AWARD NUMBER: W81XWH-18-1-0108

TITLE: Glycocalyx Mechanoregulation of Angiopoietin-2 and Post-Traumatic Lung Injury

PRINCIPAL INVESTIGATOR: Jillian R. Richter, PhD

CONTRACTING ORGANIZATION: University of Alabama at Birmingham
Birmingham, Alabama 35294

REPORT DATE: May 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
May 2019	Annual	1 May 2018 - 30 Apr 2019

4. TITLE AND SUBTITLE

Glycocalyx Mechanoregulation of Angiopoietin-2 and Post-Traumatic Lung Injury

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-18-1-0108

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Jillian Richter, PhD

E-Mail: jillianrichter@uabmc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Alabama at Birmingham
Birmingham, Alabama 35294

8. PERFORMING ORGANIZATION REPORT

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Preliminary results from our clinical studies show elevated levels of activated heparanase (HPSE) in plasma collected from trauma patients upon arrival to our Level I Trauma Center. Additionally, these patients have elevated levels of Angiopoeitin-2, a well-established biomarker of endothelial cell (EC) inflammation. Results from our in vitro studies suggest that degradation of heparan sulfate causes lung microvascular EC to produce more Agpt-2, suggestive of a mechanistic linkage between HPSE enzyme activity and dysregulation of Agpt-2 levels. Since Agpt-2 increases the permeability of blood vessels and promotes lung injury, blocking the activity of HPSE and preventing Agpt-2 release could be a viable strategy for preventing lung injury in trauma patients. Preliminary data from animal studies show that blocking HPSE activity with the drug Roneparstat may attenuate pulmonary cytokine production caused by trauma-hemorrhage and resuscitation. These findings are important for the field of trauma research since they address a clinical need for developing therapeutic strategies that target mechanisms responsible for vascular dysfunction and organ failure in trauma patients.

15. SUBJECT TERMS

Heparanase, angiopoietin dysregulation, hemorrhagic shock, resuscitation

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
Unclassified	Unclassified	Unclassified	UU	14	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

Page

1. INTRODUCTION:

The overall objective of this research is to study the role of trauma-activated heparanase (HPSE) and endothelial glycocalyx layer damage (e.g., loss of Syndecan-1 from endothelial cell surface) in mediating upregulation of the vascular destabilizing cytokine, angiopoietin-2 (Agpt-2). Glycocalyx damage and increased Agpt-2 production result in endotheliopathy and pulmonary injury that promotes late-stage mortality in trauma patients. Our Aim 1 studies are focused on determining whether glycocalyx damage via loss of syndecan-1 (Syn1)-mediated Akt signaling leads to increased synthesis of Agpt-2. In Aim 2, we are (a) characterizing HPSE activity and Agpt-2 levels in plasma collected from injured trauma patients, (b) demonstrating the role of HPSE in mechanoregulation of Agpt-2, and (c) demonstrating the therapeutic efficacy of HPSE inhibitor, Roneparstat, for preventing HPSE-mediated GCX damage in human lung microvascular EC and lung injury in mice.

2. KEYWORDS:

trauma, hemorrhagic shock, lung injury, glycocalyx, syndecan, heparan sulfate, heparanase, angiopoietin, endothelial barrier dysfunction, Akt

3. ACCOMPLISHMENTS:

What were the major goals of the project?

- Task 1: Measure Akt activation, Agpt-2 synthesis/release and endotheliopathy in human lung microvascular endothelial cells (MVEC) in response to hemorrhagic/resuscitative flow and pressure. Target Completion: Months 1-4; Current Progress: 90%
- Task 2: Using the developed mouse model of trauma-hemorrhage and resuscitation (THR), measure pulmonary Akt signaling, Agpt-2 and lung injury in Syn1 KO mice compared to C57Bl/6 wildtype mice at 0.5, 4 and 24 h after resuscitation compared to baseline (non-hemorrhaged) levels. In parallel studies, some mice will be treated with the Akt activator SC79 at resuscitation. Target Completion: Months 3-6; Current Progress: 50%
- Task 3: Measure Akt activation, Agpt-2 synthesis/release and endotheliopathy in human lung MVEC following treatment with patient plasma from trauma patients or healthy controls. Target Completion: Months 6-12; Current Progress: 75%
- Task 4: Using the *in vivo* trauma-hemorrhage and resuscitation model, measure glycocalyx shedding and lung injury in C57Bl/6 mice after treatment with Roneparstat at 0.5, 4, and 24 h post-resuscitation. Target Completion: Months 16-18; Current Progress: 50%

What was accomplished under these goals?

