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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

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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	14	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 hextend, 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

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)	
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		Completed
Subtask 1: Obtain local IACUC approval for mouse studies. (Estimated total number of animals: 245)	0-2 Kozar	6-27-2017
Subtask 2: Obtain ARUCO approval for mouse studies	0-4 Kozar	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 Kozar	11-8-2017
Subtask 2: : Perform LD100 experiments	5-7 Kozar	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 ; harvest lung tissue and collect blood. The 4 groups include: sham, HS+FDP, HS+FFP, HS+hextend	7-12 Kozar	8-1-2018
Subtask 2: Analyze lung tissue for injury, inflammation, and permeability	10-15 Kozar	
Subtask 3: Analyze tissue for junctional integrity	10-15 Pati	Not completed see below
Subtask 4: Analyze blood and BAL for cytokines	10-15 Pati	Not completed see below
Milestone *Complete short term mouse surgeries and analysis for HS and PHR*	15	

Major Task 4: **Conduct short term studies of HS and TAS in mice**		
Subtask 1: Optimize TAS model (18 mice)	13-15	Completed** see note below
Subtask 2: Perform short term HS and TAS (4 groups of 20 mice) ; harvest lung tissue and collect blood. The 4 groups include: sham, TAS+FDP, TAS+FFP, TAS+ hextend	15-22	completed
Subtask 3: Analyze lung tissue for injury, inflammation, neutrophil activity, permeability, and junctional integrity	22-24	In progress
Subtask 4: Analyze blood and BAL for cytokines	22-24	
Subtask 5: Analyze lung and blood for syndecan1	22-26	
Milestone: *Complete short term mouse surgeries and analysis for HS and TAS*	26	

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

Specific Aim 1.
Major Task 1: Approvals-Completed year 1
Major Task 2: Cecal slurry-Completed year 1
Major Task 3: Conduct short term study of HS and prolonged hypotensive resuscitation (PHR)
Subtask 1: Animal surgeries completed year 1.

Year 2: By year 2 we successfully developed, to our knowledge, the first rodent model of hemorrhagic shock with prolonged hypotensive resuscitation.

Subtask 2-5 At the end of year 1, we had done preliminary analysis which demonstrated that FFP, but not lyophilized plasma, provided protection to the endothelium. As we have extensive experience in our hemorrhagic shock model and have used different lyophilized products, the results of the LP group were unexpected and caused concern. We concluded that these findings were attributable to the pH of the French product we were using, as it is not pH balanced. After discussion with the DOD, it was decided to hold off on more detailed analysis as it was clear that the product we were using was not effective. Results of the analysis performed are shown below:

Lung histopathology is shown in Fig.1

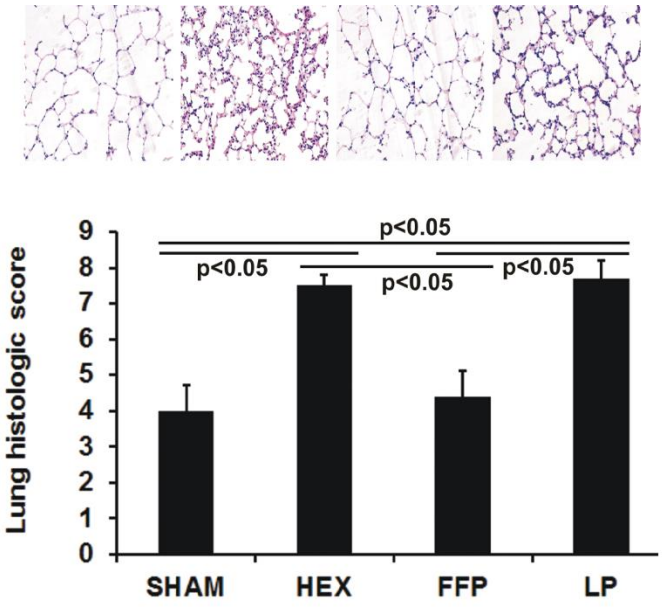


Figure 1. Lung histopathology after hemorrhagic shock and prolonged hypotensive resuscitation. *There was a significant increase in lung injury after HS and resuscitation with hextend and lypophilized plasma which was decreased back to sham levels with FFP.*

