individual time points means using Bonferroni bounds. Two-sample *t* tests were performed on the computed net change in fluorescence compared with baseline for all time points within each region. Comparisons for area under the curve (AUC) and peak interstitial EACA concentrations after infusion within each region were made using ANOVA. All statistical procedures were performed using STATA statistical software (Intercooled STATA 8.0; STATA Corp, College Station, TX). Results are presented as mean ± SEM, with *p* values less than 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 and 30, 60, 90, and 120 minutes after 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, 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 (Fig 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 after 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 signif-

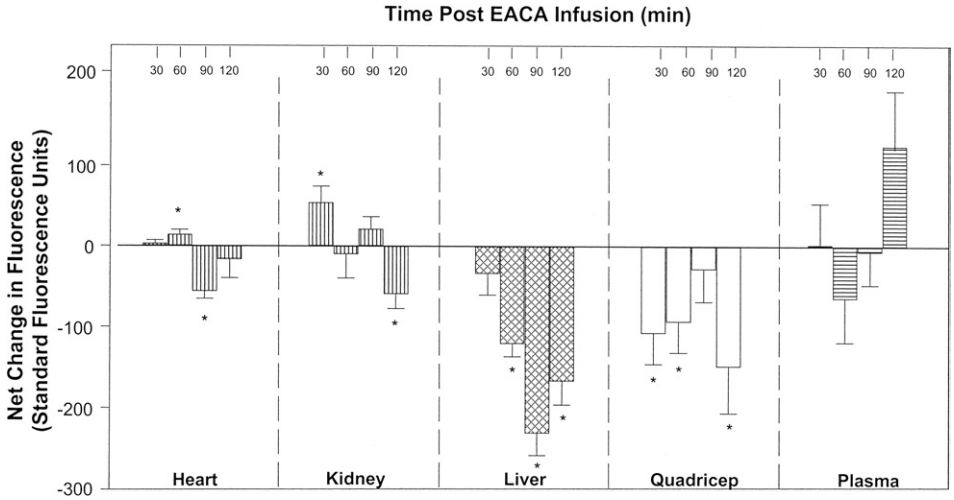


*Fig 2. The computed net change in mean fluorescence emission, reflective of changes in PLact, with respect to time-matched vehicle values after epsilon aminocaproic acid (EACA) infusion for selected compartments demonstrates the unique temporal and regional differences in the affects of EACA on PLact (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.*

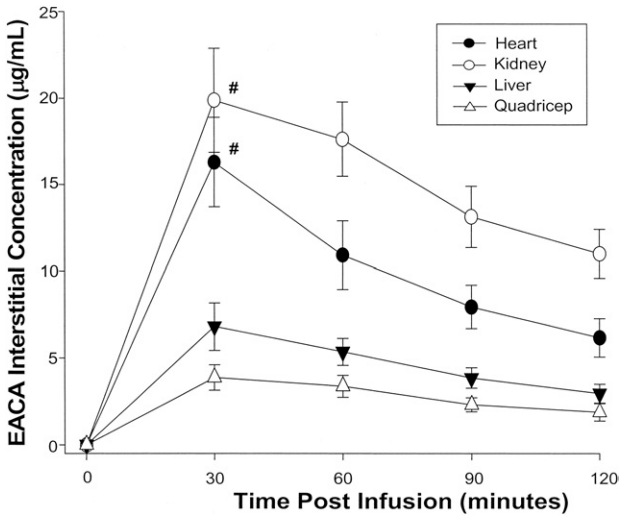


Fig 3. Epsilon aminocaproic acid (EACA) interstitial concentrations were determined by high-performance liquid chromatography/mass spectrometry techniques obtained at baseline (time 0), 30, 60, 90, and 120 minutes after EACA infusion. Peak EACA interstitial concentrations for all tissue compartments occurred at 30 minutes after EACA infusion, with a general decline in values in a time-dependent manner. Peak EACA and AUC EACA values for the kidney (open circles) and heart (solid circles) were significantly higher when compared with the liver (solid triangles) or quadricep (open triangles) compartments (plotted values are mean ± SEM; $p < 0.05$).

icant 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 after EACA infusion are shown in Figure 3. The peak EACA interstitial concentrations for all tissue compartments occurred at 30 minutes after 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 with 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 after EACA infusion are shown in Figure 4. The peak EACA plasma concentration occurred at 30 minutes after EACA infusion and subsequently decreased in a negative logarithmic time-dependent manner consistent with first-order elimination pharmacokinetics.

