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TITLE: Dried Plasma to Improve Outcomes in Polytrauma, Hemorrhage, and Trauma-Associated Sepsis (TAS): Novel Solutions for the Prolonged Field Care Environment

PRINCIPAL INVESTIGATOR: Dr. Rosemary Kozar

CONTRACTING ORGANIZATION: University of Maryland, Baltimore

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Hemorrhagic shock (HS) remains the leading cause of early death among the severely injured in both the civilian and military settings, and patients that survive HS are prone to sepsis**.** As the treatment of trauma-associated sepsis (TAS) on the battlefield will be unique to prolonged field care, new therapeutic strategies that are feasible and readily translatable are urgently needed. We are proposing the novel use plasma as a primary resuscitative fluid for TAS as we anticipate that the endothelial protective effects seen after HS will also be present after TAS. However, there are logistical challenges and safety issues with the use of fresh frozen plasma (FFP) in the battlefield. We therefore will study the use of pathogen-reduced freeze dried plasma (FDP) and hypothesize that FDP- based resuscitation after TAS will be equivalent to FFP, superior to hextend, and will reduce the endotheliopathy of sepsis (EOS), mitigate vascular and end organ injury, and decrease mortality, in clinically relevant models of TAS. This hypothesis will be tested first in a rodent model to examine systemic, vascular, organ-specific pathophysiology and survival in a mouse model of HS and prolonged hypotensive resuscitation with TAS and then confirmed and expanded using a swine model of TAS to determine the modulatory effects of SDP compared to hextend on hemodynamics, end-organ function, coagulopathy and survival in a swine model of TAS.

15. SUBJECT TERMS

Hemorrhagic shock, trauma, trauma-associated sepsis, sepsis, prolonged field care, endotheliopathy of trauma, hextend, fresh frozen plasma, freeze dried plasma

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	Unclassified	21	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

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

The goal of the current project is to determine if plasma is an ideal fluid for resuscitation in the prolonged field care environment for trauma/hemorrhagic shock and trauma-associated sepsis (TAS). Additionally, use of a freeze dried(FD) plasma product and compared to fresh frozen plasma will be tested. We hypothesize that FD plasma- based resuscitation after TAS will be equivalent to FFP, superior to Lactated Ringers, and will reduce the endotheliopathy of sepsis (EOS), mitigate vascular and end organ injury, and decrease mortality, in clinically relevant mice and swine models of TAS.

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

Hemorrhagic shock, trauma, trauma-associated sepsis, sepsis, prolonged field care, endotheliopathy of trauma, hextend, fresh frozen plasma, freeze dried plasma, lactated Ringers

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.

	Timeline (Months)	Completed
Specific Aim 1: To determine the effects of spray- dried plasma compared to fresh frozen plasma and hextend on systemic, vascular, organ-specific pathophysiology and survival in a rodent model of hemorrhage shock and prolonged hypotensive resuscitation with trauma associated sepsis.		
Major Task 1: **Obtain approval for mice experiments**		
Subtask 1: Obtain local IACUC approval for mouse studies. (Estimated total number of animals: 245)	0-2	6-27-2017
Subtask 2: Obtain ARUCO approval for mouse studies	0-4	9-18-2017
Milestone*: IACUC/ARUCO approvals*	4	
Major Task 2: Preparation and testing of cecal slurry		
Subtask 1: Harvest cecal slurry (25 mice)	4-5	11-8-2017
Subtask 2: Perform LD100 experiments (30 animals)	5-7	11-28-2017
Milestone: *Complete cecal slurry preparation and testing*	7	

Major Task 3: Conduct short term study of HS and prolonged hypotensive resuscitation (PHR)		
Subtask 1: Perform short term hemorrhagic shock and PHR	7-12	8-1-2018
Subtask 2: Analyze lung tissue for injury, inflammation, and permeability	10-15	12-15-2018
Subtask 3: Analyze blood and BAL for cytokines	10-15	12-21-2018
Milestone: *Complete short term mouse surgeries and analysis for HS and PHR*	15	

Major Task 4: Conduct short term sepsis studies in mice		
Subtask 1: Optimize cecal slurry model	13-15	3-2020
Subtask 2: Perform short term experiments of sepsis	22-29	ongoing
Subtask 3: Perform analysis on tissues from cecal slurry animals	25-30	ongoing
Milestone: *Complete short term mouse surgeries and analysis for sepsis*	30	

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.