Lung myeloperoxidase (MPO) as an indicator of lung inflammation is shown in Fig 2

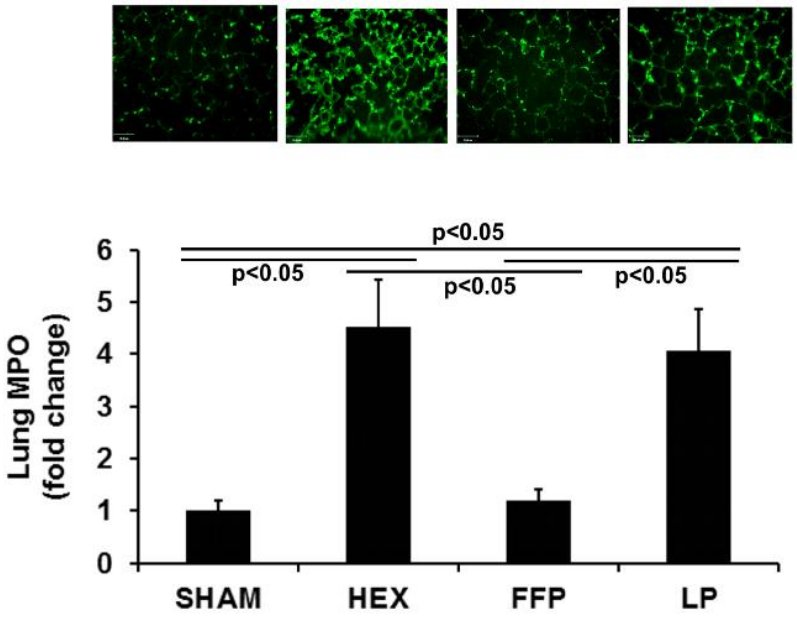


Figure 2. Lung myeloperoxidase after hemorrhagic shock and prolonged hypotensive resuscitation. *There was a significant increase in lung MPO after HS and resuscitation with hextend and lypophilized plasma which was decreased back to sham levels with FFP.*