Task 1: *In vitro* evaluation of Syn1-mediated Akt activation, Agpt-2 release and inflammation in MVEC in response to changes in flow that mimic hemodynamics of hemorrhagic shock and resuscitation

- Major Activities: In vitro studies were performed using an Endothelial Cell Perfusion System (ECPS) to expose human lung MVEC to fluid dynamic conditions that mimic changes in vascular hemodynamics caused by hemorrhagic shock and fluid resuscitation. MVEC were grown to confluence on the luminal surface of polydimethylsiloxane (PDMS) tubes and then perfused with culture medium for 24h prior to exposure to 1h of low flow (to mimic conditions of hemorrhagic

shock) followed by restoration of flow (to mimic conditions of permissive hypotensive resuscitation). For endpoint analyses, cells were fixed for immunostaining or processed for protein/RNA isolation.

- Specific Objectives:
  1. Measure Akt activation, Agpt-2 production and inflammation in lung MVEC after exposure to 1h of low flow followed by restoration of hypotensive flow.
  2. Treat MVEC with MMP9 prior to low flow to cause Syn1 shedding in order to elucidate the role of Syn1 in mediating cellular response to changes in flow.
- Significant Results/Key Outcomes:
  1. Exposure to 1h of low flow followed by restoration of normal flow decreases Akt phosphorylation and causes loss of cellular Agpt-2 (Fig. 1).

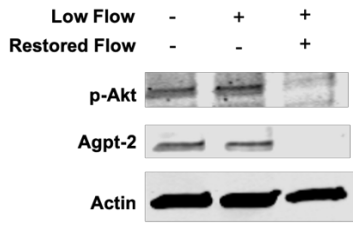

Figure 1. Protein expression of p-Akt and Agpt-2 in lung MVEC in response to changes in flow that mimic hemorrhagic shock and resuscitation. MVEC were conditioned to a dynamic flow environment for 24h, then exposed to 1h of low flow followed by restoration of flow over a 15min period. Protein was isolated from cells prior to low flow (control), immediately after low flow or 30min after restoration of flow.

2. Exposure to 1h of low flow followed by restoration of normal flow results in increased phosphorylation and nuclear localization of p65-NFkB (Fig 2).

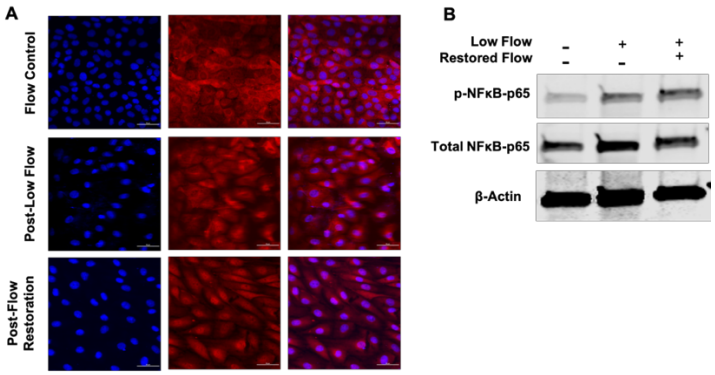

Figure 2. NFkB activation in lung MVEC in response to changes in flow that mimic hemorrhagic shock and resuscitation. MVEC were conditioned to a dynamic flow environment for 24h, then exposed to 1h of low flow followed by restoration of flow over a 15min period. Cells were either (**A**) fixed for NFkB-p65 immunostaining and confocal imaging or (**B**) protein was isolated from cells prior to low flow (control), immediately after low flow or 30min after restoration of flow.

- Other Achievements: Using the ECPS, we have also shown that changes in flow lead to increased activation of HPSE (Fig. 3). Additionally, degradation of heparan sulfate from MVEC results in increased release of Agpt-2 (Fig. 4). Together, these data indicate that activation of HPSE in response to hemorrhagic shock and loss of heparan sulfate may act to regulate Agpt-2 release and subsequent endotheliopathy.

