Postinjury fibrinolysis shutdown: Rationale for selective tranexamic acid

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Postinjury systemic fibrinolysis has been recognized as a biologic process for more than 200 years, but the mechanisms of regulation and their clinical implications remain unclear. In 1794, John Hunter from Edinburgh observed that the last blood exiting from fatal gunshot wounds did not clot.[1] Albert Dastre from Paris proposed the term *fibrinolysis* in 1893 (*Archives de Physiologie*) based on experimental work demonstrating digestion of fibrin. In 1927, interest in fibrinolysis was piqued by a Russian report that victims of sudden death were preferred as blood donors because their blood "reliquified" within a few hours, permitting transfusion without an anticoagulant. Scientific knowledge of physiologic fibrinolysis improved rapidly during the ensuing two decades and, by the 1950s, the plasminogen (PLG)-plasmin-antiplasmin system was established as critical in preserving microvascular patency during clotting to maintain hemostasis.[1,2] Thus, in parallel to the highly regulated clot formation system, clot stabilization and physiologic degradation by the fibrinolysic system was also appreciated to be highly regulated.

THE CHALLENGES OF MODIFYING FIBRINOLYSIS

In 1963, Starzl et al.[3] identified systemic fibrinolysis by thrombelastography (TEG) during the anhepatic phase of liver transplantation and advocated routine antifibrinolytics (aminocaproic acid). Three years later,[4] however, this Colorado transplant team reversed their recommendation when three of their four transplant survivors given aminocaproic acid developed multiple pulmonary emboli. Interestingly, during the same period, Hardaway et al.[5] demonstrated the benefits of fibrinolytic administration to prevent irreversible experimental hemorrhagic shock. During the ensuing three decades, fibrinolytic therapy became the standard for arterial thromboemboli in the coronary, cerebral, mesenteric, and peripheral vasculature, with selective use in the venous system. By the late 1980s, recombinant tissue PLG activator (tPA) became the fibrinolytic of choice. On the other side, with the widespread availability of TEG, excessive fibrinolysis was incriminated in post–coronary artery bypass grafting mediastinal bleeding presumably because of contact activation. But the enthusiasm for antifibrinolytics was dampened after the BART (Blood Conservation using Antifibrinolytics in a Randomized Trial) indicated increased renal failure, myocardial infarction, and mortality after coronary artery bypass grafting when a plasmin inhibitor (aprotinin) was given.[6]

ENTHUSIASM FOR TRANEXAMIC ACID IN TRAUMA MANAGEMENT

Acknowledging the potential role of the PLG-plasmin system in trauma is a relatively recent event and largely caused by the implementation of TEG[7] and ROTEM (rotational thromboelastometry).[8] The stage was set by Hoffman and Monroe[9] in 2001 who proposed the cell-based model of hemostasis. Based on this construct, Brohi et al.[10] introduced the provocative concept that trauma-induced coagulopathy was mediated via the activation of protein C (aPC), resulting in the degradation of clotting factors V and VIII. Embedded within this novel proposal was the consumption of PLG activator inhibitor-1 (PAI-1) by aPC, thus, indirectly enhancing fibrinolysis.[11] Within a year, our group in Denver documented systemic hyperfibrinolysis by TEG in 18% of acutely injured patients requiring a massive transfusion.[12] These data were further supported by contemporary reports from the United States[13] and Europe.[14] The CRASH-2 (Clinical Randomization of an Antifibrinolytic in Significant Hemorrhage) trial, reported in 2010,[15] provided the ultimate impetus for the widespread adoption of an antifibrinolytic (tranexamic acid [TXA]) for trauma management.

However, the significant limitations of this prospective randomized trial were soon emphasized by a number of groups.[16,17] Although 20,211 patients were enrolled in this study designed to reduce mortality caused by coagulopathy, only half of the patients required a red blood cell (RBC) transfusion. Furthermore, there was no reduction in transfused blood products; each group received six units of RBCs. Finally, an additional analysis of the data indicated a 1.44 increased risk for mortality when TXA was given more than 3 hours after injury.[18] While the MATTERS (Military Application of Tranexamic Acid in Trauma Emergency Resuscitation Study) suggested a benefit of TXA in combat casualty care, this was a retrospective analysis confounded by the administration of fibrinogen.[19] Recently, Valle et al.[20] in a retrospective analysis

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of civilian data, using propensity score matching , found an increased mortality in severely injured patients administered TXA, and Harvin et al.[21] in a retrospective study of civilian patients confirmed to have fibrinolysis by TEG observed no benefit from TXA.

