Temporally and Regionally Disparate Differences in Plasmin Activity by Tranexamic Acid

Daryl L. Reust, MD,* Scott T. Reeves, MD,* James H. Abernathy, III, MD,* Jennifer A. Dixon, MD,† William F. Gaillard, II, BS,† Rupak Mukherjee, PhD,† Christine N. Koval, BS,† Robert E. Stroud, MS,† and Francis G. Spinale, MD, PhD†‡

BACKGROUND: A major complication associated with cardiac surgery is excessive and prolonged bleeding in the perioperative period. Improving coagulation by inhibiting fibrinolysis, primarily through inhibition of plasmin activity (PLact) with antifibrinolytics such as tranexamic acid (TXA), has been a pharmacological mainstay in cardiac surgical patients. Despite its almost ubiquitous use, the temporal and regional modulation of PLact profiles by TXA remains unexplored. Accordingly, we developed a fluorogenic-microdialysis system to measure *in vivo* dynamic changes in PLact after TXA administration in a large animal model.

METHODS: Pigs (25–35 kg) were randomly assigned to receive TXA (30 mg/kg, diluted into 50 mL normal saline; $n = 9$) or vehicle (50 mL normal saline; $n = 7$). Microdialysis probes were placed in the liver, myocardium, kidney, and quadriceps muscle compartments. The microdialysate infusion contained a validated plasmin-specific fluorogenic peptide. The fluorescence emission (standard fluorogenic units [SFU]) of the interstitial fluid collected from the microdialysis probes, which directly reflects PLact, was determined at steady-state baseline and 30, 60, 90, and 120 min after TXA/vehicle infusion. Plasma PLact was determined at the same time points using the same fluorogenic substrate approach.

RESULTS: TXA reduced plasma PLact at 30 min after infusion by >110 SFU compared with vehicle values ($P < 0.05$). Specifically, there was a decrease in liver PLact at 90 and 120 min after TXA infusion of >150 SFU ($P < 0.05$) and 175 SFU ($P < 0.05$), respectively. The decrease in liver PLact occurred 60 min after the maximal decrease in plasma PLact. In contrast, kidney, heart, and quadriceps PLact transiently increased followed by an overall decrease at 120 min.

CONCLUSIONS: Using a large animal model and *in vivo* microdialysis measurements of PLact, the unique findings from this study were 2-fold. First, TXA induced temporally distinct PLact profiles within the plasma and selected interstitial compartments. Second, TXA caused region-specific changes in PLact profiles. These temporal and regional differences in the effects of TXA may have important therapeutic considerations when managing fibrinolysis in the perioperative period. (Anesth Analg 2010;110:694–701)

Excessive perioperative bleeding is a major complication associated with cardiothoracic, major vascular, liver transplantation, orthopedic spine, and trauma surgeries. Blood products and antifibrinolytics have been effectively used to achieve needed hemostasis in these clinical scenarios.[1–6] Antifibrinolytics have been the pharmacological mainstay with proven efficacy in reducing blood loss and blood product transfusion requirements, particularly in relation to cardiac surgery.[1,3] Common clinically used antifibrinolytics affect plasmin activity (PLact) primarily by inhibiting the enzymatic interaction of plasminogen/plasmin with fibrinogen/fibrin and can be classified as either serine protease inhibitors or lysine analogues.[7] The serine protease inhibitor aprotinin significantly inhibits fibrinolysis, but this drug has been removed from clinical use.[7] As a consequence, lysine analogues, such as tranexamic acid (TXA), have now become the major class of pharmacological intervention in which antifibrinolytic therapy is indicated for the management of excessive perioperative bleeding and has likely resulted in an increased use of TXA for this purpose. However, the basic regional and temporal PLact profiles after TXA administration remain unexplored. Accordingly, the primary goal of this study was to characterize the effects of TXA on the regional and temporal PLact profiles in plasma and selected tissue compartments.

