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TITLE: Prothrombin Complex Concentrate for Prolonged Field Care of War Casualties

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CONTRACTING ORGANIZATION: Oregon Health & Science University, Portland, OR

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14. ABSTRACT

Patients who initially survive from traumatic thoracic injury are at risk for Acute Respiratory Distress Syndrome (ARDS). The only proven treatments available once ARDS has developed are low tidal volume ventilation (ARDSnet) and proning, but there is no existing treatment strategy to prevent the onset of ARDS following traumatic injury. As a potential solution, recent evidence suggest that prothrombin complex concentrate (Kcentra) acts similarly to plasma to prevent vascular leak and edema, but this has not been investigated in the trauma setting. Therefore, the purpose of this project is to conduct a series of in vitro and in vivo studies to determine if the therapeutic administration of Kcentra prevents the development of ARDS following pulmonary contusion and hemorrhagic shock.

15. SUBJECT TERMS

None Listed

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1. INTRODUCTION:

Patients who initially survive from traumatic thoracic injury are at risk for Acute Respiratory Distress Syndrome (ARDS). The only proven treatments available once ARDS has developed are low tidal volume ventilation (ARDSnet) and proning, but there is no existing treatment strategy to prevent the onset of ARDS following traumatic injury. As a potential solution, recent evidence suggest that prothrombin complex concentrate (Kcentra) acts similarly to plasma to prevent vascular leak and edema, but this has not been investigated in the trauma setting. Therefore, the purpose of this project is to conduct a series of *in vitro* and *in vivo* studies to determine if the therapeutic administration of Kcentra prevents the development of ARDS following pulmonary contusion and hemorrhagic shock.

2. KEYWORDS:

Swine, shock, pulmonary contusion, mesenchymal stem cells, acute respiratory distress syndrome, liver injury, endotheliopathy.

3. ACCOMPLISHMENTS:

What were the major goals of the project?
The major tasks listed in the SOW include:

1) Obtain regulatory approval, run Rat HS model
2) Run mouse model of hemorrhagic shock to test doses of Kcentra
3) Tissue and Molecular Analysis of Rat Model of HS
4) Run randomized study in swine model of lung injury and hemorrhagic shock
5) Obtain regulatory approval and test swine model.
6) Begin randomized study in swine
7) Assess blood and tissue samples for inflammation
8) Submit abstracts, publications, and final report to Army

What was accomplished under these goals?
The cumulative achievements for each year of the project are as follows:
Calendar Year 2017: Attain ACURO approval and IACUC approval for all studies
Calendar Year 2018: Perform model development in both rats and swine. Being randomized study in rats and swine. Complete rat experiments.
Calendar Year 2019: Complete swine experiments. Complete analysis of rat samples. Begin running 24-hour model in mice.
Calendar Year 2020: Complete analysis of swine samples. Complete 24-hour mouse model.

Major activities and specific objectives accomplished in this project include:
Major Task #1: Obtain regulatory approval, Rat HS model (UCSF) – 100% complete
UCSF acquired IACUC approval on 10/24/2017 and ACURO approval on 1/10/2018.

Rat model of hemorrhagic shock 100% completed.

Major Task #2: Model of HS, 24 hour survival (Changes planned for a switch from rats to mice) (UCSF) (70% complete)
We have received IACUC and ACURO approval to switch from rats to mice for the 24 hour model. Due to COVID-19 stay in place order, we were delayed in starting this study. We ran the 24 hour model in mice and are analyzing the effect of PCC on lung pathology, inflammation and inflammatory gene expression. These studies will continue until 9/22.

We have submitted an abstract to shock which has been accepted for poster presentation in June, 2022 in Toronto. These data suggest that in this particular model recovery from shock and trauma in the lungs occurs by 24 hours. This is of importance to the field since the model is depicting acute but not chronic injury.

