Fibrinolysis shutdown phenotype masks changes in rodent coagulation in tissue injury versus hemorrhagic shock

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Introduction. *Systemic hyperfibrinolysis (accelerated clot degradation) and fibrinolysis shutdown (impaired clot degradation) are associated with increased mortality compared with physiologic fibrinolysis after trauma. Animal models have not reproduced these changes. We hypothesize rodents have a shutdown phenotype that require an exogenous profibrinolytic to differentiate mechanisms that promote or inhibit fibrinolysis.*
Methods. *Fibrinolysis resistance was assessed by thrombelastography (TEG) using exogenous tissue plasminogen activator (tPA) titrations in whole blood. There were 3 experimental groups: (1) tissue injury (laparotomy/bowel crush), (2) shock (hemorrhage to mean arterial pressure of 20 mmHg), and (3) control (arterial cannulation and tracheostomy). Baseline and 30-minute postintervention blood samples were collected, and assayed with TEG challenged with taurocholic acid (TUCA).*
Results. *Rats were resistant to exogenous tPA; the percent clot remaining 30 minutes after maximum amplitude (CL30) at 150 ng/mL (*P *= .511) and 300 ng/mL (*P *= .931) was similar to baseline, whereas 600 ng/mL (*P *= .046) provoked fibrinolysis. Using the TUCA challenge, the percent change in CL30 from baseline was increased in tissue injury compared with control (*P *= .048.), whereas CL30 decreased in shock versus control (*P *= .048). tPA increased in the shock group compared with tissue injury (*P *= .009) and control (*P *= .012).*
Conclusion. *Rats have an innate fibrinolysis shutdown phenotype. The TEG TUCA challenge is capable of differentiating changes in clot stability with rats undergoing different procedures. Tissue injury inhibits fibrinolysis, whereas shock promotes tPA-mediated fibrinolysis. (Surgery 2015;158:386-92.)*

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One in 4 patients with severe injury experience changes in coagulation within 30 minutes of injury, which is associated with a 5-fold risk of mortality.[1] Coagulation derangements after trauma have been reported to have 2 predominant components: (1) impairment of blood clot formation (hypocoagulation), and (2) increased rate of clot degradation (hyperfibrinolysis).[2] These coagulation changes in animal experiments were originally attributed to a combination of tissue injury and hemorrhagic shock. Animal models attempting to recreate trauma-induced coagulopathy (TIC) have been able to demonstrate impaired clot formation,[3-6] but have not been able to replicate the increased levels of clot degradation (systemic hyperfibrinolysis) seen in humans with TIC.

As the study of postinjury coagulopathy has intensified, hypocoagulation and hyperfibrinolysis seem to be distinct mechanistically. Principal component analyses suggest that hyperfibrinolysis does not correlate with impaired clot formation.[7,8] In a human study, patients with nontraumatic cardiac arrest had a high prevalence of hyperfibrinolysis.[9] Although significant hypotension in injured

Supported in part by National Institute of General Medical Sciences grants: T32-GM008315, P50-GM049222 1, and UM-1HL120877. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIGMS, NHLBI or National Institutes of Health.

Presented at the 10th Annual Academic Surgical Congress in Las Vegas, Nevada, February 3-5, 2015.

Accepted for publication April 5, 2015.

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0039-6060/$ - see front matter

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http://dx.doi.org/10.1016/j.surg.2015.04.008

patients is associated with hyperfibrinolysis, there is no correlation with injury severity.[10] Rodents may have an inherent resistance to fibrinolysis, which would explain failure to reproduce changes in clot degradation in animal models. To reduce resistance of fibrinolysis, a novel assay was developed that challenged the stability of whole blood with taurocholic acid (TUCA), a known profibrinolytic agent.[11] We hypothesize that shock promotes fibrinolysis, and that tissue injury has an inhibitory effect that can be differentiated using a modified thrombelastography (TEG) technique challenging whole blood with TUCA.