Common clinically implemented weight-based TXA dosing regimens are largely empirically derived and, as such, there is no consensus as to appropriate dosing to provide optimal perioperative control of fibrinolysis.[8] This lack of established clinical dosing regimens suggests that the modulation of fibrinolysis by TXA may be enhanced by regional and temporal measurements of PLact. Accordingly, we used a common weight-based TXA dosing scheme to investigate the effects of TXA on regional and temporal PLact profiles.[9] To explore the regional dynamics of PLact, we used a large animal model using established microdialysis techniques.[10,11] Such microdialysis techniques, utilizing a fluorogenic substrate, allowed the detection of interstitial enzymatic activity, such as plasmin.[12] Accordingly, the objectives of this study were 2-fold. The first objective was the validation and calibration of a fluorogenic peptide that could be used to assess PLact *in*

From the *Department of Anesthesiology and Perioperative Medicine, Medical University of South Carolina; ‡Ralph H. Johnson Veterans Affairs Medical Center; and †Division of Cardiothoracic Surgery, Medical University of South Carolina, Charleston, South Carolina.

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Address correspondence and reprint requests to Francis G. Spinale, MD, PhD, Department of Cardiothoracic Surgery, Strom Thurmond Research Building, 114 Doughty St., Room 625, Medical University of South Carolina, Charleston, SC 29403. Address e-mail to wilburnm@musc.edu.

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vivo. The second objective was the development of a porcine model to measure PLact in plasma and interstitial regions of clinical relevance using this validated fluorogenic approach.

METHODS

This study was conducted in 2 stages. First, *in vitro* validation studies were performed to develop a PLact measurement system using a plasmin-specific fluorogenic substrate.[12] This validated PLact measurement system was used to perform *in vivo* PLact measurements, via microdialysis probes, within targeted regions. TXA was then infused IV and PLact was continuously monitored within these regions. Finally, plasma TXA and D-dimer concentrations were measured.

In Vitro Validations

Several *in vitro* validation studies were performed using a plasmin-specific fluorogenic substrate[12] (Cat. #A8171, Sigma-Aldrich, St. Louis, MO). In particular, this substrate contained a validated fluorogenic peptide that, when specifically cleaved by plasmin, yielded a coumarin fluorescent moiety with excitation/emission wavelengths of 365/440 nm, respectively.[12] The first *in vitro* validation study determined the response of the fluorogenic substrate to increasing concentrations of plasmin. Briefly, 6.25 μM of plasmin substrate was injected into a 96-well polystyrene plate (Nalge Nunc, Rochester, NY) with increasing concentrations of plasmin (0–31.25 μg/mL; Cat. #P1867, Sigma-Aldrich). After a 5-min 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 was performed using a solution of reference normal porcine plasma, which determined the TXA plasma concentration inhibition curve. Specifically, plasmin (31.25 μg/mL) and diluted control porcine plasma (1:32) were incubated with increasing concentrations of TXA (0–62.2 mg/mL) and subjected to the same fluorescence measurement procedure previously described. As shown in Figure 1B, the fluorescence emission, reflective of PLact, decreased in response to increasing concentrations of TXA in a classic, logarithmic, concentration-dependent manner.[13] A logarithmic equation was matched to these data using regression analysis.

Therefore, these *in vitro* studies established the optimal substrate concentration, demonstrated specificity of the substrate for plasmin, and determined the fluorescence emission inhibition curve for TXA in porcine plasma. 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 = 16, 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).