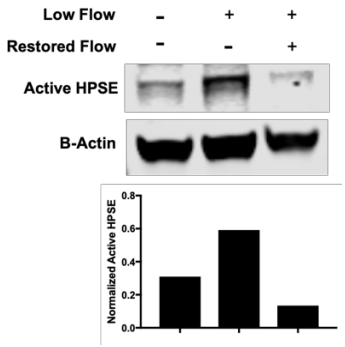

Figure 3. HPSE activation in lung MVEC in response to changes in flow that mimic hemorrhagic shock and resuscitation. MVEC were conditioned to a dynamic flow environment for 24h, then exposed to 1h of low flow followed by restoration of flow over a 15min period. Protein was isolated from cells prior to low flow (control), immediately after low flow or 30min after restoration of flow, and active (50kDa) HPSE was detected via Western blot.

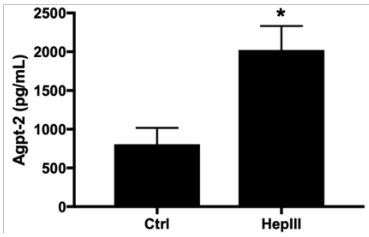

Figure 4. Agpt-2 release from MVEC following degradation of heparan sulfate with the bacterial heparanase (heparitinase-III; HepIII). Lung MVEC were treated with HepII for 30min to degrade heparan sulfate. Following treatment, cells were washed and incubated in complete growth medium. At 24h, Agpt-2 levels were measured in culture medium via ELISA. *p<0.01. Data reported as mean+/- standard error.

- Stated Goals Not Met: We have not yet demonstrated the role of Syn1 in mediating the cellular response to low flow/restored flow shown in Figures 1 and 2. The above experiments will be repeated with the addition of MMP9 to cause Syn1 degradation prior to changes in flow.

Task 2: *In vivo* analysis of Akt and Agpt-2 in WT mice compared to Syn1 KO mice in order to demonstrate the role of Syn1 in Agpt-2-mediated lung injury after THR

- Major Activities: *In vivo* studies were performed using a mouse model of THR. Briefly, anesthetized mice underwent a laparotomy followed by 60 min period of hemorrhagic shock (MAP = 25+/-5 mmHg) and 20 min period of resuscitation with packed red blood cells and fresh frozen plasma (1:1). Sham animals underwent laparotomy and cannulation but were not hemorrhaged or resuscitated. Mice were sacrificed at 30 min or 4 h after resuscitation, and plasma and lungs were processed for endpoint measurements.
- Specific Objectives:
  1. Measure pulmonary levels of phosphorylated and total Akt and Agpt-2 in WT and Syn1 KO mice after THR
  2. Measure systemic Agpt-2 levels in WT and Syn1 KO mice after THR
  3. Demonstrate whether restoration of Akt signaling prevents lung injury in WT and Syn1 KO mice using the Akt activator SC79
- Significant Results/Key Outcomes:
  1. Pulmonary phospho-Akt levels decrease after THR in WT mice (Fig. 5).

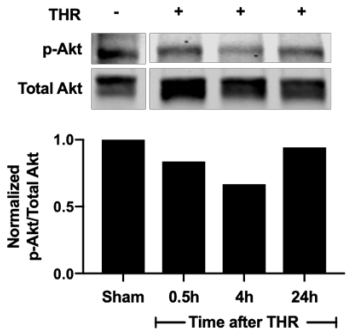

Figure 5. Pulmonary levels of Akt phosphorylation in WT mice following THR. Total and phosphorylated Akt were measured in lung homogenate isolated from mice at 0.5, 4, or 24h after hemorrhagic shock and resuscitation and compared to Sham mice.

