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AWARD NUMBER: W81XWH-17-2-0066

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

PRINCIPAL INVESTIGATOR: Martin Schreiber, MD

CONTRACTING ORGANIZATION: Oregon Health & Science University
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5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5e. TASK NUMBER

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

Oregon Health & Science University
Portland, OR 97239

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13. SUPPLEMENTARY NOTES

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

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
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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

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:** Provide a brief list of keywords (limit to 20 words).

Swine, shock, pulmonary contusion, prothrombin complex concentrate, acute respiratory distress syndrome, liver injury, endotheliopathy

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

The major tasks listed in the SOW include:

1) Obtain regulatory approval and run tail snip model and also start Rat HS model
2) Run rat 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?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include

pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Task #1: Obtain regulatory approval, Rat HS model

UCSF acquired IACUC approval on 10/24/2017 and ACURO approval on 1/10/2018.

Rat model of hemorrhagic shock (UCSF). 44% completed with 31 animals run as of 11/28/2018.

Work on the rat 3 hour acute model of hemorrhagic shock has proceeded on schedule and is almost complete with 31 animals. Although not complete, Kcentra treated animals appear to be no different from LR treated rats in terms of lung permeability. However, lactate readings do seem to be lower in Kcentra treated animals. This lack of an effect maybe due to the fact that Kcentra is of human origin and derived from human plasma. Further studies are underway with human FFP to test if human FFP is active in rats. Due to these results, after completion of all animals in the 3 hour study in rats, we propose changes to the 24-hour time point to mice. We found no discernable differences in the ROTEM results between any of the groups.

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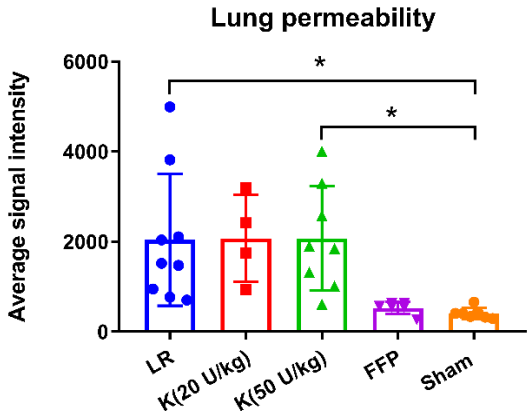

Figure 1: Average fluorescence intensity of the lungs. Each dot represents a single animal. Groups are LR treated animals (LR), Kentra treated animals with (20 U/kg) and (50 U/kg), Rat FFP treated animals and Sham animals with no injury * =p<0.05 via one way ANOVA tukey post hoc test. Shock + LR vs FFP group p value is currently at 0.057.

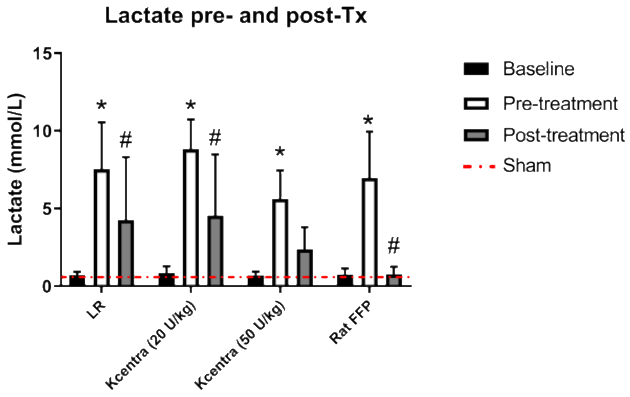

Figure 2. Compared to LR-treated animals, lactate is lower post HS in animals treated with rat plasma or Kcentra. * = p<0.05 significantly different from baseline, # = p<0.05 significantly different from Pretreatment

Major Task #2: Model of HS, 24 hour survival (Changes planned for a switch from rats to mice)

We have submitted protocol changes to the UCSF IACUC for approval and discussed these changes with Dr. Patrick Regan. We will be submitting a revised SOW, Budget and Justification from UCSF. We plan to run the 24 hour model in mice between years 2 and 3 of this grant.

Major Task 3: Tissue and Molecular Analysis of Rat Model of HS

We are currently sectioning and staining the lung tissue from the 3 hour rat model of HS. We are optimizing staining for markers of inflammation and vascular integrity. We are also optimizing quantitation and analysis of the markers.

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.

We are ahead of schedule described in the SOW in terms of model development and implementing the randomized study. We are using the same model for another project, so the model development we proposed to conduct in Year 1 is complete.

In preparation for the randomized study, we conducted two preliminary experiments to examine the safety of the Kcentra treatment. Two control animals (no injury) were given Kcentra and monitored for 48 hours. No adverse events were reported. We also collected plasma from 5 donor animals for the group that received FFP.

Major Task #5: Run randomized study in swine model of lung injury and hemorrhagic shock (OHSU). 5% completion.

We started the blinded randomized study on 10/16/2018.

Major Task 6: Assess Blood and Tissue Samples for Inflammation. 0% completion.

These samples will be analyzed at the end of the randomized study.

Major Task #7: Submit abstracts, publications, and final report to Army. 0% completion.

This task will be completed at the end of the study.

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

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of

expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to Report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

For Year 2, our goals are to start the following tasks:

1) Continue randomized study (Schreiber)
2) Start mouse HS mechanistic studies (Pati), Histopathological analysis of the rat 3 hour tissue.
3) Histopathological analysis of swine lung tissue for vascular markers and inflammation (Schreiber)

4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to Report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

UCSF: Our data indicates that Kcentra does not decrease lung permeability in rats subjected to HS as we have previously seen in mice (Pati et al, 2015). Our positive control, rat fresh frozen plasma, however does indeed result in reduced lung permeability after treatment. While we plan to complete the

current cohort of rats for the 3 hour model of HS, we propose to switch the overnight time point to mice since they are known to respond to Kcentra. We also plan to add in animals with human plasma to this study to help us understand if it is a xenocompatibility issue that is the reason behind a lack of an effect in rats. Kcentra is derived from human plasma.