Comment

Perioperative hemorrhage constitutes an important risk factor for morbidity and mortality in most major surgical procedures, notably cardiovascular surgery [4, 23–25]. Accordingly, blood product transfusions, coagulation factor delivery, and pharmacologic strategies targeted at the coagulation/fibrinolytic mechanisms are important clinical maneuvers to reduce blood loss in the perioperative setting [1–3, 23]. However, such interventional modalities can be associated with untoward outcomes that may be due to diverse dosing regimens, dynamic coagulation/fibrinolytic states, off-target effects, temporal alterations in organ function, and genetic polymorphisms [4–16]. One commonly utilized antifibrinolytic is EACA, which can modulate the fibrinolytic pathway by inhibiting local 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 [27–29]. This lack of established clinical dosing guidelines 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 after EACA administration are quantifiable. The present study addressed this issue through the use of a validated fluorogenic-microdialysis approach in a large animal model to provide direct serial measurements of interstitial PLact on a regional basis, after a common standardized dose of EACA [29]. The unique finding from this study was that interstitial and plasma PLact is differentially affected after EACA infusion in both a region- and time-dependent manner. For example, EACA induced temporally distinct PLact profiles within the plasma and selected interstitial

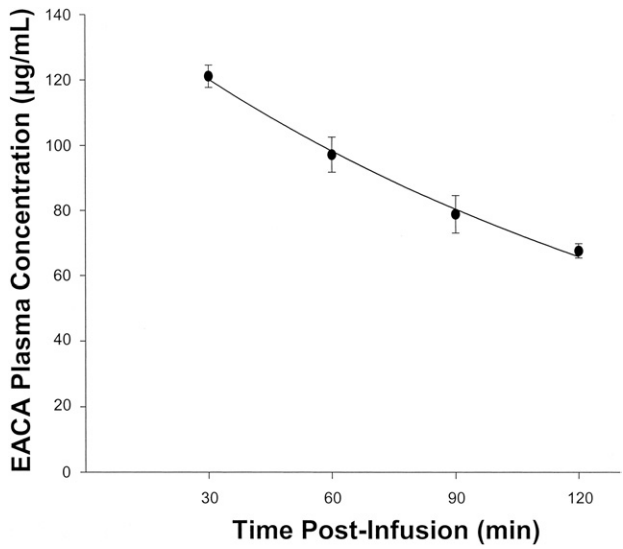


Fig 4. Epsilon aminocaproic acid (EACA) plasma concentrations determined by high-performance liquid chromatography/mass spectrometry techniques obtained at time intervals 30, 60, 90, and 120 minutes after EACA infusion decreased in a negative logarithmic time-dependent manner consistent with first-order elimination pharmacokinetics (plotted values are mean ± SEM; regression, $y(x) = 146.90*e^{-0.067*x}$, $r^2 = 0.995$, $p = 0.0024$).

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 (deep vein, 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, 7–12]. The primary mechanism of elimination of EACA is 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 variables that must be considered when attempting to balance the extensively dynamic and sensitive coagulation/fibrinolytic states of cardiac surgical patients in the perioperative period. The continuous monitoring of 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 that 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 [27–29]. 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 has developed 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 after 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 [4–6]. For example, after 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. Although this acute study could not address this issue directly, the disparate effects on PLact may in turn affect hepatic and renal function, the latter of which has been identified as a potential consequence of antifibrinolytics such as aprotinin [4–6]. 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 twofold. 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 pharmacologic 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 [27–29]. 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 pharmacologic relevance. Moreover, the occur-

rence for the peak plasma PLact inhibition after the peak EACA plasma concentration at 30 minutes demonstrates the pharmacologic efficacy of the EACA within the vascular compartment. Furthermore, the time of the peak interstitial EACA concentrations for all tissue compartments at 30 minutes after 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 after a single computed EACA dose (Fig 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 with the respective PLact profiles, both regional and temporal heterogeneity exists between the pharmacokinetics and pharmacodynamic effects of EACA (Figs 2 and 3). For example, among all of the interstitial compartments, the kidney had the highest EACA concentration at 30 minutes after 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 after 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

There are two primary potential limitations in the current study. 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 to examine the compartment specific temporal dynamics of EACA on PLact profiles that 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, to limit the complexity of hemostatic interactions that 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.