METHODS

Subjects. The University of Colorado Institutional Animal Care and Use Committee approved this animal study under protocol #90814. Male Sprague Dawley rats (n = 27) aged 14–16 weeks weighing between 350 and 400 g were used for the experiment. Female rodents were not used owing to their reported differences in immune–inflammatory responses to injury. In addition, males were selected owing to this gender being more prevalent in severely injured human trauma patients, and an appropriate subject for an initial approach to identify regulators of fibrinolysis.

Animal induction and cannulation. Anesthesia was induced with pentobarbital (5 mg/kg, intraperitoneal) followed by tracheostomy and femoral artery cannulation. The femoral artery cannula was used for invasive measurement of blood pressure, and for blood withdrawal for controlled hemorrhage and sampling. The animals were allowed to recover from these initial procedures for airway and vascular access, and then randomized into one of the 3 groups (control, shock, and tissue injury).

Stage II hemorrhagic shock and mild tissue injury (control). Mild tissue injury from tracheotomy and femoral artery cannulation was performed. A 2-mL baseline blood sample was drawn through the femoral artery. This quantity of blood was required for multiple TEG assays and for plasma protein analysis. The animal was observed for 30 minutes before a second (final) blood draw was performed (2 mL). The total blood obtained for assays was approximately 15% of the total estimated blood volume and representative of stage II shock.[12]

Stage IV hemorrhagic shock and mild tissue injury (shock). Mild tissue injury (from tracheotomy and femoral artery cannulation) has been described. After baseline blood draw (2 mL), the animal was hemorrhaged sequentially with 0.5- to 1.0-mL blood draws at 30-second intervals to obtain a mean arterial pressure (MAP) of 20 mmHg within 5 minutes. This pressure-driven procedure was designed to target a blood loss of >40% the estimated blood volume (representative of stage IV shock) based on an objective metric of shock severity (MAP).[12] Animals were kept at MAP of 20 ± 2 mmHg for 30 minutes. The 30-minute time frame was designed to replicate the clinical course (in terms of time from injury to arrival at the hospital) of a cohort of the most severely injured patients who undergo a resuscitative emergency department thoracotomy (median, 24 minutes; interquartile range, 20–30; data not published). This severe degree of shock was selected based on our prior work with rodents, which demonstrated that lesser degrees of hemorrhagic shock (MAP > 30 mmHg) did not result in changes in coagulation.[13] Additional blood was removed during the 30 minutes of shock if the MAP exceeded 25 mmHg. At the end of 30 minutes, a final blood draw of 2 mL was obtained.

Stage II shock and major tissue injury (tissue injury). Tracheotomy and femoral artery cannulation were performed as described. Baseline blood samples were obtained as described, followed by a midline incision of the abdomen. After evisceration, a 10-cm section of small intestine was isolated proximal to the cecum and run gently through a clamp covered with silastic tubing to cause a mild crush injury. The intestines were returned to the peritoneal cavity, and the abdominal incision closed. Intestinal injury was employed because of previous data demonstrating that postinjury mesenteric lymph drives remote multiple organ failure.[14] This injury type could reasonably promote impairment of fibrinolysis, because it has been observed that multiple organ failure is associated with fibrinolysis shutdown.[15] After 30 minutes of observation, a final blood draw was performed (2 mL).

Blood samples and rodent TEG. Whole blood was collected in 3.2% sodium citrate tubes at a 1:10 ratio, based on a standardized model of performing TEG in rodents.[16] Individual microcentrifuge tubes were prefilled with citrate and marked to an appropriate fill level to ensure reproducible ratios of whole blood to citrate. Citrated native TEG assays were recalcified and run according to the manufacturer's instructions on a TEG 5000 TEG Hemostasis Analyzer (Haemonetics, Niles, IL). The percent clot remaining 30 minutes after maximum amplitude (CL30) parameter was used to quantify fibrinolysis. Blood not used for TEG was spun to yield plasma for protein analysis. Whole blood was centrifuged at 6,000 g for

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