POSTINJURY HYPERFIBRINOLYSIS, PHYSIOLOGIC FIBRINOLYSIS, AND FIBRINOLYSIS SHUTDOWN

Principal component analyses of patients with trauma-induced coagulopathy performed by the Denver group[22] and the San Francisco General group[23] indicated that clotting factor deficiencies and systemic hyperfibrinolysis are mechanistically distinct. These analyses stimulated us to investigate the mechanistic regulation, and consequent manifestation, of fibrinolysis in our animal shock/trauma models. Although systemic fibrinolysis is difficult to replicate in animal models, we developed a tPA challenge assay to unmask latent hyperfibrinolysis versus fibrinolysis shutdown.[24] We define *shutdown* as a relative resistance to tPA caused by a physiologic dysregulation of the PLG-plasmin system. Interestingly, in both our rodent and swine models, shock (ischemia/reperfusion) produced consistent systemic hyperfibrinolysis, whereas tissue injury (thoracotomy/laparotomy/femur fracture) provoked physiologic fibrinolysis shutdown.[25]

Stimulated by these experimental findings, we then interrogated our prospectively collected TEG database from 2010 to 2013. Patients were eliminated if the first TEG was obtained longer than 12 hours after injury or the patient was taking preinjury anticoagulants. A citrated kaolin TEG was conducted by standard TEG 5000 methods (Haemonetics) and used because of its greater accuracy for identifying lysis compared with the rapid TEG. Systemic fibrinolysis was quantified as the percent clot lysis at 30 minutes after maximum strength was achieved (LY30). Patients were then stratified into three groups by LY30 criteria: hyperfibrinolysis (≥3%); physiologic fibrinolysis (0.81–2.9%); and fibrinolysis shutdown (≤0.08%). The hyperfibrinolysis cutoff was based on previous clinical studies indicating increased blood product consumption and mortality in acutely injured patients.[13,26] Because there were no previos reports addressing fibrinolysis shutdown, we used a receiver operating characteristic curve for mortality in the remaining patients with an LY30 less than 3%. The point of greatest specificity and sensitivity was 0.8% based on a Youden Index. Our study population consisted of 180 severely injured patients who were 43 years old (interquartile range [IQR], 28–55 years), 70% male, and 79% sustained blunt trauma. The median Injury Severity Score (ISS) was 29 (IQR, 22–36), median initial base deficit (BD) was 9 (IQR, 6–3), and the mortality was 20%, with two thirds occurring within 24 hours. Fibrinolysis shutdown (as previously defined) was the most common phenotype, accounting for 64% (n = 115) with physiologic fibrinolysis (n = 32) and hyperfibrinolysis (n = 33) representing 18% each. Interestingly, the three phenotypes could not be distinguished by age, sex, ISS, or BD. When considering patients who required RBCs, the systemic hyperfibrinolysis patients required more RBCs and fresh-frozen plasma, and this correlated with an increased need for massive transfusion (Fig. 1).

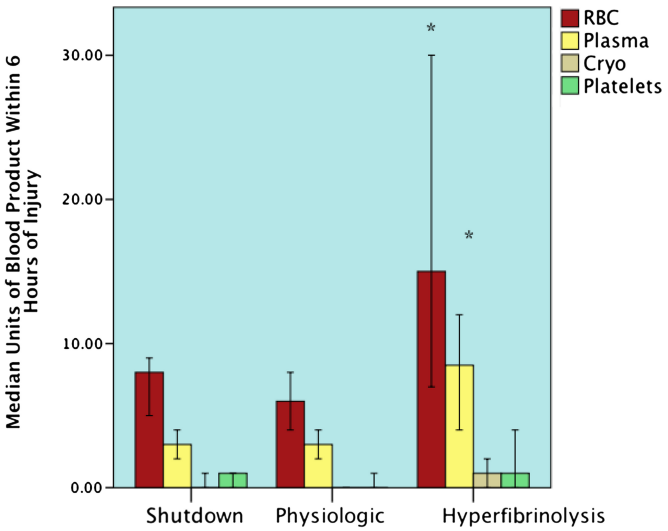

Figure 1. Blood product transfusions between systemic fibrinolysis phenotypes. The blood component transfusion among the systemic fibrinolysis phenotypes summarized; *y* axis represents the number of units within the first 6 hours after injury. RBC and plasma transfusions were higher in the systemic hyperfibrinolysis phenotype. Cyro indicates cryoprecipitate. *$p < 0.05$ after pairwise adjustment. Reproduced from Moore et al.[24] with permission from Lippincott Williams & Wilkins.