In conclusion, the present study provided a proof of concept approach for continuous and regional measurement of PLact, which is a fundamental pharmacologic target for antifibrinolytic therapy. The present study also demonstrated in a clinically relevant large animal model that regional and temporal heterogeneity in PLact exists after a single computed dose of EACA, a prototypical antifibrinolytic. Although commonly utilized, EACA and similar antifibrinolytics are not approved by the Food and Drug Administration 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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References

1. Laupacis A, Fergusson D. Drugs to minimize perioperative blood loss in cardiac surgery: meta-analyses using perioperative blood transfusion as the outcome. Anesth Analg 1997;85:1258–67.
2. Hardy JF, Bélisle S. Natural and synthetic antifibrinolytics in adult cardiac surgery: efficacy, effectiveness and efficiency. Can J Anesth 1994;41:1104–12.
3. Ferraris VA, Ferraris SP, Saha SP, et al, for the Society of Thoracic Surgeons Blood Conservation Guideline Task Force. Society of Cardiovascular Anesthesiologists Special Task Force on Blood Transfusion. Perioperative blood transfusion and blood conservation in cardiac surgery: the Society of Thoracic Surgeons and the Society of Cardiovascular Anesthesiologists clinical practice guideline. Ann Thorac Surg 2007;83(Suppl):27–86.
4. Fraser IS, Porte RJ, Kouides PA, Lukes AS. A benefit-risk review of systemic haemostatic agents. Part 1: in major surgery. Drug Safety 2008;31:217–30.
5. Fergusson DA, Hébert PC, Mazer CD, et al, for the BART Investigators. A comparison of aprotinin and lysine analogues in high-risk cardiac surgery. N Engl J Med 2008;358:2319–31.
6. Kincaid EH, Ashburn DA, Hoyle JR, Reichert MG, Hammon JW, Kon ND. Does the combination of aprotinin and angiotensin-converting enzyme inhibitor cause renal failure after cardiac surgery? Ann Thorac Surg 2005;80:1388–93.
7. Mutter WP, Stillman IE, Dahl NK. Thrombotic microangiopathy and renal failure exacerbated by epsilon-aminocaproic acid. Am J Kidney Dis 2009;53:346–50.
8. Wymenga LF, van der Boon WJ. Obstruction of the renal pelvis due to an insoluble blood clot after epsilon-aminocaproic acid therapy: resolution with intraureteral streptokinase instillations. J Urol 1998;159:490–2.