This past year we have focused on **Task 4.**

Methods: Creation of Cecal slurry

A separate set on animals were used to create the cecal slurry. We chose this model of sepsis as it has been shown to be both reproducible and consistent and to mimic the battlefield scenario of an intestinal injury. After appropriate anesthesia, mice were sacrificed and the entire cecum dissected from each mouse and the collected cecal contents combined, weighed and mixed in 10% glycerol in phosphate buffered saline with the ratio of 1-mL buffer for 100-mg of stool. The cecal slurry was serially strained using meshes of progressively smaller pore sizes: 860, 380, 190, and finally 74 microns. The slurry was aliquoted and frozen at -80°C. An aliquot was thawed prior to use.

Results: After doing a number of experiments to confirm lethality of cecal slurry we proceeded with experiments.

As discussed at the end of year one annual report, we were consistently having high mortality with our combined model of trauma/hemorrhagic shock and then sepsis. Mortality for FFP and lyophilized plasma (LP) was higher than Hextend. To attempt to explain this we tried using different donor units of FFP, pooling FFP, and buying new FFP to no avail. We concentrated this initial efforts on FFP as we have vast experience using FFP in our trauma/hemorrhagic shock model. After discussion with members of the DOD and revision of SOW, proceeded with a sepsis only (no trauma/hemorrhagic shock) model to help differentiate the problems. There was concern that giving a second dose of human plasma to mice may be causing some type of transfusion reaction.

Methods for sepsis: Mice receive an intraperitoneal dose of cecal slurry. After six hours, animals were anesthetized, femoral vessels cannulated, then animals resuscitated with a bolus of 30 cc/kg of Lactated

Ringers (LR), FFP, or lyophilized plasma. Six hours was chosen as consistently being associated with clinical sepsis (personal communication with Dr. Saito). Twenty four hours later animals were euthanized and lungs and blood obtained for analysis.

Results:

Previously, we did not see a difference in lung injury between groups with a cecal slurry dose of 200ug and resuscitation with 30cc/kg (either FFP or lyophilized plasma (LP) or lactated ringer's (LR)) and we found that mortality was very high (68%) with a cecal slurry dose of 300ug and the same resuscitation regimen. We then opted to test a cecal slurry dose of 250ug and a modified strategy for resuscitation of 10cc/kg of LP or FFP and 30 cc/kg LR.

	BP cannulation	**BP resus t=0**	**BP resus t=10**	**BP resus t=30**	**mortality**	**Sepsis score**	**Sepsis score post resus**	**Lung Injury Score**
LP (n=10)	69±24.6	70±20.1	64±19.1	69±22.1	40%	1.4±0.53	1.2±1.2	1.33±0.977
LR (n=8)	60±9.8	68±9.4	66±16.3	68±9.2	25%	1±0	2.3±1.8	1.39±0.68

Given that we did not see a difference in lung injury with this modified resuscitation strategy, we felt that the interval between cecal slurry injection, resuscitation and euthanasia should be adjusted to better capture lung pathology and followed the only report of using plasma for sepsis in rodents

(Chang R et al Shock 2017) so opted for a modified resuscitation strategy of 10cc/kg of LP and 30 cc/kg of lactated Ringers (LR) and the adjusted interval of 22 hours post injection of cecal slurry.

