AWARD NUMBER: W81XWH-20-1-0406

TITLE: Novel Dried Cryoprecipitate-Based Intervention to Improve Outcomes from Trauma and Hemorrhagic Shock: Applicability for Multidomain Operations

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REPORT DATE: July 2021

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2021	Annual	15Jun2020 - 14Jun2021

4. TITLE AND SUBTITLE

Novel Dried Cryoprecipitate-Based Intervention to Improve Outcomes from Trauma and Hemorrhagic Shock: Applicability for Multidomain Operations

5a. CONTRACT NUMBER
W81XWH-20-1-0406

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

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8. PERFORMING ORGANIZATION REPORT NUMBER

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

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

10. SPONSOR/MONITOR'S ACRONYM(S)

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Hemorrhagic shock (HS) remains the leading cause of early death among the severely injured in both civilian and military settings. The major objective of this effort is to define the role of 5PRC and LPRC using two different small animal models of HS to examine the effect of these novel products on both hemostasis and endothelial protection. We HYPOTHESIZE that pathogen reduced cryoprecipitate-based interventions will decrease both early hemorrhagic deaths and later multiple organ failure (MOF) through their dual effects on hemostasis and endothelial stability. To test this hypothesis, the early use of 5PRC and LPRC will be compared to CC, FFP and standard of care Lactated Ringers in 2 rodent models of trauma/HS applicable for multi-domain operations in the following Specific Aims: SA1. Determine the effect of early cryoprecipitate on hemostasis, organ function, and mortality in a short- term mouse model of trauma and uncontrolled hemorrhage (UCH). A well-established mouse liver transection model of UCH will be utilized followed by resuscitation with the described blood products or lactated Ringers and compared to shams. Hemostasis, coagulation, lung function, endothelial integrity and short-term mortality will be assessed. SA2. Determine the effect of early cryoprecipitate on endothelial protection, MOF and mortality in a mouse model of sustained hypotensive resuscitation (SHR). Our established mouse model of trauma/HS and SHR with the described blood products or Hextend will be used and compared to shams. Animals will be followed for 72 hours to track survival. Lung specific indicators of injury and function, inflammation, and leak will be assessed.

15. SUBJECT TERMS

hemorrhage shock, cryoprecipitate, uncontrolled hemorrhage

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

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1. INTRODUCTION:

The major ***objective*** of this effort is to define the role of a five day shelf life pathogen reduced cryoprecipitate (5PRC) and lyophilized cryoprecipitate (LPRC) using 2 different small animal models of hemorrhagic shock (HS) to examine the effect of these novel products on both hemostasis and endothelial protection. The ***long-term goal*** is FDA approval of these products for clinical and field use after HS. We ***HYPOTHESIZE*** that pathogen reduced cryoprecipitate-based interventions will decrease both early hemorrhagic deaths and later MOF through their dual effects on hemostasis and endothelial stability. To test this hypothesis, the early use of LPRC and 5PRC will be compared to conventional cryoprecipitate (CC), fresh frozen plasma (FFP) and standard of care Lactated Ringers (LR) in two different rodent models of trauma/HS applicable for multi-domain operations in the following ***specific aims: SA1***. ***Determine the effect of early cryoprecipitate on hemostasis, organ function, and mortality in a short- term mouse model of trauma and uncontrolled hemorrhage (UCH).*** A well-established mouse liver transection model of UCH will be utilized followed by resuscitation with the described blood products or LR and compared to sham animals. Hemostasis, coagulation, lung function, endothelial integrity and short-term mortality will be assessed. ***SA2. Determine the effect of early cryoprecipitate on endothelial protection, organ failure and mortality in a mouse model of sustained hypotensive resuscitation.*** Our established mouse model of trauma/HS and sustained hypotensive resuscitation with the described blood products or LR and compared to shams. Following pronged hypotensive resuscitation (PHR) animals will be followed for 2 days to track survival. Lung specific indicators of injury and function, endothelial integrity, leak and activation measured along with biomarkers of kidney and liver injury. The proposed research and downstream investigations will improve treatment of HS by providing critical information about cryoprecipitate-based resuscitation that would be of benefit to both military and civilian populations.