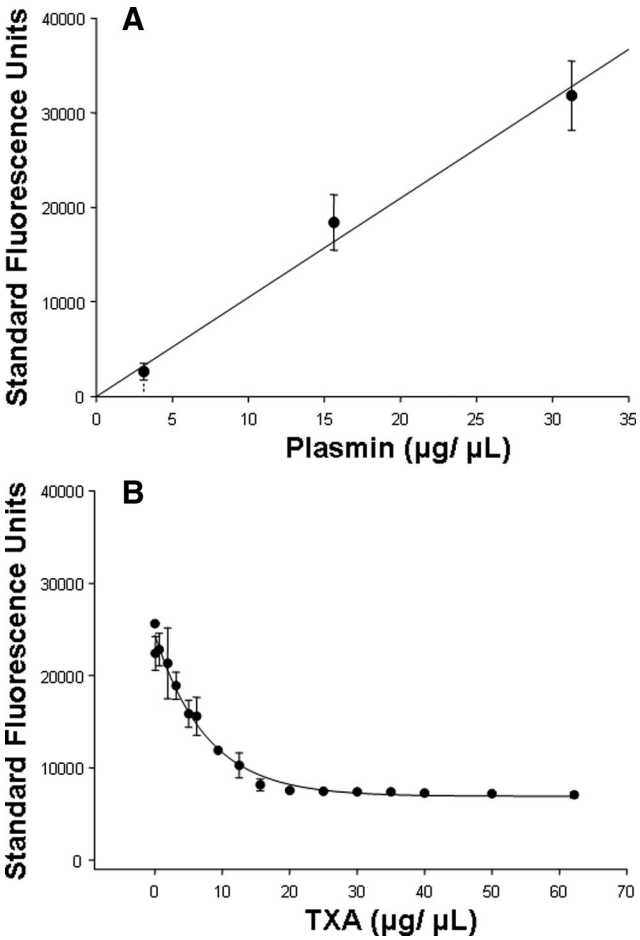

Figure 1. A, Fluorescence emission of the plasmin-specific substrate (6.25 μg/mL), reflective of plasmin activity (PLact), increased with increasing concentrations of plasmin (0–31.25 μg/mL) in a linear concentration-dependent manner (n = 3, plotted values are mean ± SEM; linear regression, $y(x) = 1048.8 \times x$, $r^2 = 0.996$, $P = 0.002$). B, Fluorescence emission of the plasmin-specific substrate (6.25 μg/mL), reflective of PLact, in the presence of plasmin (31.25 μg/mL) and control porcine plasma (1:32) decreased in response to increasing concentrations of tranexamic acid (TXA) (0–62.2 mg/mL) in a classic logarithmic concentration-dependent manner[13] (n = 3, plotted values are mean ± SEM, regression, $y(x) = 23{,}280 \times e^{-0.063} \times x$, $r^2 = 0.964$, $P < 0.001$).

After sedation with diazepam (100 mg *per os*, Elkins-Sinn, Cherry Hill, NJ), general inhaled anesthesia was induced using isoflurane (3%, Baxter Healthcare, Deerfield, IL) mixed with oxygen and nitrous oxide (67%:33%) and peripheral IV access was obtained. A stable surgical plane of anesthesia was established and maintained throughout the protocol using sufentanil (2 μg/kg IV, Elkins-Sinn), etomidate (0.1 mg/kg IV, Elkins-Sinn), vecuronium (10 mg IV bolus, 0.5 $mg \cdot kg^{-1} \cdot h^{-1}$ IV infusion, Ben Venue Laboratories, Bedford, OH), morphine sulfate (3 $mg \cdot kg^{-1} \cdot h^{-1}$ IV, Elkins-Sinn), and isoflurane (1%, Baxter Healthcare). Tracheal intubation was achieved via tracheostomy, and mechanical ventilation was established (Narkomed 2B, North American Drager, Telford, PA). Intravenous fluids (lactated Ringer's solution) were administered per 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-min baseline and stabilization period, each pig was assigned to receive TXA (30 mg/kg, diluted into 50 mL normal saline; Pharmacia & Upjohn, New York, NY) or vehicle (50 mL normal saline) over a 10-min period using a prespecified randomization protocol. This anesthesia regimen and surgical preparation provided a physiologically and hemodynamically stable experimental model for up to 6 h as previously reported.[11]

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 quadriceps muscle compartments. Placement of the microdialysis probes required a median sternotomy, a subxiphoid intraabdominal 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 isoosmotic dialysis was performed. Dialysate was infused for 30 min to allow for equilibration with each of the respective tissue compartments. The microdialysate infusion contained the validated fluorogenic peptide (10 μM, Cat. #A8171, Sigma-Aldrich). Preliminary studies demonstrated that this microdialysate concentration yielded a steady-state fluorescence emission within 30 min 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 and 30, 60, 90, and 120 min after TXA/vehicle infusion, using fluorescence measurement techniques as previously described.

Plasma Sampling

Arterial blood samples (50 mL) were collected immediately after a 30-min stabilization period. 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-min intervals throughout the protocol, coinciding with the microdialysis samples, arterial blood samples (10 mL) were collected. All blood samples were collected in EDTA tubes, centrifuged, and the plasma was decanted and frozen for subsequent measurement of PLact using the previously described fluorescence measurement system.

TXA Plasma Concentration Measurements

An Acquity UPLC coupled to a Quattro Premier XE mass spectrometer (Waters, Milford, MA) was used to measure TXA plasma 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 eluted isocratically over 5 min, and 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 a capillary voltage of 3.1 kV, source temperature of 120°C, desolvation temperature of 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). TXA plasma concentrations were determined from precalibrated TXA standards (0.5–40 μg/mL).