Figure 3 demonstrated lung cell surface syndecan-1 immunostaining

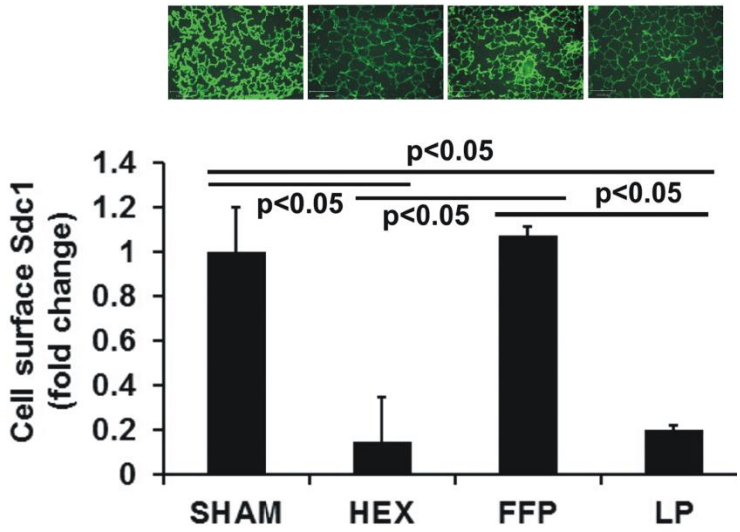
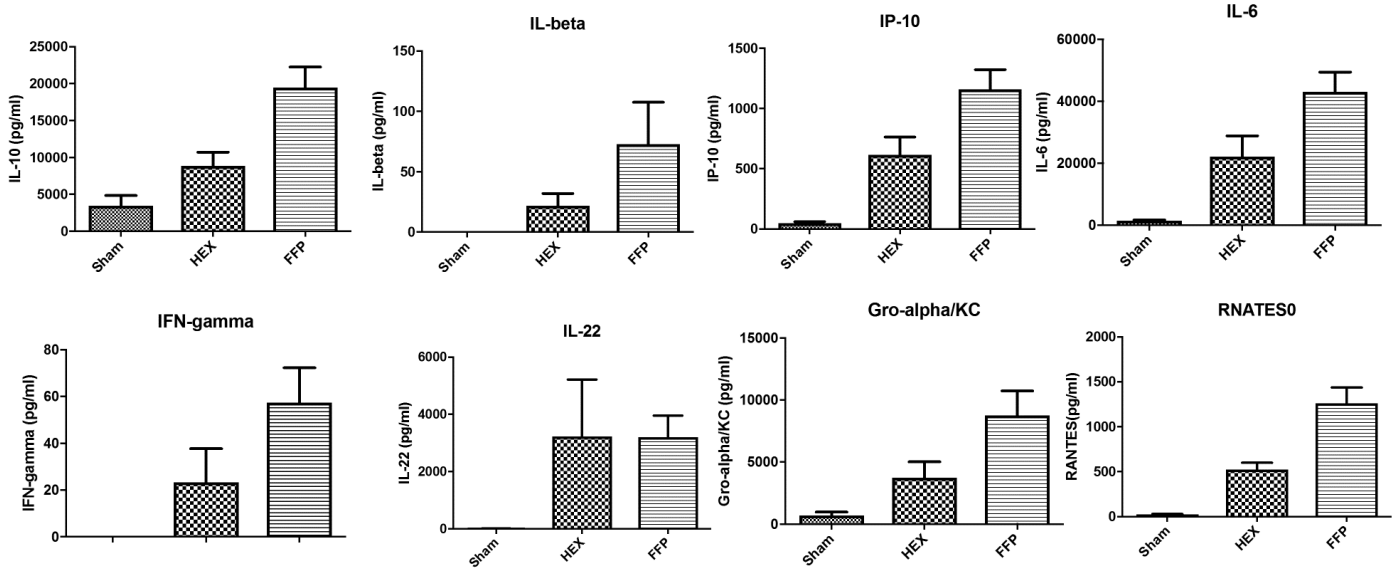


Figure 3. Lung cell surface syndecan-1 immunostaining after hemorrhagic shock and prolonged hypotensive resuscitation. *There was a significant decrease increase in lung synecan-1 staining after HS and resuscitation with hextend and lyophilized plasma which was increased back to sham levels with FFP*

Systemic cytokine production following HS and resuscitation is shown in Figures 3 and 4.



