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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Background: Trauma remains the leading cause of death and disability in patients under 40. Coagulopathy is common following trauma and is associated with poor outcome. Our group has identified an Acute Traumatic Coagulopathy, which this grant seeks to characterize.
Purpose: Our preliminary human data indicate that there is a close correlation between the development of coagulopathy and the activation of the protein C pathway. Thus, in this work, we are testing the hypothesis that acute traumatic coagulopathy is primarily caused by tissue hypoperfusion resulting in a complement-mediated activation of the protein C pathway.
Major Findings: In the first objective, a single center, prospective cohort study examined the timing and causes coagulation derangements after severe trauma and hypoperfusion. We found an activated protein C mediated coagulopathy after injury and shock. The second and third objectives continue to mechanistically define the role of the protein C pathway and complement in a mouse model in the development of these coagulation abnormalities.
Relevance: During this grant cycle we identified that that activation of the anticoagulant protein C is a critical mechanism driving early posttraumatic coagulopathy. Thus, further research into the mechanisms and treatment of coagulation dysfunction after trauma will continue to prevent early and late deaths in severely injured patients.

15. SUBJECT TERMS
Coagulopathy, Injury, Shock, Protein C, Complement

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Introduction: Trauma remains the leading cause of death and disability in patients less than 40 years old, eclipsing ischemic heart disease, stroke and HIV/AIDS. Coagulopathy is common following severe trauma and is associated with poor outcome. Classically, traumatic coagulopathy has been thought to be due to acidosis and hypothermic inhibition of clot formation as well as dilution of coagulation factors from intravenous fluid therapy. However, two recent studies have reported that one quarter of major trauma patients are coagulopathic upon arrival in the emergency department before any fluid resuscitation. This coagulopathy was dependent on concomitant hypoperfusion and hence unlikely to be exclusively due to tissue injury and coagulation factor consumption. Despite the understanding of a strong link between injury, hypoperfusion and bleeding, the mechanisms for hypoperfusion-induced coagulopathy after trauma are unknown.

Our preliminary human data indicate that there is a close correlation between the development of this coagulopathy and the activation of the protein C pathway early after severe trauma. Furthermore, the inhibition of the protein C pathway completely corrects that early posttraumatic coagulopathy in our mouse model of trauma-hemorrhage that mimics the clinical findings. Thus, in this application, we are testing the overall hypothesis that acute traumatic coagulopathy is primarily caused by tissue hypoperfusion resulting in a complement-mediated activation of the protein C pathway.

Body:

Task 1. To determine the relationship between the activation of the protein C pathway, the complement cascade and the development of early coagulopathy and later end-organ injury associated with severe trauma in humans

To date and in the current funding period we have continued the sample collection and measurement at UCSF. To date we have successfully enrolled and collected samples on the 200 patients promised in the project milestones. Serial blood samples were drawn at the arrival in the emergency department, 6 12 and 24 hours after admission to the hospital for analysis of partial thromboplastin and prothrombin times, coagulation factors V and VIII activities, plasma levels of protein C, activated protein C, tissue plasminogen activator, and D-Dimers. Base deficit (BD) was used as a measure of tissue hypoperfusion.

Our results show that patients with tissue hypoperfusion and severe traumatic injury showed a strong activation of the protein C pathway in the bloodstream that was associated with a coagulopathy characterized by a deactivation of the coagulation factors V and VIII and a derepression of the fibrinolysis with high plasma levels of plasminogen activator and high D-dimers. Elevated plasma levels of activated protein C were significantly associated with increased mortality, organ injury, increased blood transfusion requirements, and reduced ICU ventilator-free days. Finally, the inability to recover physiologic plasma levels of protein C within 24 hours after trauma is associated with an increased risk to develop post-traumatic ventilator associated pneumonia. We have met and exceeded our recruitment goals as specified in the SOW. During our no cost