2. KEYWORDS:

Hemorrhagic shock, prolonged hypotensive resuscitation, cryoprecipitate, endotheliopathy of trauma, pathogen reduced 5-day shelf life cryoprecipitate, pathogen reduced lyophilized cryoprecipitate

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1. Determine the effect of early cryoprecipitate on hemostasis, endothelial protection, organ(lung) function, and mortality in a short-term mouse model of trauma and uncontrolled hemorrhage (UCH)	**Timeline**	**Site 1**
Major Task 1	Months	
Subtask 1. Obtain local IACUC approval (estimated total number 159)	0-2	Completed
Subtask 2. Obtain ARUCO approval	0-4	Completed
Milestone Achieved: HRPO/ACURO Approvals	4	*Completed*
Major Task 2		
Subtask 1 Develop/optimize mouse model UCH (n=10)	4	Completed
Subtask 2. Complete mouse surgeries UCH (n=72)	4-10	In Progress
Subtask 3. Coagulation, hemostasis, shed syndecan	10-12	In Progress
Subtask 4 Lung histology, MPO, syndecan immunostaining and BAL protein	10-12	In Progress
Subtask 5: Lung VE-cadherin and vWF staining	10-12	In Progress
Subtask 6: Data Analysis	13-14	
Milestone(s) Achieved: Completion of SA1	14	
Specific Aim 2. SA2. Determine the effect of early cryoprecipitate on endothelial protection, organ failure and mortality in a mouse model of sustained hypotensive resuscitation (SHR).		
Major Task 3		
Subtask 1 Develop/optimize mouse model SHR (n=5)	12	Completed
Subtask 2: complete mouse studies SHR	13-20	In progress

What was accomplished under these goals?

Major Task 2 Basic Methodology:

C57BL/6 WT mice (8–12 weeks) of both sexes underwent anesthesia with inhaled oxygen and isoflurane. A rectal thermometer was inserted and mice placed on a heating pad to maintain normothermia. The femoral artery and vein are cannulated for continuous blood pressure monitoring and blood draws or solution infusion. Baseline blood pressure is obtained then a midline laparotomy made. Pre-weighed gauze pads are placed in the peritoneal cavity away from the liver.

The liver is isolated and an injury created by sharply transecting 75% of the left lobe. The lacerated segment is removed and then weighed. The abdomen is quickly closed. UCH is carried out for 60 minutes. If during this time MAP increases > 40 mm Hg, additional blood is withdrawn from the femoral line to achieve a MAP ≤ 40 mm Hg. To prevent excess mortality, if MAP decreases < 30 mm Hg, blood will be returned to maintain MAP ≥ 30 mm Hg. At the conclusion of UCH, animals are randomly assigned to one of five resuscitation regimens to include FFP, CC, 5PRC, LPRC or standard of care LR and compared to sham animals. Fluid or product are administered, and volume measured to achieve a hypotensive resuscitation MAP of 55 ±5 mm Hg which is maintained over the next 3 hours by repeat hemorrhage &/or volume infusion as needed.

Results:

Subtask 1: Develop model

Eleven mice were used in model development where the process of catheterization, laparotomy, liver resection, maintaining hypotension, resuscitation, and tissue harvesting was optimized.

Subtask 2: Mouse surgery

1. Results from original model of uncontrolled hemorrhage.

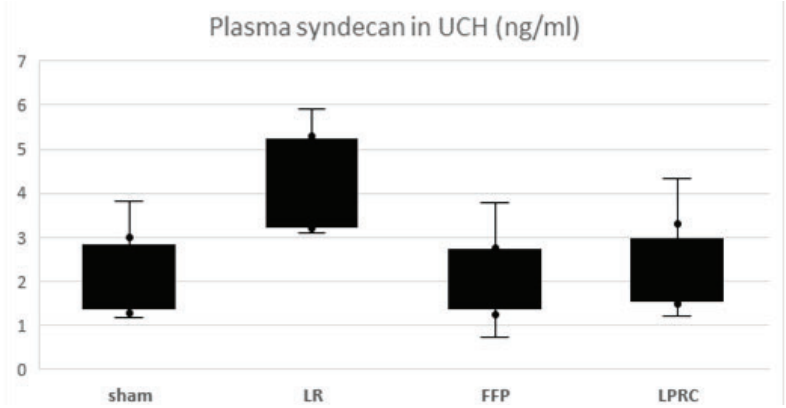
The initial mouse surgeries were completed for sham, LR, FFP, and LPRC. Of the LR and FFP mice, 9 survived out of 12. Of the LPRC mice, 10 survived. Nine shams were completed at this point. The average mouse weight was 27 +/- 2.5g. Average weight of liver resected was 0.11 +/- 0.03g. Average blood removed to achieve MAP of 35 was 332 +/- 152ul. Average weight of gauze collecting blood from abdominal cavity measured after euthanasia was 0.54 +/- 0.15 and total blood loss was 836 +/- 144ul which were all similar across groups.