D-Dimer Measurements

D-dimer measurements were made on plasma collected at baseline (time 0) and 120-min time intervals for vehicle and TXA treatment groups using an enzyme-linked immunosorbent assay (Cat. #602, American Diagnostics, Stamford, CT).

Data Analysis

Comparisons for baseline steady-state as well as for net change in fluorescence for all time points within each region were made using an analysis of variance followed by pairwise tests of individual time points means using Bonferroni bounds. The net change in fluorescence compared with baseline for all time points within each region was determined using a 2-sample *t*-test. Comparisons of D-dimer concentrations at baseline (time 0) and 120-min intervals were performed using a 2-sample *t*-test. All statistical procedures were performed using STATA statistical software (Intercooled STATA 8.0, StataCorp, College Station, TX). Results are presented as mean ± SEM with P values <0.05 considered to be statistically significant.

RESULTS

After successful placement of microdialysis probes in all tissue compartments, respective steady-state baseline fluorescence emission measurements, reflective of PLact within each compartment, were obtained (Fig. 2A). There was no significant difference in baseline fluorescence emissions between groups, randomized to either vehicle or TXA treatment, for each tissue compartment, reflective of equivalent PLact before initiation of treatment. Figure 2B illustrates the representative fluorescence emission for a selected tissue compartment (i.e., the liver) for both a representative vehicle and TXA pig preparation. Respective fluorescence emission measurements were obtained at baseline (time 0) and 30, 60, 90, and 120 min after either vehicle (saline) or TXA (30 mg/kg) infusion. The differences in fluorescence emission values between the vehicle and TXA groups at each of the respective time intervals are reflective of changes in PLact induced by the administration of TXA. Therefore, to directly examine the effects of TXA on PLact, the absolute fluorescence emission values were transformed to yield a net change in mean fluorescence emission with respect to mean vehicle values for each of the selected compartments at the specified time intervals (Fig. 3). Compared with vehicle values, TXA significantly reduced plasma PLact at 30 min after infusion. However, in the interstitial compartments, temporal and regional differences in PLact were observed after TXA administration. Specifically, there was a significant decrease in liver PLact at 90 and 120 min, which occurred 60 min after the maximal decrease in