We first needed to do a dose response experiment to determine the optimal dose of cecal slurry with this new time point. To make the dose more standardized, we also switched to a dose/gram of body weight rather than strictly a volume.

Dose of slurry	Mortality with LR %	Mortality with LP %	Sample size
5ul/g	0	0	5
8ul/g	100	60	7
11ul/g	100	100	3

Based on this data, chose 8 ul/g as optimal dose. However, by this time we had now used up our entire batch of cecal slurry (done as a large batch then aliquots frozen). We then created a new batch and had to test it.

We also wanted to rule out that human plasma was an issue. We have used human plasma in mice countless times with no problem but not in sepsis. We therefore used mouse plasma to compare it to Lactated Ringers in a dose response experiment using the new 22 hour timepoint.

Dose of slurry	**Mortality with LR %**	**Mortality with MP %**	**Sample size**
5 ul/g	**0**		**5**
10 ul/g	**38%**	**100%**	**8**
20 ul/g	**100%**	**100%**	**3**

For reasons unclear 100% of the mice resuscitated with mouse plasma died even with 10 ul/g which resulted in only a mortality of 38% with lactated Ringers. We therefore used 8 ul/g of cecal slurry in the following experiment:

Group	BP after sepsis MAP	BP after resuscitation	Mortality at the end of resuscitation %	Mortality at 24 hours %
LR (n=8)	60 ± 6	60 ± 8	0	31
MP (n=5)	61 ± 4	48 ±8	38	100

No additional studies with mouse plasma were done as it was clear that human plasma was not the issue

Using 8 ug/kg and the new cecal slurry we proceeded to test the Teleflex LP product

	BP baseline	**BP after sepsis**	**BP resus t=30**	**BP resus t=60**	**Mortality 48 hrs**	**Sepsis score pre-resus**	**Sepsis score post resus**

LR n=4	79±14.7	80±19.1	75±16.4	71±18.6	25%	0.20±0.45	0±0
LP n=5	82±10.2	71±25.4	78±13.6	74±15.4	60%	0.25±0.5	0.67±1.2

In this set of experiments, mice were given the initial bolus of either 10cc/kg (LP) or 30cc/kg (LR). This fluid is given over a 15 minute interval. If the mice subsequently have hypotension with a MAP<60mmHg, they are given 0.1cc of additional fluid in 15 minute increments to maintain a MAP>60mmHg. None of the 5 LP mice required any additional resuscitation, but one of the LR mice did require additional fluid (0.3cc). Therefore, on average LP mice required 10cc/kg to maintain MAP>60mmHg, whereas LR mice required 33±5.8cc/kg to maintain a normal blood pressure. Catheters were removed at 60 minutes post resuscitation and mice were allowed to recover from anesthesia. All mortalities occurred after the mice received resuscitation and recovered from anesthesia.

In more closely looking at the data, there was a question as to why the blood pressure was not lower than baseline and hypothesized that in some animals the cecal slurry may not have been injected completely intraperitoneal. We therefore adjusted our method of injection and have completed the following mice thus far. Baseline MAP for mice is approximately 80. Now all mice that have received the slurry are hypotensive prior to the onset of resuscitation.

Sample size	**BP Resus t=0 min**	**BP Resus t=60 min**	**Mortality 24 h post resus**	**Sepsis score pre-resus**	**Sepsis score post resus**
LR n=8	**60±6**	**60±8**	**31%**	**1.08±0.18**	**1.82±0.26**
LP n=4	**57±2**	**56±5**	**62%**	**1.02±0.2**	**2.22±0.18**

We plan on completing this set of experiments using the same methodology then performing analysis on these animals. However, once again we ran out of cecal slurry with all the additional experiments we needed to do. When I rewrote the new IACUC in June, I did not include mice/methods for additional slurry. Therefore we had to submit an amendment which was done and approved. The amendment is now under review by ACURO.