Resuscitation volume needed to achieve and maintain MAP of 55 +/- 5mmHg was 1218 +/- 325ul for LR, 318 +/- 117ul for FFP, and 304 +/- 130ul for LPRC, approximately a 75% reduction in fluid requirements.

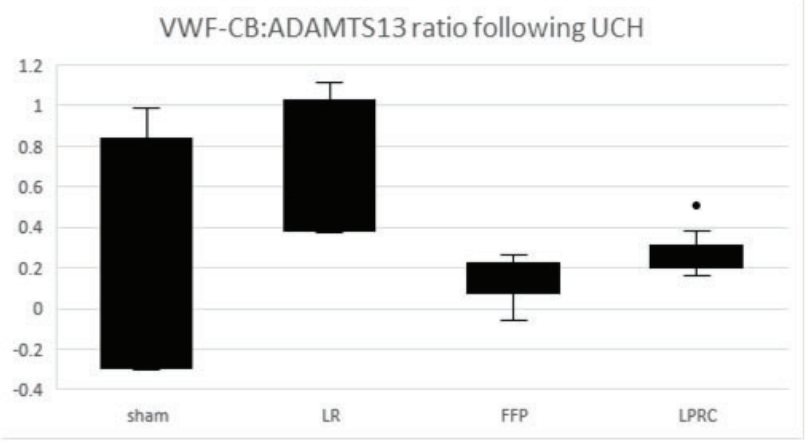
Baseline BP was similar across groups 94 +/- 8mmHg. Similarly, pre-resuscitation BP was 35 +/- 5 mmHg on average and similar across groups. At 180 minutes, average BP was 92 +/- 9mmHg for shams, 48 +/- 8mmHg for LR, 63 +/- 10mmHg for FFP, and 66 +/- 11mmHg for LPRC.

Protein concentration in BAL collected from these mice showed no difference (sham avg 63, LR avg 54, FFP avg 60, LPRC avg 62 ug/ml).
Plasma Syndecan-1 was significantly higher in LR compared to all other groups (sham avg 2.14, LR avg 4.35, FFP avg 2.19, LPRC avg 2.32ng/ml). Collagen binding was also measured and LPRC had significantly higher values than LR or shams but not of FFP (sham avg 0.24, LR avg 0.31, FFP avg 0.34, LPRC avg 0.57U/ml). Plasma ADAMTS13 was significantly higher in FFP and LPRC compared to sham and LR (sham avg 0.3, LR avg 0.45, FFP avg 2.25, LPRC avg .35ng/ml).

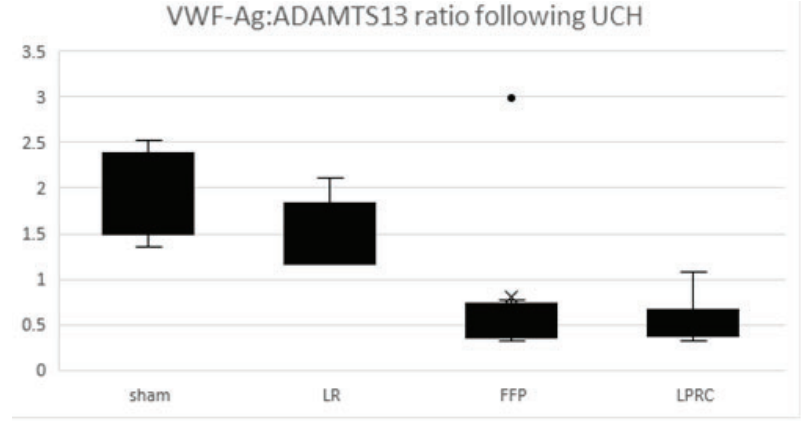
VWF-Ag : ADAMTS13 ratio was significantly lower in FFP and LPRC (sham avg 1.97, LR avg 1.45, FFP avg 0.8, LPRC avg 0.56). VWF-CB : ADAMTS13 ratio LR was significantly higher than other groups (sham avg 0.18, LR avg 0.65, FFP avg 0.13, LPRC avg 0.26).