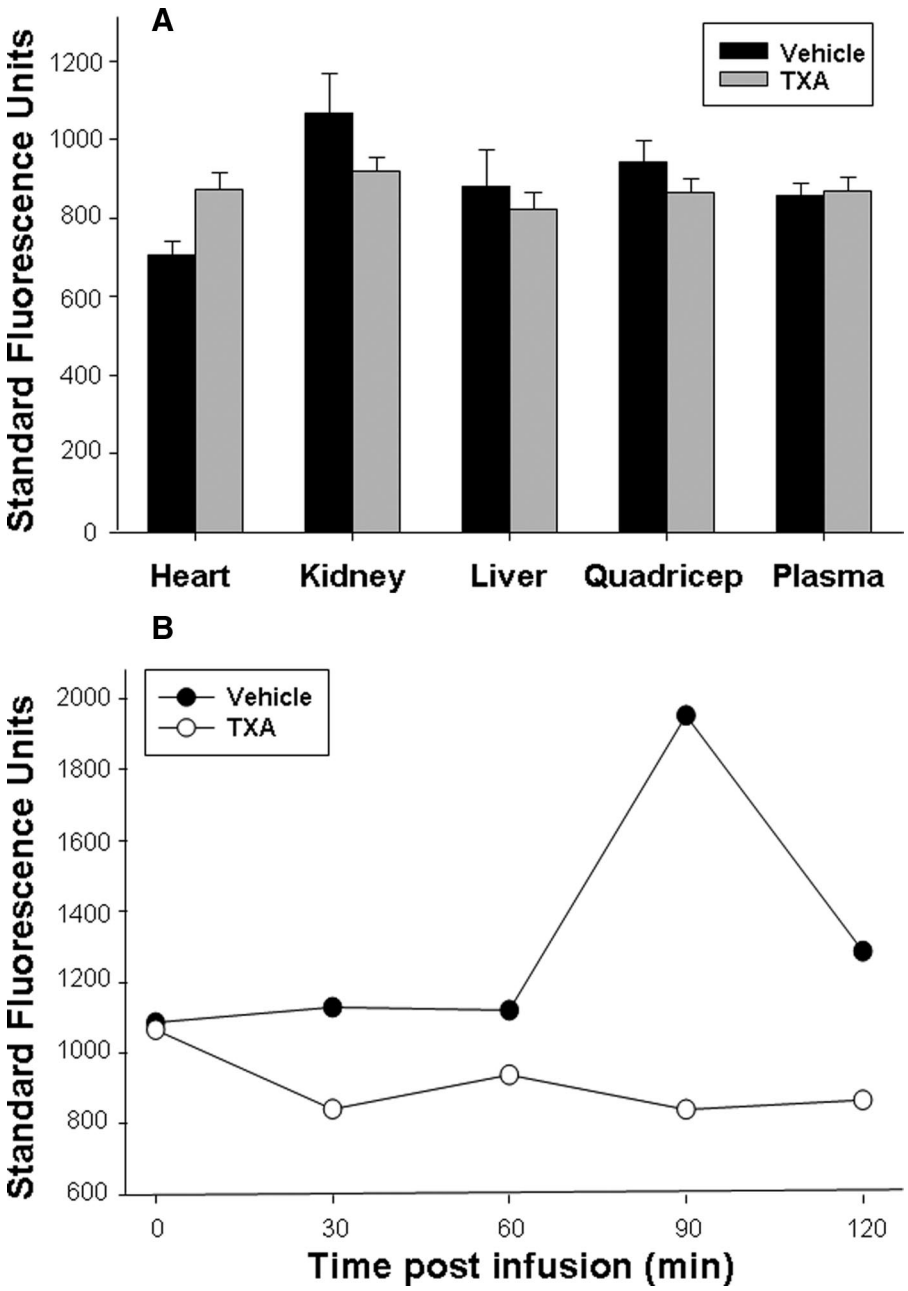

Figure 2. A, Steady-state baseline fluorescence emission, reflective of plasmin activity (PLact), within each of the target tissue compartments was equivalent in pigs randomized to either vehicle (saline) or tranexamic acid (TXA) (30 mg/kg). Thus, the baseline fluorescence emissions between the 2 groups was comparable before initiation of treatment (plotted values are mean ± SEM, $*P < 0.05$). B, Representative fluorescence emission measurements within the liver tissue compartment were obtained at baseline (time 0) and 30, 60, 90, and 120 min after either vehicle (saline) or TXA (30 mg/kg) infusion. There was a notable increase in absolute fluorescence emission over time after vehicle (saline) infusion. In contrast, there was an overall decrease in fluorescence emission over time, reflective of reduced PLact within the liver after TXA administration. The summary data reflective of PLact across each target compartment and all time intervals are shown in Figure 3.

plasma PLact. In contrast, kidney PLact was significantly increased at 30, 60, and 90 min. Within the myocardium, PLact remained virtually unchanged. In the quadriceps muscle, PLact decreased after TXA infusion but did not reach statistical significance at any time point ($P > 0.5$).

The TXA plasma concentrations for time intervals 30, 60, 90, and 120 min after TXA infusion are shown in Figure 4. The peak TXA plasma concentration occurred at 30 min after TXA infusion and subsequently decreased in a negative logarithmic time-dependent manner consistent with first-order elimination pharmacokinetics.[13] Plasma from baseline (time 0) and 120-min time intervals for vehicle and TXA treatment groups was subjected to D-dimer analysis. The baseline, steady-state plasma D-dimer concentration was 20 ± 8 μg/mL with no difference between vehicle and TXA groups at randomization ($P = 0.67$). The plasma D-dimer concentration at 120 min after infusion decreased slightly, but not significantly, from baseline (13 ± 3 μg/mL, $P = 0.49$) with no difference between vehicle or TXA ($P = 0.77$).

DISCUSSION

Perioperative hemorrhage is an important risk factor for morbidity and mortality in most major surgical procedures, notably cardiovascular surgery.[14–17] Accordingly, blood transfusions, blood product and coagulation factor delivery, as well as pharmacological modalities targeted at the coagulation/fibrinolytic mechanisms are important clinical maneuvers in the perioperative setting.[3,14] However, these interventional strategies, such as pharmacological approaches, can be associated with adverse outcomes, which