RECOVERY OF ENDOTHELIOPATY AT 24 HOURS IN AN ESTABLISHED MOUSE MODEL OF HEMORRHAGIC SHOCK AND TRAUMA

Mark Barry MD[1], Alpa Trivedi PhD[2], Lindsay Vivona BS[2], Jenna Chui[2], Byron Miyazawa BS[2], Praneeti Pathipati PhD[2], Martin Schreiber MD[3], Shibani Pati MD, PhD[2]*

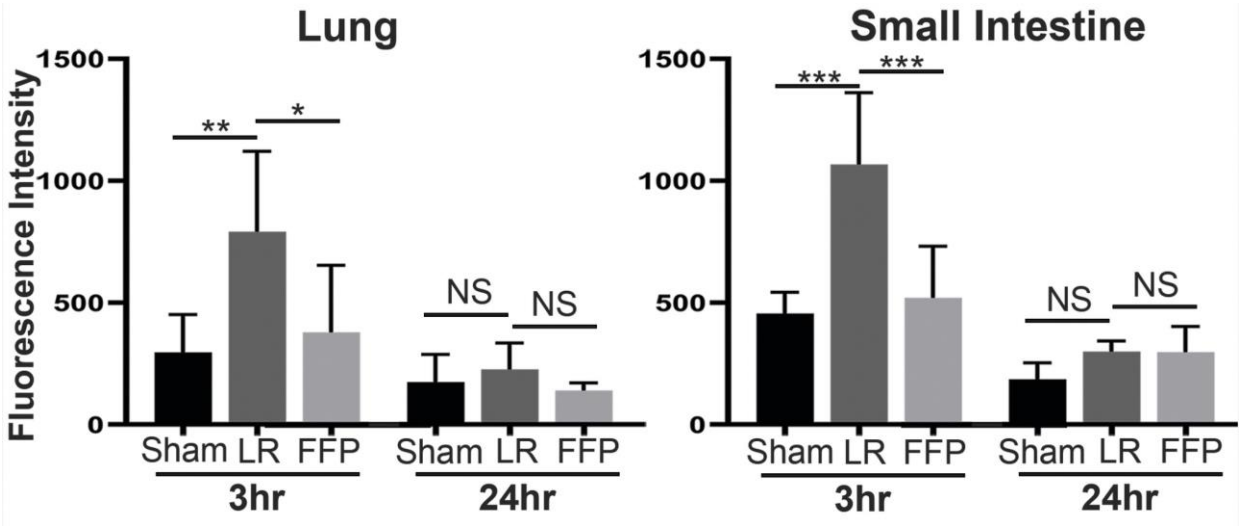
Introduction: The endotheliopathy of trauma develops early after injury and consists of increased vascular permeability, inflammation, and dysfunctional coagulation. Persistence of these abnormalities ultimately leads to multi-organ failure. We hypothesized that extending an established acute mouse model of hemorrhagic shock and trauma (HS/T) to a 24-hour survival model would allow for evaluation of persistent endotheliopathy and organ injury after HS/T.

Methods: Adult male C57BL/6J mice underwent laparotomy, femoral artery cannulation, and blood withdrawal to induce HS to a mean arterial pressure of 35mmHg for 90 minutes. Mice were resuscitated with either lactated Ringer's (LR) or fresh frozen plasma (FFP). Vascular permeability in the lung and gut were assessed by measuring extravasation of a fluorescent dextran dye. Lungs were evaluated for histopathologic injury, and immunofluorescent staining was used to evaluate neutrophil infiltration and intercellular junction integrity. Pulmonary inflammatory gene expression was evaluated using NanoString. All endpoints were evaluated at both 3 and 24 hours after initiation of shock.

Results: LR- and FFP-treated mice had an equal mortality rate of 17% in the 24-hour model. LR-treated mice demonstrated increased vascular permeability in the lung and gut at 3 hours compared to sham mice (lung $P < .01$; gut $P < .001$), which was mitigated by FFP treatment (lung $P < .05$; gut $P < .001$). Twenty-four hours after shock, however, there were no differences in vascular permeability between groups. Similarly, the lungs of LR-treated mice demonstrated significant histopathologic injury, neutrophil infiltration,

loss of tight and adherens junctions, and a pro-inflammatory gene expression profile at 3 hours, all of which recovered by 24 hours.