SPECIFIC AIM 2

There were changes in personnel related to this aim. Dr Jacob Glaser will be leaving the Navy and Dr AJ Burdette assumed a new position. Therefore:

Michael Tiller will assume Jacob Glaser's role

Leslie Neidert will assume Alexander Burdette's role

Changes were submitted and approved by the DOD.

Progress on Aim 2:

● Swine plasma was purchased and will be shipped to Teleflex the end of October to the lyophilized, a timeline requested by Teleflex.

● The IACUC is being prepared and should be submitted for local approval prior to November deadline.

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."

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.

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."

As above, we will complete the set of mice experiments with the new cecal slurry after ACURO approval and then begin analysis.

Work should begin on Aim 2 in swine after appropriate approvals are obtained.

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

- *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.*

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:*

As above: There were changes in personnel related to this aim. Dr Jacob Glaser will be leaving the Navy and Dr AJ Burdette assumed a new position. Therefore: Michael Tiller will assume Jacob Glaser's role Leslie Neidert will assume Alexander Burdette's role Changes were submitted and approved by the DOD.

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.

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.

We have encountered a number of challenges:

1. The LP and FFP groups after trauma-associated sepsis (hemorrhagic shock then sepsis) resulted in an unexplained mortality. To begin to understand these findings, we switched to a sepsis only model to first understand how plasma works in sepsis. Our hemorrhage only experiments clearly showed protection by plasma

2. We did not see significant differences between Hextend and our plasma groups. The military also changed their guidelines and LR was recommended in place of Hextend. Additionally, LR is the standard of care for sepsis resuscitation. We therefore we revised our procedures to now include LR rather than extend as a resuscitative fluid.

3. The COVID pandemic occurred and labs at the University of Maryland were closed for about 3.5 months. During that time we did need to write and submit an entirely new IACUC which was approved. We then submitted this for ACURO approval which did take awhile but we were eventually given permission to perform experiments that were included in the initial protocol only. We have subsequently received ACURO approval.

4. Because of the issues we have encountered, we ran out of cecal slurry. I did not include this in the new IACUC (written in June) so have had to submit an IACUC amendment which has been approved and is now pending ACURO approval.

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.

We have had to use additional mice with the issues discussed above but during COVID salary support and expenditures minimal so able to complete the set of mice described above.

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

New IACUC and ACURO: old IACUC expired
Now with different timepoints after sepsis, LR, and new fluid resuscitation schemes as described
IACUCU amendment (to add mice for additional cecal slurry) approved 9-28-2020
ARUCO: submitted 9-30-2020

Significant changes in use of biohazards and/or select agents

Nothing to report

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).*

Chipman AM, Wu F, Pati S, Burdette AJ, Glaser JJ, Kozar RA. Fresh frozen plasma attenuates lung injury in a novel model of prolonged hypotensive resuscitation. J Trauma Acute Care Surg. 2020 Aug;89(2S Suppl 2):S118-S125. doi: 10.1097/TA.0000000000002719. PMID: 32282752.

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

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".

Rosemary Kozar
PI
1.8 calendar months
Completed IACUC/ARUCO/HRPO and updates, assisted with planning, methods, analysis of data, trouble- shooting challenges, negotiations for plasma products, and completing all reports for DOD.

Feng Wu
Research Associate
3.0 calendar months
Assisted with IACUC protocols, performed animal experiments, tissue processing and assays

Amanda Chipman
Surgical resident
6.0 Calendar months
Assisting with animal experiments and tissue processing and assays

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.

New:
6/15/2020-6/14-2022 Kozar (PI) 25% effort
Novel Dried Cryoprecipitate-Based Intervention to Improve Outcomes from Trauma and Hemorrhagic Shock: Applicability for Multi-Domain
DOD

Closed:
04/01/2017-03/31/2020 Kozar (Co-I) 2% effort
Extracellular miRNAs: Novel Biomarker and Potential Therapeutic Target in Trauma
Air Force

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

7. 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.*

8. 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.