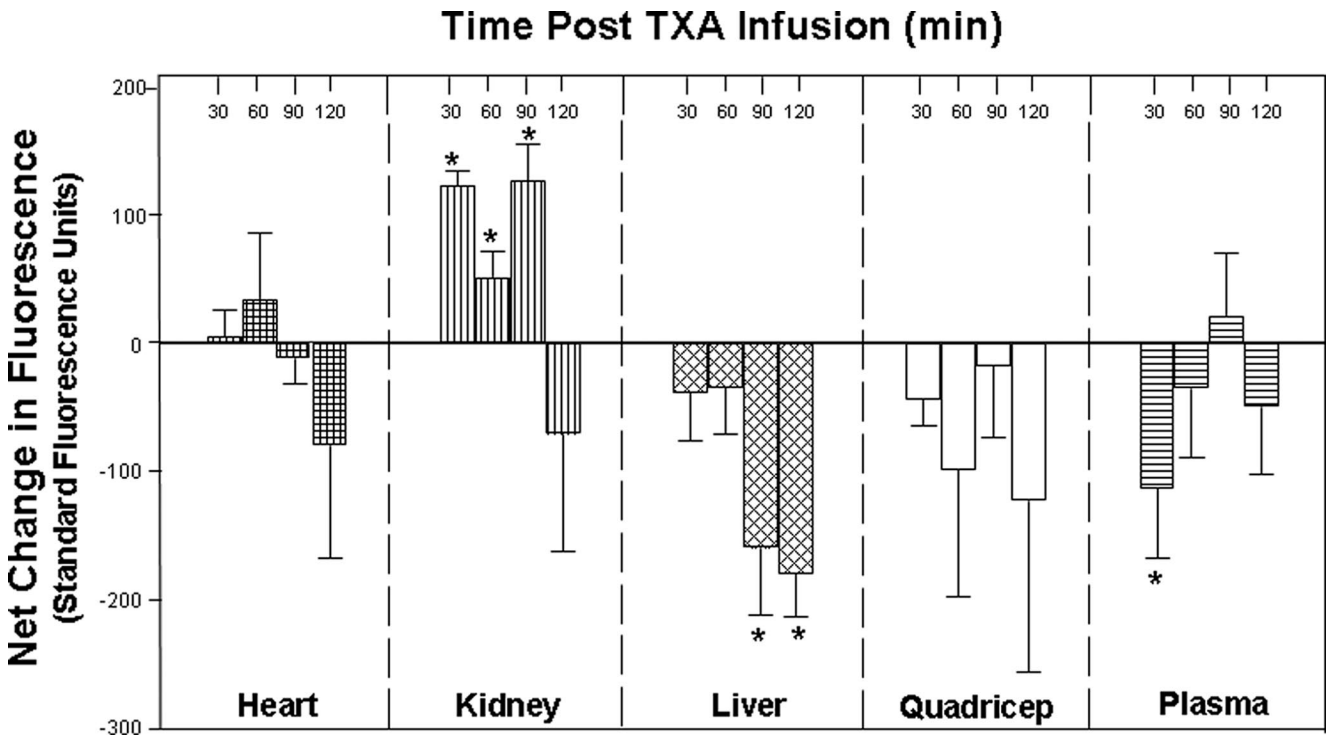

Figure 3. The computed net change in mean fluorescence emission, reflective of changes in plasmin activity (PLact), with respect to time-matched vehicle values after tranexamic acid (TXA) (30 mg/kg) infusion for selected compartments demonstrates the unique temporal and regional differences in the effects of TXA on PLact. Specifically, TXA significantly reduced plasma PLact at 30 min. In addition, there was a significant decrease in liver PLact at 90 and 120 min. In contrast, kidney PLact was significantly increased at 30, 60, and 90 min. There was no significant change in heart PLact for all time points. The PLact within the quadriceps muscle decreased after TXA infusion but did not reach statistical significance at any time point (plotted values are mean ± SEM, $*P < 0.05$ versus baseline).