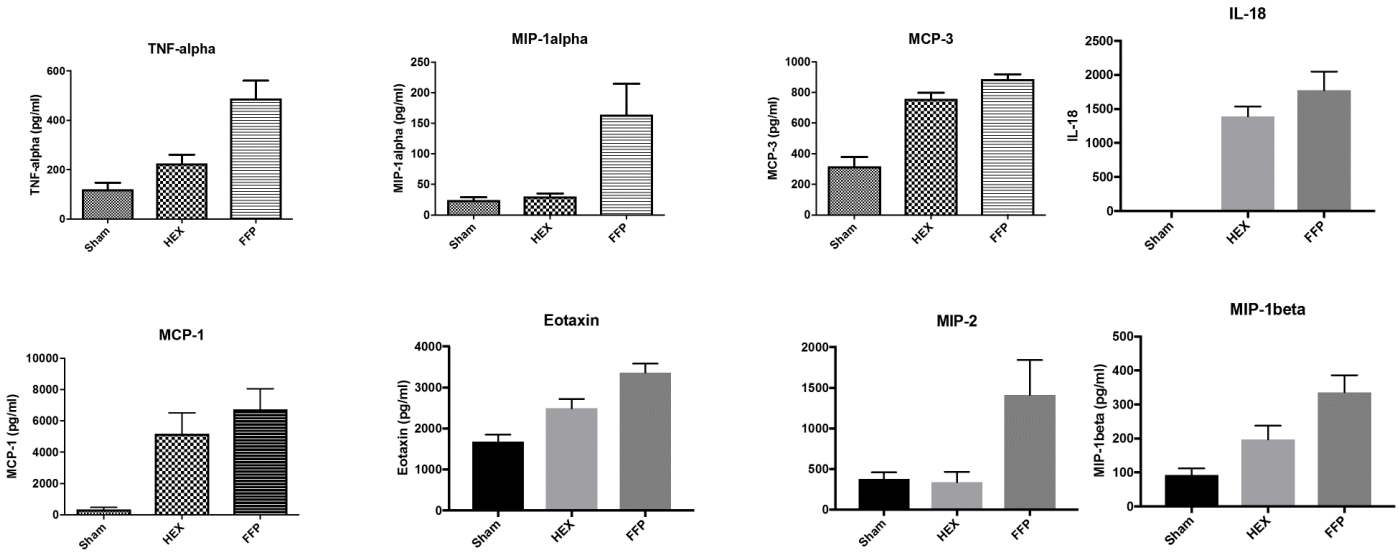


Figure 3 and 4. Systemic cytokine levels after hemorrhagic shock and prolonged hypotensive resuscitation. Both pro- and anti-inflammatory cytokines are shown after hextend and FFP resuscitation. Interestingly, FFP increased some pro-inflammatory cytokines such as TNF alpha while it also increased anti-inflammatory and protective cytokines such as IL-10.

Additional discussion with the DOD centered around how to proceed. It was agreed we would seek to find another company that would provide us with a lyophilized product. We were able to reach an agreement with Teleflex. This took a rather prolonged period of time to obtain approval from the company, get a signed MTA and then to get the product shipped to us for use. In the end, Teleflex chose to send us reconstituted product that we now have in hand.

Major Task 4:

Subtask 1: Optimize TAS model

The first step was to determine the optimal dose of cecal slurry.

Mice were injected IP with 200µL of cecal slurry. Six hours later, their femoral artery was cannulated and blood pressure was monitored. Mice then received a 30mL/kg bolus of resuscitative fluid (Hextend or FFP). The cannula was removed and the mice were allowed to recover from anesthesia. They were sacrificed 24 hours after the initial injection of cecal slurry. Sepsis scores were recorded at 6 hours after cecal slurry injection and just before sacrifice. 7 mice were treated

with Hextend, 4 of these survived to completion of experiments; 5 mice were treated with plasma, 4 of these survived to completion of experiments.

Table 1.						
Group	**Sample size**	**Mortality**	**Sepsis score-6 hr**	**Sepsis score-24 hr**	**MAP prior to resuscitation**	**MAP 30 min after resuscitation**
Hextend	7	42.9%	3.0 ±0.38	3.25±1.11	67.14±8.74	85.71±2.36
FFP	5	20%	1.8 ±0.53	1.8±1.31	80.8±10.56	82.6±.39

Based on this data, we proceeded to **Subtask 2: Perform short term HS and TAS**

Methods:

On day 1, mice were subjected to a laparotomy and hemorrhagic shock (MAP 35mmHg) for 90 minutes followed by resuscitation to a MAP of 60mmHg for 6 hours with either Hextend, FFP or LP. After 6 hours, cannulas were removed and the mice were allowed to recover from anesthesia. On day 3, mice were injected IP with 200µL of cecal slurry. Six hours after injection the opposite femoral artery was cannulated and mice were resuscitated with a 30mL/kg bolus of the same resuscitative fluid. Again, cannulas were removed and the animals were allowed to recover from anesthesia. Sepsis scores were recorded 6 hours after injection of cecal slurry and just prior to sacrifice (24 hours after cecal slurry). Mice were sacrificed on day 4 (24 hours after administration of cecal slurry). Sham animals (7 completed) underwent cannulation, but no hemorrhagic shock (and no laparotomy). Rather than cecal slurry they were injected with 200µL normal saline IP. The LP was reconstituted and aliquoted by Teleflex and shipped on dry ice. It was maintained at -20C until time of use. Approximately 15 minutes prior to administration it was placed in a 37C water bath and thawed. At time of euthanasia, animals were sacrificed by cardiac puncture and blood was collected in citrate coated tubes and centrifuged at 2000x for 10 min. The plasma fraction was collected. The right lung lobes were collected as fresh frozen tissue. The trachea was cannulated and BAL was collected. The left lung was perfused with 10% formalin and preserved in formalin. It was then treated with sucrose and ultimately saved in OCT. All specimens are maintained at -80 until they are analyzed.