extension we are continuing to enroll and measure human patients at the UCSF/SFGH site. We have done this as we believe that because the infrastructure is in place and the value of samples was high it was justified to continue to collect beyond our original goals. Preliminary data on cause of death was presented at the 2011 ATACCC meeting. An additional abstract was sent using PCA analysis of our data for the 2012 AAST annual meeting and was written as a manuscript to the Journal of Trauma, which is under review. One additional abstract is planned for the Pacific Coast Surgical Association meeting in. We have one manuscript in press at the Journal of Trauma and we are in process of two additional manuscripts, which have just been submitted. We expect additional manuscripts to come from these abstracts and the exceptional dataset that this project has produced and hope to submit these papers (regarding Traumatic Brain Injury and poly trauma related to coagulopathy) during the next year (during which the project will continue with a granted no cost extension).

Task 2. To determine the role of protein C pathway in the development of early posttraumatic coagulopathy after trauma-hemorrhage in mice. Task 3. To determine the role of the complement in causing early coagulopathy following trauma-hemorrhage in mice.

We have continued the animal work and have characterized the protein C response in mice. During this period and due to planned budget reductions we have worked primarily on characterzing the time course of the aPC response in mice. To examine the mechanistic role of protein C in the development of endogenous acute coagulopathy (EAC), we continued to utilize a mouse model of trauma & hemorrhagic shock, characterized by the combination of tissue injury and severe metabolic acidosis, which we have successfully developed in our lab. We have previously published first mouse model of traumatic coagulopathy and the contribution of the protein C system to this mechanism.. Briefly, mice were subjected to one of four treatment groups: 1) C-control, 2) T- trauma (laparotomy), 3) H- hemorrhage (MAP 35mmHg x 60 minutes), 4) TH-trauma+hemorrhage. After 60 minutes, blood was drawn and plasma spun for analysis. Compared to C-mice, the TH-mice had a significantly elevated aPTT (23.3 vs 34.5 sec) and significantly increased levels of activated protein C (aPC) (2.30 vs. 13.58 ng/mL). In contrast, T- and H and H-mice did not develop an elevated aPTT or increased aPC. Selective inhibition of the anticoagulant property of aPC prevented the coagulopathy seen in response to trauma/hemorrhage (23.5 sec vs. 38.6 sec. [inhib. mAb vs control mAb]) with no impact on survival during the shock period. However, complete blockade of both the anticoagulant and cytoprotective functions of aPC, caused 100% mortality within 45 minutes of shock, with histopathology evidence of pulmonary thrombosis and perivascular hemorrhage. (Data not shown) These results indicate that our unique mouse model of trauma/hemorrhagic shock mimics our previous observations in trauma patients and demonstrates that EAC is mediated by the activation of the protein C pathway. We have now expanded these analyses to include functional measures of coagulation and platelet function. To do so we have expanded our model to include thromboelastographic measures of coagulation.

In this time we have repeated the model and measured mouse whole blood on Rotem[TM] thromboelastography. Our results indicate that Trauma and Shock result in impaired clot formation and strength (MCF shown, other measures not shown. In addition there is enhanced fibrinolysis (Data not shown). Interestingly new data suggests a period of hypercoagulability after severe trauma. The addition of hemorrhage results in

anticoagulation likely mediated by activated protein C. These perturbations correlated strongly with activated protein C and can be blocked by aPC antibody (Data not shown). This is in keeping with human data showing strong correlations between aPC, factor Va, VIIIa. In addition, the cytoprotective effect of protein C activation appears to be necessary for survival of the initial shock injury. We are beginning to expand these initial results to test varying types and degrees of injury. Specifically we are examining laparotomy vs. hind limb fracture and various degrees of shock to determine the relative contribution of tissue injury and shock to coagulopathy after trauma (data not shown). In addition we have continued to partner with Dr. Geoff Manley's lab to add a controlled cortical impact model of TBI. Initial experiments show that TBI alone is sufficient to produce coagulopathy which seems independent of activated protein C. (Data not shown) As we have previously published in humans the addition of hemorrhagic shock seems necessary for activation of protein C and significant coagulopathy and this combination of hemorrhagic shock and TBI is currently being performed.