Of note, mortality among the fibrinolysis phenotypes had a U-shaped distribution (Fig. 2), with the nadir in the physiologic group (3%) compared with systemic hyperfibrinolysis (44%) and fibrinolysis shutdown (17%). The cause of death was also substantially different between the phenotypes (Fig. 3). Acute blood loss accounted for 66% of the mortality in the hyperfibrinolysis group compared with 15% in the fibrinolysis shutdown patients. Conversely, death because of multiple organ failure occurred in 7% of the hyperfibrinolysis group compared with 40% in the shutdown patients.

In sum, our clinical study identified three distinct phenotypes of fibrinolysis after severe injury. Unfortunately, it was not possible to predict which phenotype would be manifest based on injury severity (ISS) and magnitude of shock (BD). The majority of these patients (64%) had fibrinolysis shutdown and typically had delayed mortality from multiple organ failure. These patients should not improve with further blockade of the fibrinolytic system and may even be harmed by the untimely administration of TXA. Conversely, the 18% with documented systemic hyperfibrinolysis will presumably benefit from timely TXA delivery, as mortality in this group was early and caused by acute blood loss. We believe that these findings argue against the empiric use of TXA in acutely injured patients and support the routine monitoring for lysis with TEG or ROTEM in high-risk patients. There are limitations to our initial clinical study identifying fibrinolysis phenotype caused by the delay in acquiring the TEG analysis up to 12 hours.[24] Consequently, we have an ongoing prospective study to define the timing of fibrinolysis shutdown using our gradated tPA challenge assay. The experience to date

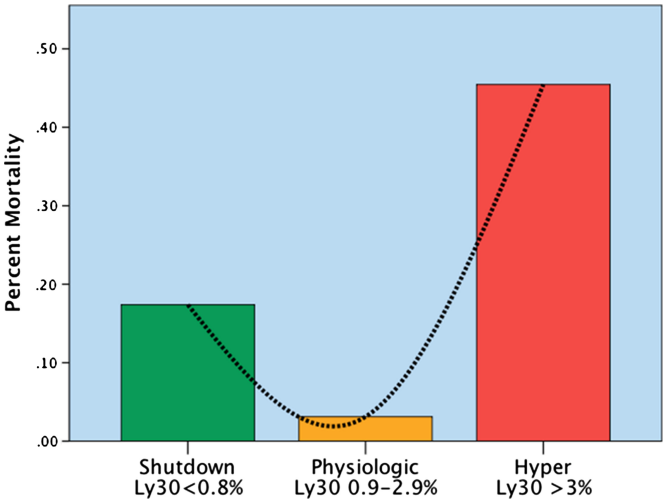

Figure 2. U-shaped distribution of mortality related to systemic fibrinolysis phenotype. The mortality among the systemic fibrinolysis phenotypes was U-shaped; *y* axis represents mortality by phenotype. *Ly30* indicates percent fibrinolysis 30 minutes after reaching maximum amplitude measured by thombelastography (*y* axis represents the percent mortality per phenotype); *shutdown*, fibrinolysis shutdown; *physiologic*, physiologic fibrinolysis; *hyper*, hyperfibrinolysis. There is a U-shaped distribution of mortality with a nadir in mortality identified in the physiologic group (Ly30 between 0.9 and 2.8%). Ly30% above and below this range had statistical increases in mortality. Reproduced from Moore et al.[24] with permission from Lippincott Williams & Wilkins.

indicates that the majority of severely injured patients manifest tPA resistance/fibrinolysis shutdown within 3 hours of injury. Considering the half-life of 2 hours for TXA, we believe that our data support our recommendation for the selective administration of TXA.[16]