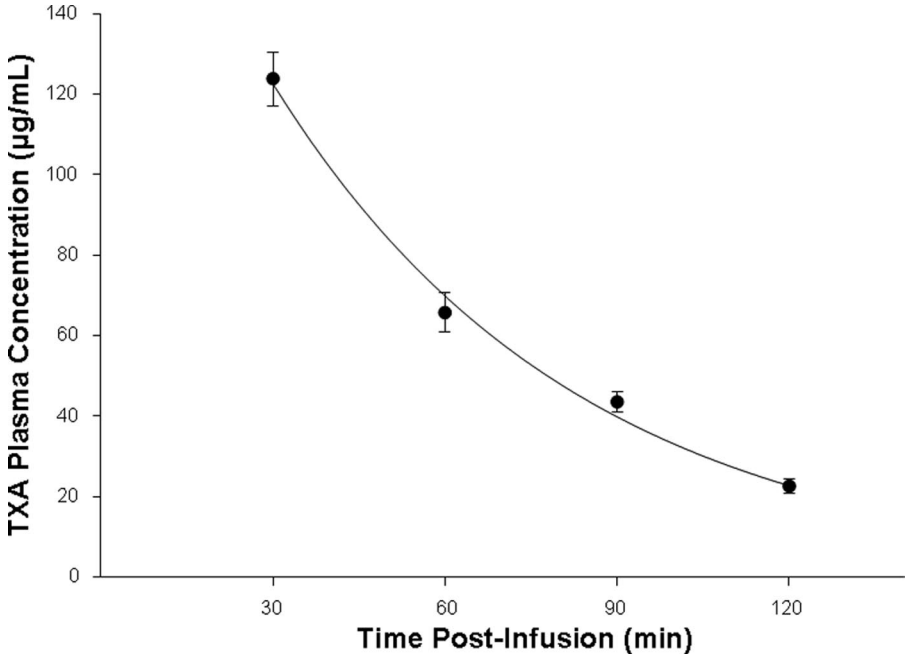

Figure 4. Tranexamic acid (TXA) plasma concentrations determined by high-performance liquid chromatography/ mass spectrometry techniques obtained at time intervals 30, 60, 90, and 120 min after TXA infusion decreased in a negative logarithmic time-dependent manner consistent with first-order elimination pharmacokinetics[13] (plotted values are mean ± SEM, regression, $y(x) = 219.37 \times e^{-0.019} \times x$, $r^2 = 0.994$, $P = 0.003$).

may be attributable to differences in dosing regimens as well as off-target effects.[14–18] One frequently used antifibrinolytic is TXA, which can modulate the fibrinolytic pathway by inhibiting local PLact.[19] However, current TXA dosing schedules are largely empirical, and the regional and temporal effects with respect to changes in PLact remain unknown.[8] This study addressed this issue through the use of a validated fluorogenic-microdialysis approach in a large animal model, to provide serial assessment of PLact on a regional basis, after a standardized dose of TXA.[9] The unique finding from this study is that interstitial PLact is differentially affected after TXA infusion in both a region- and time-dependent manner. For example, TXA induced temporally distinct PLact profiles within the plasma and selected interstitial compartments such

as the kidney and the liver. These temporal and regional differences in the effects of TXA on PLact may have important therapeutic considerations when managing fibrinolysis in the perioperative period. The prophylactic use of lysine analogue antifibrinolytics during cardiac surgery has the potential to induce a hypercoagulable prethrombotic state.[20] As such, thrombosis (deep vein, pulmonary artery, renal pelvic and artery, bladder, and cerebral vascular) with respective concomitant organ injury and dysfunction have been associated with the use of antifibrinolytics such as TXA.[17,21–26] The primary mechanism of elimination of TXA 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 such clinical scenarios in which TXA is indicated.[27] Thus, there are several temporal and regional variables 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.

Although the pharmacology of TXA has been rigorously described regarding mechanisms of action,[19] there have been no studies that have precisely quantified the effects of TXA on interstitial PLact *in vivo*, the primary target for TXA with respect to modulating fibrinolysis. Tissue plasminogen activator is synthesized and secreted by endothelial cells intraluminally and abluminally into the vascular and interstitial spaces, respectively, where it catalyzes the conversion of plasminogen to plasmin and thus facilitates fibrinolysis.[28]

This microdialysis approach provides for interstitial interrogation of PLact and thus a means to directly measure a key determinant of fibrinolysis and avoids the interference of intraluminal dynamics. Furthermore, although past basic and clinical studies have described the utility of TXA in the context of cardiovascular surgery, such as that associated with cardiopulmonary bypass, optimal dosing strategies remain a subject of debate.[8] This is the first study in which an approach was developed to continuously measure the major biological response variable relevant to TXA administration, PLact, within the plasma as well as interstitial space of critical target tissues. In this study, a microdialysis approach was used to interrogate the interstitial compartment, an approach that has been well described previously in both animal and clinical studies.[10,11] 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 TXA dosing regimens and thereby provide a basis for optimal TXA administration. This study provided the fundamental temporal and regional information necessary to move forward with studies aimed at TXA dosing optimization. Moreover, this study identified differences in PLact after TXA administration in critical target organs such as the liver and kidney, which may hold relevance in the clinical context of hepatic or renal dysfunction.[17,18,29] The continuous PLact profiling, which is described in the current study, may provide a means by which to address these issues and further optimize current and future antifibrinolytic therapies.[30]