2. Systemic levels of Agpt-2 increase after THR in WT mice (Fig. 6).

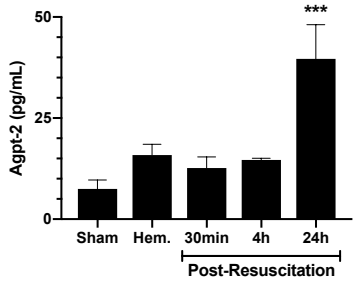

Figure 6. Plasma levels of Agpt-2 in WT mice following THR. Agpt-2 levels were measured in plasma collected from mice immediately after hemorrhagic shock or at 0.5, 4 or 24h after resuscitation and compared to Sham mice. Data reported as mean +/- standard error. N=2-4 per group. Statistical significance determined by ANOVA and Tukey's multiple comparisons test. ***P<0.001

- Other Achievements: Nothing to report.
- Stated Goals Not Met: We have not yet completed our THR studies in Syn1 KO mice or given the Akt activator, SC79, to mice during resuscitation. So, thus far, we have not accomplished the goal of demonstrating *in vivo* the role of Syn1-mediated Akt signaling in regulating Agpt-2 production and release. Further studies over the remainder of the funding period will continue to elucidate these mechanisms.

Task 3: *Ex vivo* studies of Akt signaling, Agpt-2 synthesis and inflammation in human lung MVEC in response to HPSE in trauma patient plasma

- Major Activities: We have initiated a clinical study to collect plasma from trauma patients upon arrival to our Level I Trauma Center at UAB. Preliminary analyses have been performed to measure plasma levels of HPSE activity using an assay that estimates HPSE activity based on measurements of heparan sulfate degradation. The activity levels of HPSE present in trauma patient plasma has been compared to plasma previously collected from a population of healthy controls.
- Specific Objectives:
  1. Characterize HPSE activity in plasma after traumatic injury
  2. Evaluate the utility of Roneparstat for inhibiting heparan sulfate and Syn1 shedding in MVEC
  3. Evaluate the role of plasma HPSE in causing inhibition of Akt signaling and MVEC dysfunction in response to hemorrhagic and resuscitative flow
- Significant Results/Key Outcomes:
  1. Plasma from trauma patients has elevated levels of HPSE activity compared to controls (Fig. 7)

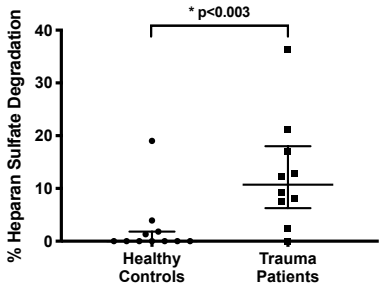

Figure 7. HPSE activity measurements in plasma from healthy controls and trauma patients. HPSE activity is indicated as a percentage of heparan sulfate degradation. More heparan sulfate is degraded by plasma from trauma patients compared to controls, indicating that HPSE activity levels are elevated after traumatic injury. N=10 per group. Data reported as median and interquartile range. Statistical significance evaluated using Mann-Whitney test.

- Other Achievements: In addition to measurements of HPSE activity, we have also measured other markers of vascular injury/dysfunction in our trauma patient plasma, including Agpt-1, Agpt-2 and Syndecan-1 (Fig. 8).

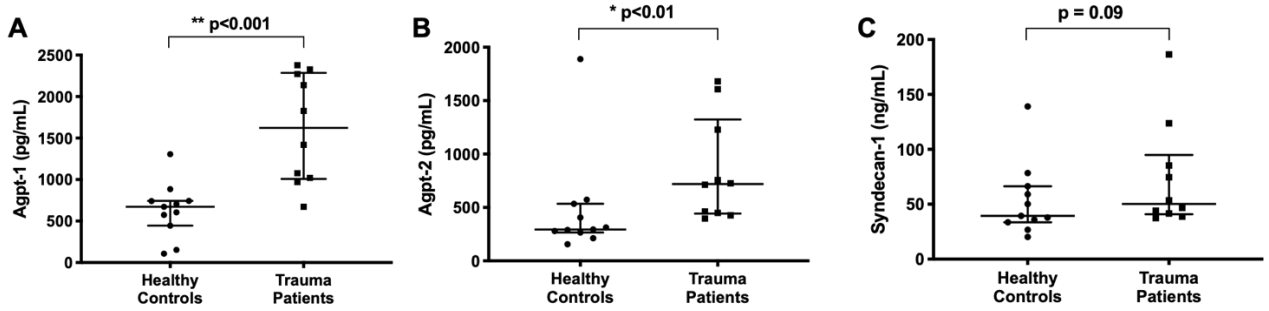

Figure 8. Biomarker measurements of endothelial cell inflammation (Agpt-1 and Agpt-2) and glycocalyx damage (Syndecan-1). Plasma levels of both (A) Agpt-1 and (B) Agpt-2 are significantly elevated after traumatic injury**.** No significant difference is observed in levels of Syndecan-1. N=10 per group. Data reported as median and interquartile range. Statistical significance evaluated using Mann-Whitney test.