Results:

Shams

Seven sham animals, 100% survival.

Hextend:

A total of 10 mice were treated with Hextend, 8 of these survived to the completion of experiments (sacrifice on day 4). The two Hextend treated mice that did not survive died sometime after PHR on day 1, but before injection with cecal slurry on day 3. Mortality of 20%

FFP

A total of 11 mice were treated with FFP, 8 of these died sometime after PHR (between day 1 and day 2), one of these died during the PHR while still under anesthesia and two died immediately after the initial administration of FFP. Several different FFP samples were used alone and in combination. Mortality 100% See challenge section.

Due to the unexpected mortality, we wanted to ensure that there was not some type of transfusion reaction. Therefore, donor mouse plasma was obtained by cardiac puncture of C57/BL6 mice and used for resuscitation. Two mice were treated with mouse plasma. They both survived to sepsis, but died while receiving the resuscitative bolus on day 3. We think this may be related to volume overload/heart failure.

LP

Three mice were treated with LP. One died very soon after the end of PHR, 1 died between day 1 and 2. One LP mouse was injected with the cecal slurry on day 3 but died once under anesthesia. A forth mouse was attempted but due to its small size, cannulation was not feasible and attempts aborted.

Results were summarized below in Table 2.

Table 2.								
Group	**Sample size**	**Mortality**	**Mortality Timepoint**	**Fluid vol during PHR (mL)**	**Sepsis score- 6 hr**	**Sepsis score- 24 hr**	**MAP prior to resuscitation (6h after cecal**	**MAP 30 min after resuscitation**

							slurry)	
Hextend	10	20%	100% after PHR	0.8±0.12	3.75±0.49	3.25±1.11	51±7.73	70.9±2.52
FFP	11	100%	100% after PHR	0.78±0.08	N/A	N/A	N/A	N/A
LP	3	100%	66% after PHR 33% after sepsis	0.7±0.25	N/A	N/A	N/A	N/A
Sham	7	0%	N/A	N/A	0	0	100.7±3.18	92±4.36

After these animals, tried one more FFP animal that died during the post sepsis resuscitation. At this time we realized that the FFP aliquots we were using were over a year old and hypothesized that this may be the issue. We therefore bought new plasma. One more mouse underwent hemorrhagic shock and then sepsis and survived. One additional LP mouse underwent HS and sepsis and also survived.

Even though the last few mice survived, we were not clear on why the high mortality. We therefore expanded our optimization studies in the **sepsis only** model described above. It is possible that plasma is not protective

Hextend: 12 mice with a 33% mortality
LP: 13 mice with a 38% mortality
FFP: 13 mice with a 33% mortality
Shams: 7 mice with a 0% mortality

Lung function:
Histopathologic injury score: no significant differences between groups
Shams: 1.72
FFP 2.14
LP 1.85
Hextend 1.75

Lung permeability as measured by BAL albumin
Shams: 0.116
FFP 0.111
LP 0.091
Hextend 0.098

At this point we were unable to demonstrate any differences between the hextend and plasma (LP or FFP) groups.

Due to these difficulties, a conference call with Mr Malloy and Dr Regan was held and challenges discussed. In addition, the military had just released their new Damage Control Resuscitation CPG ID:18 which no longer recommended Hextend as a resuscitation fluid and it was removed from the