	Average (ng/ml)
sham	2.14
LR	4.35
FFP	2.19
LPRC	2.32



	Average
sham	0.18
LR	0.65
FFP	0.13
LPRC	0.26



	Average
sham	1.97
LR	1.45
FFP	0.8
LPRC	0.56

Subtask 2

Methods for Modified protocol: Animals will undergo isoflurane anesthesia then placement of femoral artery and vein catheters. A crush injury to the contralateral limb and a mid-shaft tibia fracture will be performed using a surgical clamp. This will be done by extending the extremity, cleansing it with alternating scrubs of betadine and 70% ethanol 3 times. The middle of the tibia will be placed over a sterilized blunt edge then manual pressure applied until a transverse fracture occurs. Next a Kelly clamp will be placed along the length of the gastrocnemius muscle and tightened by closing it for 3 clicks. The tibia and gastrocnemius are not exposed for this procedure. It will remain in place for 30 minutes. A laparotomy will then be

performed and the entire length of the bowel will be examined. Pre-weighed sterile gauze will be placed in the lower quadrants to collect shed blood, then a liver laceration of 75% of the left lobe will be performed. The laparotomy will be closed quickly using sutures. Additional blood will be removed/returned using femoral catheter to maintain MAP at 35 +/- 5. At 60 minutes, the mice will be resuscitated with either 5 day shelf-life pathogen reduced cryoprecipitate(5PRC) or lyophilized pathogen reduced cryoprecipiate (LPRC) and compared to conventional cryoprecipitate (CC), fresh frozen plasma (FFP), or Lactated Ringers to maintain a MAP of 55+/- 5 At 3 hours the mice will be euthanized by cardiac puncture. Blood, lung, and gut will be collected

Results:

For model development, 11 mice were used and the model was modified from 30 minutes of muscle crush to 20 minutes, and from goal MAP of 35+/-5 mmHg during the hypotensive phase to 40+/-5 mmHg. These changes were made to decrease the initial high mortality (80%) during model development.

The modified protocol was carried out on 18 mice, 4 resuscitated with LR, 3 with FFP, 4 with CC, 3 with 5PRC and 4 with LPRC. Mortality was 17% (3 mice: 1 LR, 1 CC, and 1 LPRC).

The average mouse weight was 26.5 +/- 2.4g. Average weight of liver resected was 0.11 +/- 0.02g. Average blood removed to achieve MAP of 40 was 224 +/- 142ul. Average weight of gauze collecting blood from abdominal cavity measured after euthanasia was 0.62 +/- 0.31 and total blood loss was 657 +/- 343ul which were all similar across groups. Resuscitation volume needed to achieve and maintain MAP of 55 +/- 5mmHg was 43 +/- 11 *ml/kg* for LR, 24 +/- 10 *ml/kg* for FFP, 14 +/- 4 *ml/kg* for CC, 15 +/- 7 *ml/kg* for 5PRC, and 24 +/- 8 *ml/kg* for LPRC. Baseline BP was similar across groups 93 +/- 9mmHg. Similarly, post-shock/pre-resuscitation BP was 40 +/- 6 mmHg on average and similar across groups. At 180 minutes, average BP was 92 +/- 9 mmHg for shams, 38 +/- 16 mmHg for LR, 60 +/- 14 mmHg for FFP, 65 +/- 4 mmHg for CC, 57 +/- 19 mmHg for 5PRC, 58 +/- 5 mmHg for LPRC.

Protein concentration in BAL collected from these mice were higher than sham but thus far showed no difference between groups (sham avg 63, LR avg 142, FFP avg 125, CC avg 126, 5PRC avg 141, and LPRC avg 103 ug/ml).

In summary, results thus far demonstrate that the cryoprecipitate products require less volume and augment blood pressure better than LR alone.

Major Task 3:

Methods: C57BL/6J mice 12-16 weeks old and 22-25 grams of both sexes undergo anesthesia with inhaled oxygen and isoflurane. A rectal thermometer is inserted and mice placed on a heating pad. The femoral artery is dissected and cannulated for continuous blood pressure monitoring and blood draws for solution infusion. Baseline blood pressure is monitored and trauma mimicked by performing a 2 cm midline laparotomy, manipulating the bowel, then closing the abdomen. Blood is slowly withdrawn through the arterial catheter until MAP of 35mmHg is achieved. After 90 minutes, mice are resuscitated to achieve a MAP of 55-60mmHg. SHR is carried out for a total of 6 hours. Catheters are then removed, incisions closed, and the animals awoken from anesthesia. Blood pressure is subsequently serially monitored noninvasively. At 72 hours from the initiation of shock, animals are euthanized and blood and tissue harvested.

Results:

Subtask 1: model development
11 mice were used for model development. We found higher mortality than expected by day 1. During shock mice were kept at 35 +/- 5 mm Hg but closer to 35, we then tried to keep the MAP closer to but not> 40 mm Hg.