- Stated Goals Not Met: Studies are in progress to (1) evaluate the utility of Roneparstat for inhibiting heparan sulfate and Syn1 shedding in MVEC caused by trauma-activated HPSE and (2) evaluate the role of plasma HPSE in causing inhibition of Akt signaling and MVEC dysfunction in response to hemorrhagic and resuscitative flow.

Task 4: Evaluate therapeutic efficacy of Roneparstat for preventing glycocalyx damage and lung injury in mice after trauma-hemorrhage and resuscitation

- Major Activities: Using the THR model described above, we have evaluated the viability of Roneparstat as a therapeutic agent that can be given at the time of resuscitation to block HPSE activity and attenuate lung injury. Roneparstat was administered intravascularly to mice following hemorrhagic shock. At 4h after resuscitation, plasma was collected for Syn1 measurements of glycocalyx damage and lung tissue was processed for immunohistochemical staining of polymorphonuclear (PMN) cells as an indicator of pulmonary inflammation.

- Specific Objectives:
  1. Perform a dose response study to determine the safety of administering Roneparstat intravascularly.
  2. Assess glycocalyx damage and lung injury in THR mice treated with Roneparstat compared to untreated mice

- Significant Results/Key Outcomes:
  1. No mortality was observed when Roneparstat was administered via retro-orbital injections to normal C57Bl/6 mice at 1 and 10 mg/kg. Some toxicity/mortality was observed when Roneparstat was given to mice in hemorrhagic shock. Dose optimization studies are underway to determine a tolerable dose for animals in the THR model.
  2. Of the surviving mice, no significant difference was observed in glycocalyx damage as measured by circulating Syn1 levels (Fig. 9A). Pulmonary PMN counts were elevated after THR in both Roneparstat-treated and untreated mice compared to Sham mice (Fig. 9B).

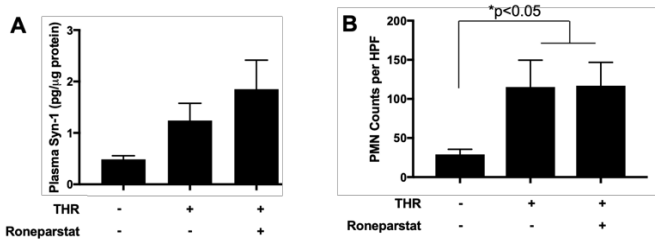

Figure 9. Glycocalyx damage and lung PMN recruitment in mice 4h after THR. (A) No significant difference in glycocalyx damage was observed between sham, THR and THR+Roneparstat treated mice as indicated by circulating levels of Syn1. **(B)** THR mice had increased numbers of pulmonary PMNs compared to Sham animals. Treatment with Roneparstat after hemorrhagic shock did not attenuate PMN recruitment into the lungs. Data reported as mean +/- standard error. N=3 animals per group.

- Other Achievements: We have also given Roneparstat to mice prior to injury to demonstrate proof-of-concept that trauma-activated HPSE plays a role in mediating glycocalyx damage and lung injury after THR. In these pilot studies, administration of Roneparstat to mice 2h prior to THR attenuated pulmonary levels of many proinflammatory/chemoattractant cytokines (Fig. 10) and systemic levels of IL-1b (Fig. 11). We will continue to develop the use of Roneparstat and evaluate different dosages and routes of delivering the drug that may prove therapeutically viable for attenuating lung injury in our THR model.