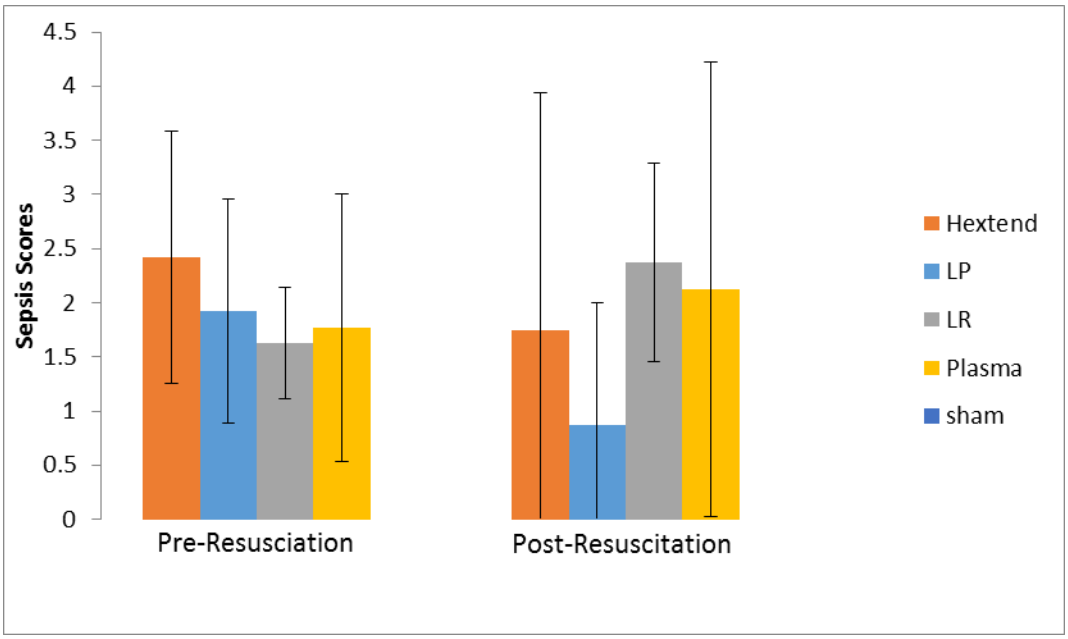
guideline. It was decided that the next step would be to try lactated Ringers (LR) as that is the new fluid being used by the military and we would do this in the sepsis only model.

We submitted an IACUC amendment and after approval obtained approval from ACURO

LR: 7 mice with a zero % mortality.

We have not yet completed any assays with LR groups.

We have calculated a post sepsis and post resuscitation sepsis score for all of the animal groups and is shown below. The sepsis score is based on the animals' overall appearance, eyes, activity, state of consciousness and respiratory rate. Shams were all zero.



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.

The results were presented as a poster at the Shock Society meeting in June and MHSRS in August 2019. We are in the process of completing a manuscript which we anticipate should be submitted in the next month. It was discussed and agreed upon with DOD that we would not include the LP group (French lyophilized plasma).

See the MHSRS abstract below

Fresh frozen plasma attenuates lung injury in a novel model of prolonged hypotensive resuscitation

Introduction

Hemorrhagic shock (HS) remains the leading cause of early death among the severely injured in both civilian and military settings. As future areas of military operations will require strategies allowing prolonged field care of the injured, we sought to develop a model of prolonged hypotensive resuscitation (PHR) and to evaluate the role of plasma-based resuscitation in this model. We hypothesized that resuscitation with fresh frozen plasma (FFP) would mitigate lung injury when compared with Hextend (current standard of care therapy in the absence of available blood products per Joint Trauma System guidelines) in a rodent model of PHR.

Methods

Male C57BL/6 mice underwent femoral artery cannulation for blood withdraw, resuscitation and hemodynamic monitoring. They then underwent laparotomy and hemorrhagic shock (MAP 35±5mmHg x 90 minutes) which was followed by PHR. During PHR, mice were resuscitated with either FFP or Hextend to maintain a MAP of 55-60mmHg for six hours. At the end of six hours animals were sacrificed and tissue harvested for further analysis. Sham mice underwent femoral artery cannulation but no laparotomy or HS.