REGULATORY MECHANISMS OF POSTINJURY FIBRINOLYSIS

At a simplistic level, the regulation of postinjury fibrinolysis can be viewed as a set of activators and inhibitors of the PLG-plasmin system (Fig. 4), but the molecular events are more complex and yet to be fully elucidated.[27] A perusal of the molecular structure of fibrinogen underscores the complexities of this regulation.[28,29] Our experimental work and clinical studies[11,24–26,30] indicate that circulatory arrest provokes systemic hyperfibrinolysis. Our experimental work further confirms that shock (ischemia) stimulates systemic fibrinolysis.[24,25] Collectively, these studies suggest that a primary mechanism for systemic hyperfibrinolysis is the release of tPA that overwhelms the counter-regulatory mechanisms. At this point, we believe a major component is that tPA release exceeds the capacity of its cognate inhibitor PAI-1 (Serpin E1). Tissue PLG activator and PAI-1 form a mutually inhibitory covalent complex with 1:1 stoichiometry and are cleared by the liver. PAI-1 activity may be further impaired because of the actions of other proteolytic enzymes, including activated protein C (aPC) and neutrophil elastase, which are known to be upregulated by acute injury and inflammation. While the endothelium is a major source of tPA, the precise mediators in trauma are unclear and production is organ specific (unpublished data). Furthermore, there may be other direct contributors such as neuronal tissue.[27] Regulation of the PLG-plasmin system involves a myriad of molecular events at multiple steps, including overt and covert protein domains that modulate the binding of tPA and PLG to fibrinogen.[27,31] For example, our experimental work, using proteomics, indicates that partners bearing accessible lysine residues enhance plasmin fibrinolysis independent of tPA and PAI-1 levels.[30,31] Furthermore, our ongoing investigation with metabolomics indicates a number of potential modifiers of the plasmin system (unpublished data).

The pathogenesis of postinjury systemic fibrinolysis shutdown remains even further mysterious. There are a number of potential regulatory events after severe trauma. Plasminogen activation on fibrin is initiated when tPA binds to fibrin followed by the binding of PLG. Once this trimolecular complex is formed, plasmin cleaves fibrin and exposes carboxy terminal lysine residues. Kringle 2 of tPA and kringles 1 and 4 of PLG contain lysine-binding sites. Their counterbinding sites in fibrin (Aa 148–160 and c 312–324) are cryptogenic in fibrinogen

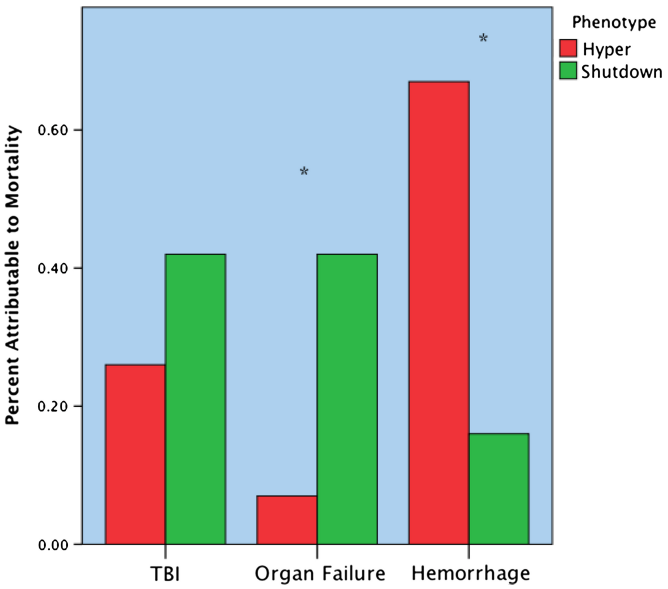

Figure 3. Distribution of mortality according to systemic fibrinolysis phenotype. The distribution of mortality among the systemic fibrinolysis phenotypes was substantially different; *y* axis represents percentage of total mortality per systemic fibrinolysis phenotype. The systemic hyperfibrinolysis phenotype died primarily because of hemorrhage, whereas the systemic fibrinolysis shutdown phenotype succumbed to multiple organ failure. *TBI* indicates traumatic brain injury; *hyper*, hyperfibrinolysis; *shutdown*, fibrinolysis shutdown. *$p < 0.05$. The *y* axis represents the percent of total mortality per phenotype. The hyperfibrinolytic phenotype had a high frequency of mortality associated with hemorrhage. The shutdown phenotype has a high frequency of organ failure–related death. TBI did not reach statistical difference between phenotypes but was more common in the shutdown cohort. Reproduced from Moore et al.[24] with permission from Lippincott Williams & Wilkins.