Conclusions: In an established mouse model of HS/T, endotheliopathy and lung injury are evident at 3 hours but recover by 24 hours. Larger animal models allowing for more severe injury coupled with supportive care are likely necessary to evaluate endotheliopathy and organ injury outside of the acute period.



Major Task #3: Tissue and Molecular Analysis of Rat Model of HS (UCSF). 100% completion
We have completed the acute model of HS in rats and have published our work. Our work demonstrates critical variability in rodent models for testing of human products.

Potter, Daniel R. PhD; Trivedi, Alpa PhD; Lin, Maximillian BA; Miyazawa, Byron Y. BA; Vivona, Lindsay R. BA; McCully, Belinda PhD; Nair, Alison MD; Schreiber, Martin A. MD; Pati, Shibani MD, PhD The effects of human prothrombin complex concentrate on hemorrhagic shock-induced lung injury in rats: Implications for testing human blood products in rodents, Journal of Trauma and Acute Care Surgery: December 2020 - Volume 89 - Issue 6 - p 1068-1075 doi: 10.1097/TA.0000000000002890

Major Task #4: Obtain regulatory approval and test swine model (OHSU). 100% completion.
OHSU acquired their most recent IACUC approval on 8/31/2018 and ACURO approval on 10/2/2018.

The model development was completed in October 2018. The details of this model development are describe in the 2018 annual report.

Major Task #5: Run randomized study in swine model of lung injury and hemorrhagic shock (OHSU). 100% completion.
Utilizing our revised protocol developed in 2018, the randomized study is 100% complete as of February 2020. Physiologic data (hemodynamic variables, thrombelastography parameters, blood gases/chemistries) were collected during the protocol and recorded in a database. Plasma and tissue (lung, spleen, kidney, heart) samples were banked for future analysis. Following completion of the experimental work, analysis of the plasma and tissue samples was initiated. The lab at OHSU was closed due to the coronavirus during the spring of 2020, so assays were delayed but progress has been made in histology, PCR and luminex assays.

In the 2018 annual report, we reported that the use of an aggressive resuscitation regimen counteracts the development of hyperkalemia following pulmonary contusion and hemorrhagic shock. Dr. Sawyer Smith presented these findings at the 2019 North Pacific Surgical Association meeting. This paper won the Resident Prize for the Best Basic Science paper, and was published in May of 2020 by the American Journal of Surgery.

Dr. Alexandra Dixon presented her abstract on physiological study results, "FFP maintains normal coagulation while PCC induces a hypercoagulable state in a porcine model of pulmonary contusion and hemorrhagic shock", to American College of Surgeons, Committee on Trauma, Region X, winning the top prize for basic science. Her paper under the same title, will be published next year.

Major Task 6: Assess Blood and Tissue Samples for Inflammation. 100 % completion.
The analysis process was initiated in February 2020 following the completion of the animal experiments. We experienced some delays and limited lab access due to coronavirus restrictions, but were able to increase analysis in 2021. As of January 2022, all assays have been run and the final step of statistical analyses is ongoing.

Major Task #7: Submit abstracts, publications, and final report to Army. 50% completion.
This task is ongoing. Upon completion of Major Task 6 the final results of the data can be submitted.

What opportunities for training and professional development has the project provided?
Nothing to Report.

How were the results disseminated to communities of interest?
In September of 2020, Dr. Schreiber presented results from the study to MRMC Grant Update Panel.

Additionally, as indicted above, Dr. Alexandra Dixon, presented results from this study and won the basic science competition for Region X, CoT.

What do you plan to do during the next reporting period to accomplish the goals?
The next step for this project is to take our candidate product into a study using human patients that mirror the models used in this project. The lead candidate product, Kcentra, is a biologic

product that has already been given FDA approval. So, it already is a tremendous aid to the general population. We aim to expands it purview and demonstrate that it can be an aid to patients with traumatic thoracic injury and hemorrhage.

Follow-on-funding has been secured to implement Kcentra in a pre-hospital setting. The funding source is the Department of Defense office via the Congressionally Directed Medical Research Programs (CDMRP).

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?Nothing to Report

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:

Changes in approach and reasons for change

Nothing to Report.