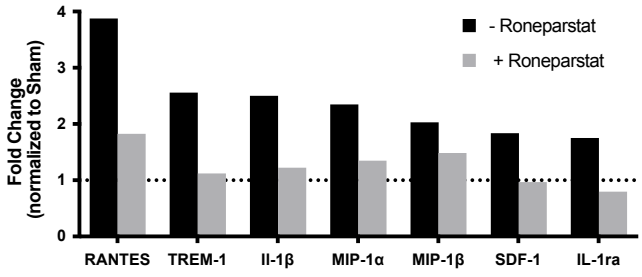

Figure 10. Pulmonary levels of proinflammatory cytokines and chemokines at 4h after THR. Mice were injected i.p. with Roneparstat or normal saline 2h prior to THR. Lungs were isolated and homogenized 4h after resuscitation, and a Proteome Profiler Mouse Cytokine Array Kit (R&D Systems) was used to assess levels of 40 different cytokines, chemokines and acute phase proteins. Protein expression levels were quantified with densitometry and are reported as a fold change relative to Sham animals (dotted line) for each analyte. The top 7 analytes with the largest difference between groups are presented, which indicate that inhibition of HPSE with Roneparstat prior to THR results in decreased levels of many proinflammatory mediators. (n=1 array per group)

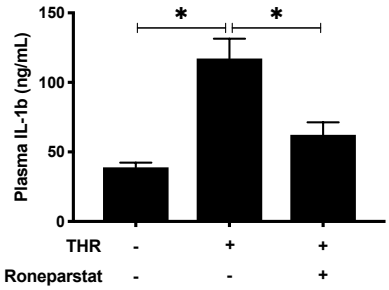

Figure 11. Plasma levels of IL-1b in mice 4h after THR. Mice were injected i.p. with Roneparstat or normal saline 2h prior to THR. Plasma was collected from mice 4h after resuscitation, and IL-1b levels were measured via ELISA. Compared to Sham, THR significantly increased circulating levels of IL-1b, which was significantly attenuated by pre-treatment with Roneparstat. *P<0.01. Data are reported as mean +/- standard error. N=3-4 per group.

- Stated Goals Not Met: Our primary goal of this Task is to evaluate the therapeutic efficacy of Roneparstat as a HPSE inhibitor that is given at the time of resuscitation from hemorrhagic shock. We have not yet determined a safe and effective dose that can administered to mice in shock. Our current studies are focused both on (1) demonstrating that HPSE plays a role in lung injury after THR (by inhibiting HPSE prior to THR) and (2) determining a tolerable dose of Roneparstat that can been given to mice after hemorrhagic shock as a treatment to attenuate inflammation post-THR.

What opportunities for training and professional development has the project provided?

Training in basic science research for residents and students

How were the results disseminated to communities of interest?

UAB Department of Surgery, Works in Progress Seminar Series
UAB Department of Surgery, Resident Research Symposium
Military Health System Research Symposium

What do you plan to do during the next reporting period to accomplish the goals?

- A major goal of the *in vivo* studies is to attenuate THR-induced lung injury caused by HSPE activity. We will continue to evaluate different Roneparstat dosages and administration routes in order to develop a viable strategy for inhibiting HPSE activity caused by THR.
- For the clinical study, we will continue to recruit and enroll trauma patients admitted to our trauma center until we achieve our target enrollment. In addition to biomarker measurements and correlations with clinical outcomes, we will perform ex vivo experiments to demonstrate the role of trauma plasma HPSE in mediating Agpt-2 release and barrier dysfunction in MVEC.
- *In vitro* studies will involve elucidating the role of Syn1 in mediating MVEC response to changes in fluid flow/pressure. We will silence Syn1 expression in MVEC and evaluate Akt activation, Agpt-2 release and inflammation in the ECPS model of hemorrhagic shock and resuscitation compared to the data reported above.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

A primary finding of the funded research, thus far, is that the HSPE activity and Agpt-2 levels are elevated in trauma patients upon arrival to hospital. Preliminary evidence from our in vitro studies also suggest that HPSE's biological function of degrading heparan sulfate from the surface of endothelial cells causes lung MVEC to produce more Agpt-2. Since Agpt-2 increases the permeability of blood vessels and promotes lung injury, blocking the activity of HPSE and preventing Agpt-2 release could be a viable strategy for preventing lung injury in trauma patients. Preliminary data from animal studies show that blocking HPSE activity with the drug Roneparstat improves pulmonary cytokine production caused by THR. However, more work is needed to optimize the dose, administration route and timing of Roneparstat to observe differences in functional measures related to lung injury after trauma. These findings are important for the field of trauma research since they address a clinical need for developing therapeutic strategies that target mechanisms responsible for vascular dysfunction and organ failure in trauma patients.