Key Research Accomplishments

a. Demonstration that the early post-traumatic coagulopathy is mediated by the activation of the protein C pathway.

b. Demonstration that the inhibition of both the anticoagulant and cytoprotective functions of protein C is associated with poor outcome after trauma-hemorrhage, indicating an important protective role for the cytoprotective, PAR-1-dependent domain of the protein C in preventing systemic intravascular coagulation in the microcirculation after severe trauma-hemorrhage.

c. Demonstration that the anticoagulant aPC response and later inflammatory response after injury are linked..

c. Demonstration that aPC results in functional coagulation perturbations in mice which mimics functionally the ATC response in humans. In addition we have shown that complement can activate the protein C pathway after severe trauma-hemorrhage via its lectin pathway.

Reportable Outcomes
See below for a list of presentations and publications germane to the award.

Cohen MJ, Call M, Rahn P, Calfee C, Esmon C, Brohi K, Baccheti P, Pittet JF. Critical Role of activated protein C in early coagulopathy and later organ failure, infection and death in trauma patients. Ann Surg (in press), 2011.

Chesebro BB, Rahn P, Carles M, Esmon CT, Xu J, Brohi K, Frith D, Pittet JF, Cohen MJ. Increase in activated protein C mediates acute traumatic coagulopathy in mice. Shock 32:659-65, 2009.

Kutcher ME, Xu J, Vilardi RF, Ho C, Esmon CT, **Cohen MJ**. Extracellular histone release in response to traumatic injury: Implications for a compensatory role of activated protein C. **J Trauma Acute Care Surg**. 2012 Dec;73(6):1387-92.

Kutcher ME, Cripps MW, McCreery RC, Crane IM, Greenberg MD, Cachola LM, Redick BJ, Nelson MF, **Cohen MJ**. Criteria for empiric treatment of hyperfibrinolysis after trauma. **J Trauma Acute Care Surg**. 2012 Jul;73(1):87-93.

Kutcher ME, Redick BJ, McCreery RC, Crane IM, Greenberg MD, Cachola LM, Nelson MF, **Cohen MJ**. Characterization of platelet dysfunction after trauma. **J Trauma Acute Care Surg**. 2012 Jul;73(1):13-9.

Narayan R, Curd, V, Singh T, Dailey A, McCreery R, Xu D, Crane I, Greenberg MD, Nelson MR, Knudson MM, Fiebig E, Kutcher ME, **Cohen MJ:** Timing and Clinical Accuracy of Massive Transfusion Protocol Activation: In Press (**J Trauma Acute Care Surgery)**

Cripps MW, Kutcher ME, McCreery RC, Crane IM, Greenberg MD, Cachola LM, Redick BJ, Nelson MF, **Cohen MJ:** Cause and timing of death in massively transfused trauma patients. **J Trauma Acute Care Surg,** In Press

Conclusion:

Clinical study:

Our clinical outcomes initially reported (as below) have been codified and strengthened in the second year of the award.

a. Early traumatic coagulopathy occurs only in the presence of tissue hypoperfusion and severe traumatic injury and is associated with the activation of the protein C pathway. Plasma levels of activated protein C at the admission to the hospital are predictive of clinical outcomes following major trauma. The inability to recover of physiologic plasma values of protein C within 24 hours after injury is associated with an increased propensity to later develop nosocomial lung infection, suggesting a role for the protein C pathway in the maintenance of the function of the alveolar-capillary barrier. This is further associated with activation of inflammatory DAMPs including HMGB1 and sRAGE and associated with the cleaving of extracellular histones.

b. The activation of complement within 30 minutes after severe injury correlates with the development of a protein C-mediated coagulopathy and fibrinolysis in trauma patients.

Mice studies:

a. The results of the first mouse study indicate that our unique mouse model of trauma/hemorrhagic shock mimics our previous observations in trauma patients and demonstrates that early traumatic coagulopathy is mediated by the activation of the protein C pathway. In addition, the cytoprotective effect of protein C activation appears to be necessary for survival from the initial shock tissue injury.

b. The preliminary results of the second mouse study indicate complement can activate protein C via its lectin pathway

References: See above for cited papers in the report.