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

We have asked for a NCE until 9/2023. The main reason for this is to finish the analysis of the mouse tissue and to correlate our findings between our mouse, rat and swine studies. We anticipate this will be complete by 9/22.

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
Nothing to report

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**
  Report only the major publication(s) resulting from the work under this award.

- **Journal publications.**
  Potter, Daniel R. PhD; Trivedi, Alpa PhD; Lin, Maximillian BA; Miyazawa, Byron Y. BA; Vivona, Lindsay R. BA; McCully, Belinda PhD; Nair, Alison MD; Schreiber, Martin A. MD; Pati, Shibani MD, PhD The effects of human prothrombin complex concentrate on hemorrhagic shock-induced lung injury in rats: Implications for testing human blood products in rodents, Journal of Trauma and Acute Care Surgery: December 2020 - Volume 89 - Issue 6 - p 1068-1075doi: 10.1097/TA.0000000000002890

  Smith S, Behrens B, McCully B, Murphy J, Bommiasamy A, Goodman A, Dewey E, Pati S, Schreiber M. Aggressive Treatment of Acute Kidney Injury and Hyperkalemia Improves Survival in a Combat Relevant Trauma Model in Swine. Am J of Surgery, May 2020, 219 (5), 860-864.

- **Books or other non-periodical, one-time publications.**
  Nothing to Report.

- **Other publications, conference papers and presentations.**
  Nothing to Report.

- **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?

OHSU
Name: Martin A. Schreiber, MD
Project Role: PI
Nearest person month worked: 1.8 calendar months
Contribution to Project: Dr. Schreiber has provided oversight and day-to-day management of the grant.

Name: James M. Murphy, MD
Project Role: Research Associate/Veterinary Technician
Nearest person month worked: 3.0 calendar months
Contribution to Project: Dr. Murphy is responsible for the designing the anesthesia/sedation regimen, surgical preparation, and overall care of the animals during surgery and recovery.

Name: Alix Dixon, MD
Project Role: Research Resident
Nearest person month worked: 3.0 calendar months
Contribution to project: Dr. Dixon is the lead resident on the project. She prepares and performs the swine surgery, monitors the experiment, organizes data and prepares data for presentation.

Name: Andrew Goodman
Project Role: Coordinator
Nearest person month worked: 6.0 calendar months
Contribution to project: Andrew performs various roles in administration, animal sedation, surgery, and sample processing.

Name: Maria Luisa Appleman, PhD
Project Role: Coordinator
Nearest person month worked: 6.0 calendar months
Contribution to project: Dr. Appleman performs various roles in administration, animal sedation, surgery, protocol management and sample processing.

Name: Brianne Madtson
Project Role: Coordinator
Nearest person month worked: 4.5 calendar months
Contribution to project: Brianne performs various roles in administration, protocol management, treatment preparation, ordering, and sample processing.

Name: Joseph Garay, PhD
Project Role: Senior scientist
Nearest person month worked: 1.5 calendar months
Contribution to Project: Dr. Garay has aided in analysis of samples and tissue. He also leads in administration of the basic science work.

Name: S. James El-Haddi, MD
Project Role: Analyst and writer
Nearest person month worked: 1.0 calendar months
Contribution to project: James has analyzed data produced by the study and written abstracts for submission.

Name: Elizabeth Rick, BS
Project Role: Research Assistant
Nearest person month worked: 1.5 calendar months
Contribution to project: Beth runs the proposed assays for the project.

UCSF
Name: Shibani Pati MD PhD
Project Role- PI UCSF
Nearest person month worked: 1.8 calendar months
Contribution to project: Supervised design and execution of all work and studies. Review data and coordinates groups.

Name: Alpa Mahuvakar, PhD
Project Role: Scientist
Nearest person month worked: 5.4 calendar months
Contribution to project: Involved in planning and execution of studies, coordination with OHSU, running and coordination of in vivo mice experiments.

Name: Byron Miyazawa B Sc.
Project Role: Scientist
Nearest person month worked: 6.6 calendar months
Contribution to project: In vitro assays and in vivo work.

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

What other organizations were involved as partners?
None

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

Not applicable

9. APPENDICES:

Not applicable