What was the impact on other disciplines?

Nothing to Report

What was the impact on technology transfer?

Nothing to Report

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS:

- Given the mortality of THR mice treated with Roneparstat intravascularly, we have chosen to administer Roneparstat subcutaneously and intraperitoneally prior to THR in order to establish a basal level of HPSE inhibition that will allow us to demonstrate the proof-of-principle role of HPSE in mediating post-traumatic lung injury. Simultaneously, we are also evaluating safe therapeutic strategies for inhibiting HPSE via intravascular treatments since we feel that this the most clinically viable strategy for acute inhibition of HPSE in trauma patients.
- Our collaborator and provider of the Syndecan-1 KO mice, Dr. Randal Dull, is no longer at the University of Illinois at Chicago. Therefore, we have been unable to complete the proposed *in vivo* experiments aimed at studying the role of Syn-1 in mediating post-traumatic lung injury. As an alternative, we will rely on *in vitro* evaluations in which we silence Syn1 in MVEC.

Actual or anticipated problems or delays and actions or plans to resolve them

- Given the lack of availability of Syndecan-1 KO mice, we will evaluate the role of Syn1 in mediating Agpt-2 release with *in vitro* studies using lung MVEC.

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to Report

Books or other non-periodical, one-time publications.

Nothing to Report

Other publications, conference papers and presentations.

R.M. Uhlich, L. Zheng, P. Hu, J.O. Jansen, J.D. Kerby, J.R. Richter. Trauma-Associated Glycocalyx Damage and Angiopoietin Dysregulation: Is There a Link? Accepted for presentation at Military Health Services Research Symposium. August 2019.

- **Website(s) or other Internet site(s)**

Nothing to Report

- **Technologies or techniques**

Nothing to Report

- **Inventions, patent applications, and/or licenses**

Nothing to Report

- **Other Products**

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Jillian Richter
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	4
Contribution to Project:	Oversee project; experimental design; data analysis; interpretation of findings
Funding Support:	Departmental Funds

Name:	Lei Zheng
Project Role:	Research Technician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Conducts experiments
Funding Support:	Departmental Funds

Name:	Rindi Uhlich
Project Role:	Research Fellow
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	Conducts experiments
Funding Support:	Departmental Funds

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

- Nothing to report for Jillian Richter (PI)
- Changes in *Current Active Support* for Jean Francois Pittet (Co-Investigator) (previously not listed or listed as "Pending"):

Pittet, Jean Francois
ACTIVE

R01HL139563 (PIs: Ding/Pittet) NIH/NIHLB Molecular Mechanisms of GBD Switch-Mediated Lung Barrier Dysfunction The goal of this grant is to understand how N-WASP regulates lung endothelial and epithelial barrier function after infection with *Pseudomonas aeruginosa*.	Dates of Approved/Proposed Project 06/01/2018-05/31/2022 Annual Direct Costs $1,764,385 TDC	Person Months 2.4 calendar

Pittet, Jean Francois ACTIVE		
R01HL141473 (PI: Richter, W.) NIH/NHLBI Regulation of phosphodiesterases and cAMP signaling during the host-pathogen interaction in the pulmonary endothelium This project will test the hypothesis that distinct bacterial virulence factors trigger changes in the activity and function of endothelial Type 4 cAMP-phosphodiesterases (PDE4s), leading to a desensitization and dysregulation of cAMP signaling and endothelial barrier disruption.	Dates of Approved/Proposed Project 04/01/2018-03/31/2022 Annual Direct Costs $1,250,000 TDC	Person Months 0.6 calendar
PR160349 (PI: Zmijewski) Department of Defense Therapeutic Recovery of Immune and Lung Tissue Bioenergetic Homeostasis in Sepsis-Induced Immunosuppression The major goal of this project is to provide (1) significant mechanistic insights into the understanding AMPK regulation and function during sepsis-induced immunosuppression; (2) motivate the re-purposing of AMPK activator metformin and GSK3β inhibitor Tideglusib, as novel therapeutics interventions to diminish a high risk of secondary bacterial lung infections, injury and respiratory failure among sepsis-immunosuppressed patients.	Dates of Approved/Proposed Project 10/01/2017-09/31/2020 Annual Direct Costs $400,000 TDC	Person Months 1.2 calendar

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Not Applicable.