Subtask 2; Animal surgeries
We have completed 10 mice using this strategy: 4 shams, 3 LR (2 survived), 3 FFP (2 survived). Average weight was 25.6 +/- 2.4g. Total blood removed to achieve shock was 610 +/- 120 ul. Resuscitation volume was 900 +/- 200ul for LR, and 425 +/- 25ul for FFP. Baseline BP was 101 +/- 12 and pre-resuscitation BP was 38 +/- 4mmHg and similar between FFP and LR. BP at 6 hours was 56 +/- 4 for LR and 64 +/- 3 for FFP. BP was similar between FFP and LR over the next 3 days of study (POD 1, 96 +/- 5, POD 2 89 +/- 7, POD 3 89 +/- 12).

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

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to report

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

We will complete the mice experiments in Aim 1 to achieve an adequate sample size and then complete the blood and tissue analysis.

4. **IMPACT:**

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

Not directly related , but the pathogen-reduced 5 day shelf-life cryoprecipitate that is being tested in the current project is now FDA approved (https://intercept-cryoprecipitation.com)

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to date

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

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

We had several problems/delays during the reporting year that have been included in prior reports:

● There was an initial delay in getting product from Cerus as our MTA had expired and there was very challenging legal issues that arose this time. This had been solved.

● Delays in receiving the 5-day shelf live cryoprecipitate product. Due to COVID and constraints on blood availability, cryoprecipitate was not available to the company to produce the product for us. This prohibited us from doing experiments using this product or its comparison with conventional cryoprecipitate. We now have product in hand.

● Unable to arrange to use a ROTEM for coagulation studies: Two options were explored. There was an investigator here at Univ MD that we approached, however he has left the institution. We then reached out the company that makes the machine, and they agreed to allow us to use a machine free of charge. However, COVID imposed a number of issues related to needed hands on training that was not possible and the person that trained took maternity leave. We eventually had to abandon this technology. We are going to use an accepted assay for coagulation.

● The lack of robustness of our original hemorrhage model: We have extensive experience with controlled hemorrhage but this was a model of uncontrolled hemorrhage which we anticipated would be a more severe model. However, this was not the case. We use bronchoalveolar lavage (BAL) fluid protein as an indicator of lung permeability. When we measured BAL protein, there was no difference between groups. We therefore have recently amended on model to include two

additional insults, a tibial fracture and a gastrocnemius muscle crush and results using this model are presented above.

Changes that had a significant impact on expenditures

Nothing to report

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

Significant changes in use or care of human subjects

Not applicable

Significant changes in use or care of vertebrate animals

An amendment was submitted and approved by our IACUC and ACURO related to a modification in our model as described above. This involved the addition of a tibial fracture and a gastrocnemius crush. We also submitted and got approved a change to allow the earlier administration of resuscitative fluids although have not done this yet.

6. **PRODUCTS:**

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

  Journal publications.

Nothing to report

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

Nothing to report

Other publications, conference papers and presentations.

The work from Major Task 2 using the original model was submitted and accepted to AAST as a poster. The same abstract was accepted at MHSRS but had to be withdrawn due to AAST rules prohibiting prior presentation. We intend on submitting a manuscript.

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

- **Technologies or techniques**

Nothing to report

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

Nothing to report

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Rosemary Kozar
Project Role: Principal Investigator
Researcher Identifier:
Nearest person month worked: 3.0 calendar month
Contribution to Project: Designed study, oversees all aspects and conduct of the study

Name: Feng Wu
Project Role: Research Associate
Researcher Identifier:
Nearest person month worked: 1.0 calendar months
Contribution to Project: Assisting with assays

Name: Ahmad Zeineddin
Project Role: Post-Doctoral Fellow
Researcher Identifier:
Nearest person month worked: 12.0 calendar months
Contribution to Project: Responsible for the overall conduct of studies including performing all animal surgeries and assays.

Name: Brooke Dorman
Project Role: Laboratory Assistant
Nearest person month worked: 2.0 calendar months
Contribution to Project: Assisting with tissue sectioning and assays

Name: Shibani Pati
Project Role: Co-Investigator
Researcher Identifier:
Nearest person month worked: 1.0 calendar months
Contribution to Project: Advising and assisting in trouble shooting

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

Nothing to report

What other organizations were involved as partners?

Organization Name: The Regents of the University of California, San Francisco (Dr. Shibani Pati)
Location of Organization: San Francisco, CA
Partner's contribution to the project (identify one or more):

- Other: Advisory

8. **SPECIAL REPORTING REQUIREMENTS**

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. **APPENDICES:**