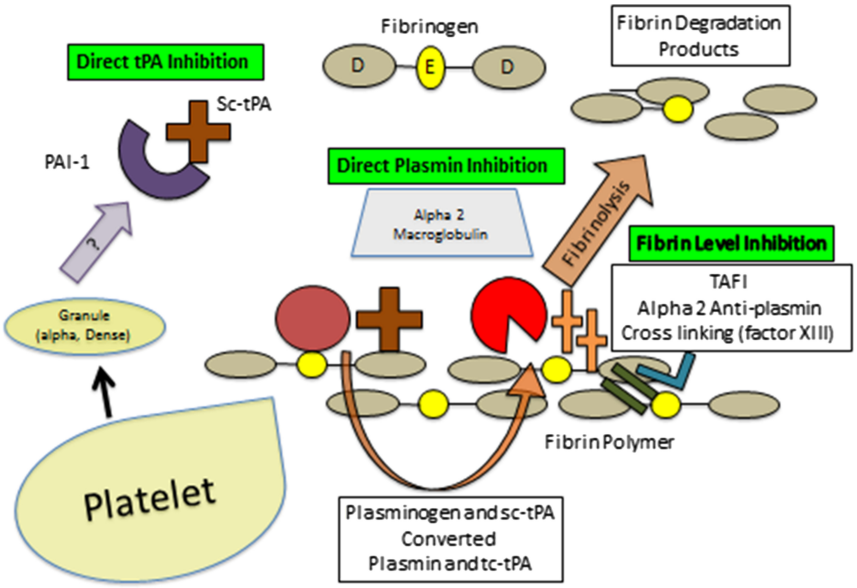

Figure 4. Regulation of postinjury systemic fibrinolysis. The control of systemic fibrinolysis driven by tPA is controlled at multiple levels, including the direct inhibition of tPA, the direct inhibition of plasmin, and the inhibition at the fibrin cross-linking stage.

but become exposed during fibrin cross-linking because of intermolecular D:E interactions that result in conformational changes in the D region. The Aa 148–160 site binds tPA and PLG with similar affinity, whereas the c 312–324 site binds tPA exclusively. Fibrin polymerization occurs with considerable diversity, and the resulting viscoelastic properties are generally referred to as clot stability. Fibrin structure affects the rate of fibrinolysis; thinner fibrin strands with frequent branch points are more resistant to plasmin disassembly. Disaggregation of the fibrin fibers proceeds by lateral transection rather than surface erosion. When plasmin is generated, it converts single-chain tPA to a double-chain form that has much greater activity. In degrading cross-linked fibrin, plasmin initially cleaves the C termini of the α and β chains within the D region, resulting in a variety of fibrin degradation products. Degradation of fibrin cross-linked by factor XIII releases the fibrin degradation product known as D-dimer, which consists of fragments containing two D regions and one E region.

Once plasmin is generated, there are a number of inhibitors that can attenuate its activity. The most active is α2-antiplasmin (α2-AP), a 70-kD single-chain glycoprotein that is a serpin. The α2-AP is made in the liver and has a circulating half-life exceeding 2 days. The α2-AP forms a lysine-binding site dependent α2-PI plasmin complex, which is cleared by the liver. The α2-AP can a be cross-linked to fibrin α chains, and this further enhances resistance to fibrinolysis. Factor XIII is also capable of incorporating α2-AP into fibrinogen. The α2-macroglobulin is a 72-kD dimeric protein synthesized by endothelial cells and macrophages. The α2 macroglobulin, like the α2-AP, is also in the platelet granule. Thrombin-activatable fibrinolysis inhibitor (TAFI), also known as procarboxypeptidase B, is a 60-kD polypeptide that is generated in the liver and present in platelets. Thrombin activation of TAFI is enhanced 1,250-fold in the presence of thrombomodulin. TAFI inhibits fibrinolysis by cleaving lysine residues on fibrin that bind tPA and PLG. There are additional proteins released after platelet activation that can influence fibrinolysis. Polyphosphate, a negatively charged polymer of inorganic phosphate, is secreted from the dense granules of platelets and promotes tighter fibrin aggregates with reduced sites for tPA and PLG binding. Enhanced interactions with the extracellular matrix by binding fibrin to fibronectin also impair fibrinolysis.

Our clinical studies indicate that the majority of severely injured patients manifest fibrinolysis shutdown within 3 hours of injury, but predicting that response based on injury pattern has been challenging. Our experimental work indicates that tissue injury provokes fibrinolysis shutdown, but the precise mechanisms remain unclear.[25] While the release of PAI-1 exceeding tPA activity is an intuitive mechanism,[32] the process seems to involve other molecular interactions. Plasmin activity on fibrin might be obstructed by differential expression of cellularly produced regulators or incur interactions with novel inhibitors released by tissue injury. Structurally, it seems that modulators of the kringle domains in plasmin and tPA can both be activators and inactivators.[27] With tissue injury as an emerging unifying stimulus, danger signaling could be another partial explanation. Recently, we have shown experimentally that lysed platelets strongly inhibit systemic fibrinolysis. Although the mechanism is unknown, data suggest that platelet activation and the release of granule contents may have a role.[30] Finally, we have now documented that fibrinolysis shutdown is prevalent in the postinjury recovery period, a dominant phenotype in the surgical intensive care unit and may explain sequelae ranging from acute lung injury to venous thromboembolism.[33]

DISCLOSURE

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