Lung tissue was harvested for assessment of histopathologic injury and inflammation. Lungs were sectioned and stained with hematoxylin and eosin and scored on a three-point scale for alveolar thickness, capillary congestion and cellularity. Infiltration of neutrophils was assessed by myeloperoxidase (MPO) immunofluorescence staining. Pulmonary syndecan-1 immunostaining was assessed as an indicator of endothelial cell integrity. For fluorescent staining, two random images were taken from each lung and quantified using Quantity One software. Results are reported as relative fluorescence units. In a separate set of animals, permeability was assessed using Evans Blue dye. Data were analyzed by one-way analysis of variance (ANOVA) with Bonferroni correction; p values < 0.05 were considered significant.

Results

Resuscitation with FFP mitigated lung histopathologic injury compared to Hextend (4.4 ± 0.74 vs. 7.5 ± 0.33, p=.002). FFP also lessened lung inflammation (2,780 ± 546 vs. 10,466 ± 2,158 RFU, p=.006) and restored pulmonary syndecan-1 when compared to Hextend treated mice (5360 ± 1024 vs. 728 ± 189 RFU, p=.001). Consistently, FFP mitigated lung hyperpermeability compared to Hextend (0.334±.023 vs. 0.651±.082, p=.007) (Figure 1).

Conclusions

We have presented a novel model of prolonged hypotensive resuscitation of military relevance to the prolonged field care environment. In this model, FFP maintains its pulmonary protective effects compared to Hextend, which supports the need for further development and implementation of plasma-based resuscitation in the forward environment.

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.

Nothing to report

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 described above, we did expand upon the sepsis only animals and added a lactated Ringers group, after approval from DOD and then IACUC and ACURO

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 problems. From year one:

1. We initially obtained FFP from Bonfils Blood center. After several animals not surviving the period of hemorrhagic shock and resuscitation with FFP, we contacted the company and they mistakenly sent us two units from female donors. They were replaced with male donors but the mice did not react as expected (we have used a mouse model of HS for years and have very consistent results). We subsequently have purchased additional units of blood from another blood center and they are functioning well.
2. There was a logistical issue in obtaining the FDP. It was shipped in liquid form from France but got caught up in customs in the US. By the time it arrived, the dry ice had melted and the plasma was defrosted. They subsequently sent us freeze dried that we reconstituted.

Year 2 challenges:

1. The French freeze dried plasma (FDP) did not performing as expected which we concluded was due to an issue with pH. We then negotiated a new arrangement with Telflex who has supplied us with reconstituted lyophilized plasma.
2. The LP and FFP groups after trauma-associated sepsis (hemorrhagic shock then sepsis) resulted in an unexplained mortality. To begin to understand these findings, no further animals have been done, rather we switched to a sepsis only model to first understand how plasma works in sepsis. Our hemorrhage only experiments clearly showed protection by plasma compared to extend.
3. With sepsis only, we saw comparable results in preliminary assays between hextend, FFP and LP, with shams showing more signs of injury than we have seen in prior experiments. We believe this is due to the fact that this is a more prolonged model and ligation of the femoral artery has been shown to cause similar findings by other investigators (personal communication by PI). We could do a naïve group to further investigate this finding. It also does not appear that the plasma groups are very protective, though we have not done any assays in the sepsis only group. I suspect this may be because animals were

resuscitated with 30 cc/kg for all groups. We likely should have used a 1:3 ratio like we have done with hemorrhagic shock. The plan is to next do some of the sepsis only assays with LR to determine the extent of injury and then decide on how to proceed.

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 with the FFP and then the FDP/LP but this as of yet have not been major changes in expenditure.

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

IACUC amendment 9/18/2019
ARUCO approval of this amendment 9/27/2019

Significant changes in use of biohazards and/or select agents

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).*

Published abstract:

Chipman AM, Wu F, Zhou Y, Pati S, **Kozar R**. Fresh frozen plasma attenuates lung injury in a novel model of prolonged hypotensive resuscitation. *Shock* 2019 June 51(1): 157-158.