In this study, TXA was used to investigate the effects of a frequently used antifibrinolytic drug on plasma and interstitial PLact profiles. The rationale for focusing on TXA with respect to PLact profiles was 2-fold. First, the objective of this study was to demonstrate the proof of concept that there is regional and temporal heterogeneity regarding 1 computed dose of an antifibrinolytic, and TXA was chosen as a prototypical example. Second, the serine protease inhibitor, aprotinin, although historically considered the first-line drug for modulating PLact, has been withdrawn from clinical use, thus leaving lysine analogues such as TXA as the pharmacological mainstay for antifibrinolytic therapy. Lysine analogues such as TXA affect PLact primarily by inhibiting the enzymatic interaction of plasminogen and plasmin with fibrinogen and fibrin, which is key to the enzymatic induction of fibrinolysis.[19] Thus, TXA 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. Comparative studies of specific antifibrinolytic drugs hold significant clinical relevance and warrant future investigation. Nevertheless, it is likely that the results from this study can be extrapolated to some degree to other lysine analogues (i.e., ϵ-aminocaproic acid) as well as aprotinin, with respect to the regional and temporal heterogeneity observed. For example, after a single bolus dose of TXA, 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 risk factor for the adverse effects of antifibrinolytics such as aprotinin.[17,18,29]

The peak TXA plasma concentrations obtained in this study are consistent with those typically reported in prior clinical investigations.[8,31,32] As such, the TXA dosing regimen used in this study is a clinically relevant dosing approach. The TXA plasma elimination profile obtained is congruent with classic first-order pharmacokinetics,[13] indicating that the large animal model used in this study holds pharmacological relevance. The time of the peak TXA plasma at 30 min coincides with the occurrence of peak plasma PLact inhibition, demonstrating the pharmacological efficacy of the TXA within the vascular compartment. Thus, the large animal preparation and TXA dosing paradigm used in this study are likely to be a clinically relevant simulation.

Study Limitations and Conclusions

One potential limitation of this study was that the TXA regimen implemented involved an initial loading dose only without a subsequent continuous infusion of TXA. In addition, the *in vivo* investigations did not include the context of cardiopulmonary bypass, which is a typical clinical scenario in which TXA is frequently used. Our primary objective was to quantify the regional and temporal effects of TXA on relevant compartment PLact profiles. Accordingly, the TXA regimen involved an initial dose only to examine the compartment-specific temporal dynamics of TXA on PLact profiles, which would have been potentially obscured by the subsequent administration of a continuous infusion of TXA. Furthermore, the context of cardiopulmonary bypass would have included requisite systemic heparinization, which could have added coagulation interactions that potentially affected *de novo* fibrinolytic processes. Indeed,

this study demonstrated that static measurements to quantify fibrinolysis (i.e., D-dimers) were stable and not different between vehicle and TXA groups. This suggests that the experimental design did not evoke a substantial fibrinolytic response. Nevertheless, using a continuous interstitial monitoring approach, this study demonstrated that there was heterogeneity in steady-state PLact in specific tissue compartments, which were differentially affected by TXA. These observations suggest that continuous PLact monitoring would be of much greater importance in the context of a heightened fibrinolytic state such as cardiopulmonary bypass. The primary focus of this preliminary study was to determine the fundamental mechanistic underpinnings of the regional and temporal effects of TXA on PLact profiles in a *de novo*, nonpathological, fibrinolytic state. Logically, one may anticipate an even greater magnitude of effect by TXA in a pathological fibrinolytic state such as that induced by cardiopulmonary bypass. The extension of the current findings will provide a basis for the pursuit of similar PLact investigations involving a clinically relevant cardiopulmonary bypass model. Nevertheless, this study demonstrated in a clinically relevant large animal model that there is regional and temporal heterogeneity in PLact after a single computed dose of TXA, a prototypical antifibrinolytic. Although TXA and similar antifibrinolytics are frequently used, they are not approved by the Food and Drug Administration for prophylactic use to reduce blood loss and blood component transfusions in patients undergoing coronary bypass surgery. Coupled with recent concerns for the adverse effects of aprotinin, the findings of this study underscore the need for more rigorous monitoring and dosing of antifibrinolytics.

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