QUAD CHARTS: Not Applicable.

9. APPENDICES

Appendix I: 2019 MHSRS Abstract

Trauma-Associated Glycocalyx Damage and Angiopoietin Dysregulation: Is there a Link?

Introduction

Hemorrhagic shock is the leading cause of preventable death on the battlefield. Hypoperfusion and inflammation associated with severe hemorrhage leads to vascular endothelial cell damage and shedding of syndecan-1 (Syn-1), a major constituent of the endothelial glycocalyx (GCX). Damage to the vascular endothelium is caused, in part, by release of angiopoietin-2 (Agpt-2), a proinflammatory cytokine that counteracts the anti-inflammatory signaling of angiopoietin-1 (Agpt-1). We recently showed that the ratio between Agpt-1 and Agpt-2 is dysregulated in pediatric trauma patients, which correlated with Syn-1 shedding and was associated with worse clinical outcomes. Based on these findings and other mechanistic studies, we hypothesized that GCX damage caused by traumatic injury may contribute to Agpt-2 release and dysregulation of the Agpt ratio. The objective of the current study is to characterize the time dependent relationship of Syn-1 shedding and Agpt dysregulation following severe trauma in an adult population.

Methods

As part of our ongoing investigation, plasma biomarkers were measured from adult trauma patients with an Assessment of Blood Consumption (ABC) score ≥ 2 admitted to our Level I trauma center. Peripheral blood samples were obtained at 0, 12, 24, and 48 hours from time of admission. For this preliminary analysis, plasma levels of Syn-1, Agpt-1, and Agpt-2 were measured by ELISA for 10 trauma patients and 12 healthy adult controls. Protein expression profiles in the injured patients were analyzed over time and compared to control levels. *In vitro* studies were performed to more directly evaluate the effect of GCX damage on Agpt-2 release from endothelial cells. Human pulmonary microvascular endothelial cells were treated with heparinase III (Hep III; 15mU/mL) for 30 minutes, after which the cells were washed and cultured in complete growth medium. Twenty-four hours later, Agpt-2 levels were measured in the conditioned medium and compared to untreated controls.

Results

Median plasma levels of Agpt-1 at 0 h were significantly higher in trauma patients than healthy controls ($p < 0.05$). Agpt-1 levels gradually returned to control levels by 48 h post-injury. Plasma expression of Agpt-2 increased over time in the injured cohort and were significantly higher than controls at 12, 24 and 48 h ($p < 0.01$). Agpt-2 to Agpt-1 ratios were similar to control ratios at the time of admission. Ratios increased over time after injury and were significantly higher at 24 and 48 h compared to Agpt ratios at 0 h ($p < 0.05$). Median Syn-1 levels at 0 h were slightly higher than controls, but this was not determined to be statistically significant. *In vitro* studies demonstrated that loss of GCX integrity after Hep III treatment resulted in increased Agpt-2 release by 24 h when compared to untreated controls ($p < 0.05$).

Conclusions

Our preliminary data suggests that an inverse and dynamic relationship between Agpt-1 and Agpt-2 protein expression exists, in a time dependent fashion following severe injury. *In vitro* findings suggest that enzymatic degradation of the endothelial GCX promotes release of Agpt-2 from vascular endothelial cells. Our future analyses will characterize the temporal profile of Syn-1

shedding after trauma and evaluate the correlation between GCX damage and Agpt dysregulation in an adult trauma population. Additionally, we will determine the role of the enzyme heparanase, a potential therapeutic target, in mediating GCX damage and Agpt-2 release in the setting of hemorrhagic shock.

Authors: Rindi Uhlich, Lei Zhang, Parker Hu, Jan Jansen, Jeffrey Kerby, Jillian Richter