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 (already listed above under dissemination of results)

- **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

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
2.4 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
6.0 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

Shibani Pati
Co-investigator
0.78 calendar months
Performing assays on lung tissue, blood and bronchoalveolar fluid

Daniel Potter
Research Associate
0.9 calendar months
Performing assays on lung tissue, blood and bronchoalveolar fluid

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

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.*

Dried Plasma to Improve Outcomes in Polytrauma, Hemorrhage, and Trauma-Associated Sepsis (TAS): Novel Solutions for the Prolonged Field Care Environment
Grant Log # DM160146
W81XWH-17-2-0054

PI: Dr. Rosemary Kozar **Org:** University of Maryland **Award Amount:** $1,101,644.00

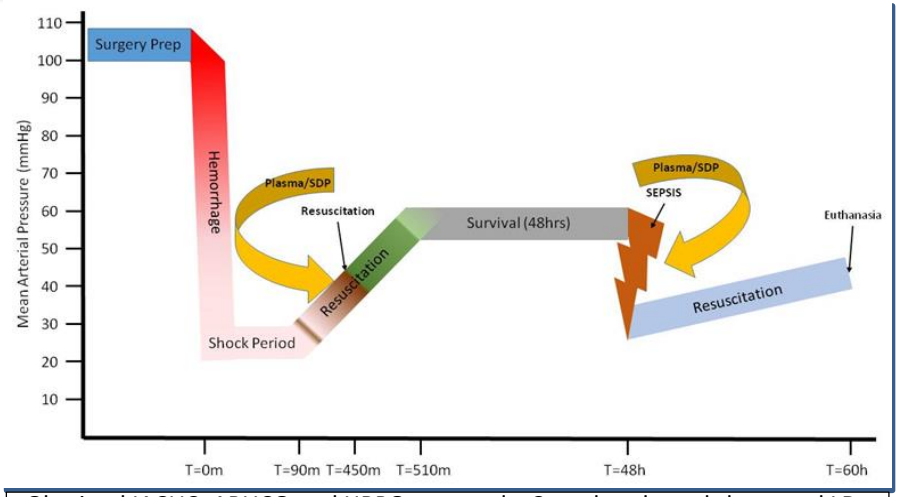
Study/Product Aim(s)

- **SA1. To determine the effects of freeze dried plasma (FDP) compared to fresh frozen plasma (FFP) and hextend on systemic, vascular, organ-specific pathophysiology and survival in a rodent model of hemorrhagic shock (HS) and prolonged hypotensive resuscitation(PHR) with trauma associated sepsis (TAS).**
- **SA2. To determine the modulatory effects of FDP compared to hextend on hemodynamics, end-organ function, coagulopathy and survival in a swine model of TAS.**

Approach

SA1.Mouse model of HS and PHR then resuscitation with FDP, FFP or hextend compared to shams then HS and PHR followed by TAS and resuscitation with similar fluids.

SA2. Swine model of HS and PHR then TAS and resuscitation with either FDP or hextend.



Obtained IACUC, ARUCO and HRPO approvals; Completed cecal slurry and LD 100 experiments; completed short term HS and prolonged field resuscitation surgeries and analysis; model developed for TAS, starting experiments

Timeline and Cost

Activities CY	17	18	19	20
Aim 1 approvals and slurry/LD100				
Aim 1 HS and PHR surgeries and analysis				
Aim 1 TAS surgeries and analysis				
Aim 2 approvals, surgeries and analysis				
Estimated Budget ($K)	**$451**	**$493**	**$157**	**$000**

October 13, 2019

Goals/Milestones

CY17 Goal – Mouse approvals

CY18 Goals – Mouse HS and PHR

☐ x Complete cecal slurry and LD 100 experiments

☐xComplete HS and PHR experiments and analysis mice-experiments and analysis complete

CY19 Goal – Complete short term mice experiments, start swine

☐ Complete TAS short term experiments and analysis: in progress

☐ Begin swine IACUC and MRMC approvals: not started

CY20 Goal complete mouse and swine studies

☐ Complete mice survival studies

☐ Complete swine studies

Comments/Challenges/Issues/Concerns

- Teleflex product arrived; issues with mortality in plasma groups

Budget Expenditure to Date

Projected Expenditure: $944,000 for Years 1-2

Expenditures To Date: $837,630 through FY19 Q4

Subcontract to UCSF: Obligated $199,283 for Years 1-2