9. Hocker JR, Saving KL. Fatal aortic thrombosis in a neonate during infusion of epsilon-aminocaproic acid. J Pediatr Surg 1995;30:1490–2.
10. Dentz ME, Slaughter TF, Mark JB. Early thrombus formation on heparin-bonded pulmonary artery catheters in patients receiving epsilon aminocaproic acid. Anesthesiology 1995; 82:583–6.
11. Hoffman EP, Koo AH. Cerebral thrombosis associated with Amicar therapy. Radiology 1979;131:687–9.
12. Seymour BD, Rubinger M. Rhabdomyolysis induced by epsilon-aminocaproic acid. Ann Pharmacother 1997;31:56–8.
13. Hunt BJ, Parratt RN, Segal HC, Sheikh S, Kallis P, Yacoub M. Activation of coagulation and fibrinolysis during cardiothoracic operations. Ann Thorac Surg 1998;65:712–8.
14. Duggan E, O'Dwyer MJ, Caraher E, et al. Coagulopathy after cardiac surgery may be influenced by a functional plasminogen activator inhibitor polymorphism. Anesth Analg 2007; 104:1343–7.
15. Slaughter TF, Faghih F, Greenberg CS, Leslie JB, Sladen RN. The effects of epsilon-aminocaproic acid on fibrinolysis and thrombin generation during cardiac surgery. Anesth Analg 1997;85:1221–6.
16. Shaw A, Swaminathan M, Stafford-Smith M. Cardiac surgery-associated acute kidney injury: putting together the pieces of the puzzle. Nephron Physiol 2008;109:55–60.
17. Mangano DT, Tudor IC, Dietzel C, and the Multicenter Study of Perioperative Ischemia Research Group. Ischemia Research and Education Foundation. The risk associated with aprotinin in cardiac surgery. N Engl J Med 2006;354: 353–65.
18. Mori T, Aisa Y, Shimizu T, et al. Hepatic veno-occlusive disease after tranexamic acid administration in patients undergoing allogeneic hematopoietic stem cell transplantation. Am J Hematol 2007;82:838–9.
19. Stensrud PE, Nuttal GA. Pharmacology of antifibrinolytic agents. Advance Cardiovasc Pharmacol 2008;8:183–204.
20. Spinale FG, Koval CN, Deschamps AM, Stroud RE, Ikonomidis JS. Dynamic changes in matrix metalloproteinase activity within the human myocardial interstitium during myocardial arrest and reperfusion. Circulation 2008; 118(Suppl):16–23.
21. Deschamps AM, Zavadzkas J, Murphy RL, et al. Interruption of endothelin signaling modifies membrane type 1 matrix metalloproteinase activity during ischemia and reperfusion. Am J Physiol Heart Circ Physiol 2008;294:875–83.
22. Smith RE, Bissell ER, Mitchell AR, Pearson KW. Direct photometric or fluorometric assay of proteinases using substrates containing 7-amino-4-trifluoromethylcoumarin. Thromb Res 1980;17:393–402.
23. Nuttall GA, Brost BC, Connis RT, et al. Practice guidelines for perioperative blood transfusion and adjuvant therapies. An updated report by the American Society of Anesthesiologists Task Force on perioperative blood transfusion and adjuvant therapies. Anesthesiology 2006;105:198–208.
24. Marietta M, Facchini L, Pedrazzi P, Busani S, Torelli G. Pathophysiology of bleeding in surgery. Transplant Proc 2006;38:812–4.
25. Goodnough LT. Risks of blood transfusion. Anesthesiol Clin North Am 2005;23:241–52.
26. Verstraete M. Clinical application of inhibitors of fibrinolysis. Drugs 1985;29:236–61.
27. Bennett-Guerrero E, Sorohan JG, Canada AT, et al. Epsilon-aminocaproic acid plasma levels during cardiopulmonary bypass. Anesth Analg 1997;85:248–51.
28. Butterworth J, James RL, Lin Y, Prielipp RC, Hudspeth AS. Pharmacokinetics of epsilon-aminocaproic acid in patients undergoing aortocoronary bypass surgery. Anesthesiology 1999;90:1624–35.
29. Ririe DG, James RL, O'Brien JJ, et al. The pharmacokinetics of epsilon-aminocaproic acid in children undergoing surgical repair of congenital heart defects. Anesth Analg 2002;94: 44–9.
30. Roelofs JJ, Rouschop KM, Leemans JC, et al. Tissue-type plasminogen activator modulates inflammatory responses and renal function in ischemia reperfusion injury. J Am Soc Nephrol 2006;17:131–40.
31. Dietrich W, Nicklisch S, Koster A, Spannagl M, Giersiefen H, van de Locht A. CU-2010: a novel small molecule protease inhibitor with antifibrinolytic and anticoagulant properties. Anesthesiology 2009;110:123–30.
32. Nilsson IM. Clinical pharmacology of aminocaproic and tranexamic acids. J Clin Pathol 1980;14(Suppl):41–7.
33. McNicol GP, Fletcher AP, Alkjaersig N, Sherry S. The absorption, distribution, and excretion of epsilon-aminocaproic acid following oral or intravenous administration to man. J Lab Clin Med 1962;59:15–24.
34. Edmunds LH. Managing fibrinolysis without aprotinin. Ann Thorac Surg 2010;89:324–31.