We originally proposed a rat model due to their size which allows us to make multiple blood draws for measuring cytokines, blood gas measures, blood chemistries and coagulation throughout the length of the experiment and planned to do four separate blood draws over the course of the 24 hour experiment. This is the one aspect of the study what will be difficult to replicate in mice. **We anticipate the entire 24 hour time point HS study can be conducted in mice and all endpoints met at no extra cost. A revised UCSF Budget, Justification and SOW will be submitted to OHSU and the DOD.** The mouse study can be completed in a timely fashion since our lab at UCSF is capable of running two mice/day. While rats are less difficult to cannulate, they require more attention during the shock period to keep them in shock between 30 to 40 mmHg MAP which makes more than one rat a day unfeasible. We have submitted protocol changes to the UCSF IACUC for approval and discussed these changes with Dr. Patrick Regan. We will be submitting a revised SOW, Budget and Justification from UCSF. We plan to run the 24 hour model in mice between years 2 and 3 of this grant.

To this end we propose performing our overnight mouse HS model as follows. **Hemorrhagic Shock Model in Mice:**

C57J-Bl6 mice will be anesthetized (5% isoflurane in 100% O_2). Both femoral arteries will be cannulated. After 15 minutes of stabilization, a laparotomy will be performed. After 10 minutes of baseline MAP and HR measurements. Blood will be removed until MAP reaches 35 mm and maintained between 30-40 mmHg for 60 minutes by periodically withdrawing blood from the femoral arterial catheter.

Resuscitation protocol: Following shock, mice will be resuscitated and infused with a bolus of LR, LR + 50U/kg Kcentra or mouse plasma according to group.

Recovery and blood samples collection time points: Following the resuscitation protocol after 30 minutes of recording blood pressure, all wounds will be sutured and the animal will be weaned off the isoflurane. As the animal recovers from anesthesia, they will be given a subcutaneous dose of an analgesic (buprenorphine, 0.05mg/kg).

Groups tested will include: 1***) Sham 2) HS+LR, 3) HS +Plasma, 4) HS + LR + 50U/kg Kcentra, 6) Naïve animals, N=10 animals/group.***

Mice will be sacrificed 24 hours post injury for via cardiac puncture. Blood will be collected and the lungs harvested. Overall outcomes will be 1) lung injury, 2) Cytokine measurements and coagulation parameters at 24 hours post shock, and 3) immunohistochemical analysis of lung tissue for markers of vascular endothelial integrity and inflammation, 4) RNA profiling by single cell Next Generation sequencing at 24 hours post shock in lung tissue.

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

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

See above on the UCSF rat studies.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable.

Significant changes in use or care of vertebrate animals

UCSF: We are switching from rats to mice for our 24 hour HS time point. We have discussed this with our program officer Dr. Patrick Regan. We are dropping the tail snip model for lack of sensitivity and response of rats to Kcentra. We will be submitting a new protocol to the UCSF IACUC and ACURO and a revised budget, budget justification and SOW to OHSU and the DOD.

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year;*

page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

None to report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

OHSU

Name: Martin A. Schreiber, MD
Project Role: PI
Nearest person month worked: 0.9 calendar months
Contribution to Project: Dr. Schreiber has provided oversight and day-to-day management of the grant.

Name: Belinda H. McCully, PhD
Project Role: Co-investigator
Nearest person month worked: 0.9 calendar months
Contribution to Project: Dr. McCully has trained new personnel and managed the daily aspects of the experiments and model development.

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

Name: Andrew Goodman, BS
Project Role: Coordinator
Nearest person month worked: 3.0 calendar months
Contribution to project: Andrew performs various roles in administration, animal sedation, surgery, and sample processing.

Name: James Russo, BS
Project Role: Coordinator
Nearest person month worked: 3.0 calendar months
Contribution to project: James performs various roles in administration, animal sedation, surgery, and sample processing.

Name: Maria-Luisa Appleman, PhD
Project Role: Coordinator
Nearest person month worked: 3.0 calendar months
Contribution to project: Luisa performs various roles in administration, animal sedation, surgery, protocol management and sample processing.

Name: Brianne Madtson, BS CVT
Project Role: Coordinator
Nearest person month worked: 3.0 calendar months
Contribution to project: Brianne performs various roles in administration, protocol management, treatment preparation, ordering, and sample processing.

UCSF
Name: Shibani Pati MD PhD
Project Role- PI UCSF
Nearest person month worked: 0.45 calendar months
Contribution to project: Supervised design and execution of all work and studies. Review data and coordinates groups.

Name: Daniel Potter, PhD
Project Role: Co-investigator UCSF
Nearest person month worked: 1.16 calendar months
Contribution to Project: Dr. Potter managed the daily aspects of the experiments, model development, surgery and sample processing.

Name: Alpa Mahuvakar, PhD
Project Role- Scientist UCSF
Nearest person month worked: 0.66 calendar months
Contribution to project: Supporting all in vitro and in vivo studies of this project.

Name: Maximilian Lin BS
Role: Research Assistant started on 2/20/18
Nearest person month worked: 0.25 calendar months
Contribution: Supporting all in vitro and in vivo studies on this project. Responsible for tissue and blood analysis by western blot, qPCR, tissue immunohistochemistry, histopathology.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

Not applicable

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Not applicable