AD _____

AWARD NUMBER: W81XWH-16-1-0545

TITLE: Adjunctive Therapy to Improve Functional Recovery after Limb Ischemia Reperfusion Injury

PRINCIPAL INVESTIGATOR: Rajabrata Sarkar, MD, PhD

CONTRACTING ORGANIZATION: University of Maryland
Baltimore, MD, 21201

REPORT DATE: October 2018

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT A: Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE *October 2018*	**2. REPORT TYPE** Annual Report	**3. DATES COVERED** **15 Sep 2017-14 Sep 2018**

4. TITLE AND SUBTITLE
Adjunctive Therapy to Improve Functional Recovery after Limb Ischemia Reperfusion Injury

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0545

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Rajabrata Sarkar, MD, PhD

email:

rsarkar@som.umaryland.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

University of Maryland Baltimore
22 South Green Street, S10B00
Baltimore, MD 21201

8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Materiel 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

Acute traumatic extremity ischemia remains a significant cause of limb loss and late limb dysfunction on the battlefield. Even with restoration of blood flow, the period of ischemia initiates a metabolic cascade that will lead to severe dysfunction of the leg and loss of the extremity in some cases. Even in cases when the blood flow is restored, additional injury to the muscle occurs, which is known as ischemia-reperfusion injury. Pyruvate is a naturally occurring metabolite that offers the promise of controlling the metabolic cascade of ischemia-reperfusion injury. This study will test whether ethyl pyruvate is protective in a pig model of limb ischemia with reperfusion. Ethyl pyruvate is one of the few agents that has shown positive results in a rodent model when administered in a clinically relevant post-ischemia protocol, e.g. the agent is effective even when administered after the leg has become ischemic. Given that small animal studies have been performed and indicate that it will likely be useful for human applications, we will combine the use of Ethyl Pyruvate with a model of perfusion-reperfusion injury in a large animal model that better simulates the way the human body responds to treatment of this important injury process.

15. SUBJECT TERMS

Ischemia-reperfusion injury, ethyl pyruvate, animal models of human disease

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
Approved for public release; distribution unlimited				12	USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

Acute traumatic extremity ischemia remains a significant cause of limb loss and late limb dysfunction on the battlefield. Even with restoration of blood flow, the period of ischemia initiates a metabolic cascade that will lead to severe dysfunction of the leg and loss of the extremity in some cases. Even in cases when the blood flow is restored, additional injury to the muscle occurs, which is known as ischemia-reperfusion injury. Pyruvate is a naturally occurring metabolite that offers the promise of controlling the metabolic cascade of ischemia-reperfusion injury. This study will test whether ethyl pyruvate is protective in a pig model of limb ischemia with reperfusion. Ethyl pyruvate is one of the few agents that has shown positive results in a rodent model when administered in a clinically relevant post-ischemia protocol, e.g. the agent is effective even when administered after the leg has become ischemic. Given that small animal studies have been performed and indicate that it will likely be useful for human applications, we will combine the use of Ethyl Pyruvate with a model of perfusion-reperfusion injury in a large animal model that better simulates the way the human body responds to treatment of this important injury process.

2. KEYWORDS:

Ischemia-reperfusion injury, ethyl pyruvate, animal models of human disease

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Task 1: Animal use approval and facility set-up. (0-4 months)

1a: Animal use approval.
Milestones achieved: Local Institutional Animal Care and Use Committee (IACUC), University of Maryland Baltimore (UMB) IACUC deferral and DoD Animal Care and Use Review Office (ACURO) (1-3 months)

1b: Model validation experiments. 6 Sus Scrofa swine to be used for model validation and set-up at UMB. (3-4) months.

Milestones Achieved: Standardized model protocol

All work related to Task 1 have been completed.

(Note: Below timeline is based on the 1-12 months of the No Cost Extension period for the award BA150585)

Specific Aim 1: To evaluate the effects and dose-response curves of EP used as a pharmacological adjunct in the treatment of severe IRI in a clinically relevant swine model.

Task 2: Determine the optimal dose of EP at 4.5 and 6 hours after induction of ischemia. Endpoints will be biochemical, histological, and electromyography. N=46 adult Sus scrofa swine. (1-8 months)

2a: Drug dosage experiments. Two different doses of EP will be tested, 75 mg/Kg, 125 mg/Kg (x dose)

Milestone(s) Achieved: Determination of the best dose of EP for treatment of IRI. (1-3 Months)

2b: Dose response experiments. Based on the injury description from the model, two time points will be tested, (1) 4.5 hours of ischemia, (2) 6.0 hours of ischemia.

Milestone(s) Achieved: Dose response curve of EP effectiveness for treatment of IRI. Optimal dosage of EP therapy determined. (4-6 months)

2c: Statistical analysis of Subtask 2a and 2b. (5-6 months)

Specific Aim 2: To evaluate the effects of combination therapy (EP with and without controlled reperfusion) on the acute neuromuscular, functional, biochemical and histological outcome in severe limb IRI of the lower extremities in this same, clinically relevant, swine model.

Task 3: Evaluate the effectiveness of EP as a combination therapy with controlled reperfusion. (7-11 months)

3a: Controlled reperfusion experiments: N = 34 adult Sus scrofa swine (15-19 months)
*Milestone(s) Achieved: Optimal therapy (*7-11 months)

3b: Statistical analysis of Subtask 3a. (10-11 months)

Task 4: Final Reporting. (11-12 months)

4a: Draft Final Report (11-12 months)

Milestone(s) Achieved: Final report (12 months)

4b: Obtain Scientific and Technical Information (STINFO) clearance. (11-12 months)

Milestone(s) Achieved: STINFO clearance. (12 months**)**

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.

In this reporting period, we were able to:

1. Received all necessary approvals from the University of Maryland Baltimore IACUC regarding the animal surgical procedures.

2. Completed the model validation experiments with 6 Sus Scrofa swine.

A) Initial experiments started February 2017, 3 surgeries completed per protocol, but progress with full approval delayed by death of two animals due to anesthetic administration causing the development of malignant hyperthermia (MH) in three animals, two of which were euthanized.

B) Issue was attributed to small size and developmental immaturity of the animals, increasing risk of MH.

C) No mortality was due to the experimental procedure or agents.

D) Several steps were taken to reduce the risk of MH in this study going forward. In addition to increasing the accepted weight range to use more mature animals that are likely to have a lower rate of symptomatic MH, we decided to obtain animals from a different vendor.

E) To address the complications associated with anesthetic administration, critical revisions to animal protocols were made at the local IACUC level, including using animals of an increased weight, 40-60 kg, and changing the medications administered for surgical anesthesia.

F) The aforementioned issues were resolved and additional animals were added to the preliminary series to demonstrate resolution of the issue to the local IACUC.

3. All modifications needed to the surgical protocol were incorporated and the University of Maryland Baltimore IACUC approved this modified protocol.

4. Modified surgical protocol was submitted to the ACURO for final approval and approval was received.

5. Trained new surgical staff in the surgical procedure needed for this study.

6. Our progress with the project was delayed due to obtaining proper IACUC and ACURO approvals of modified protocols, as these are necessary prior to implementation.

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

This project has provided opportunities in training for general surgery residents by vascular surgery fellows and attending surgeons. Techniques of carotid exposure, vascular control,

retroperitoneal dissection, and arteriotomy closure in particular were developed over time with direct supervision and input from senior surgical staff during the training phase of this project, and continue to be refined with independent study and practice by trainees.

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?

Over the upcoming year, we plan to receive IACUC approval for the full series of 86 animals outlined in the ACURO approval and initial funding request and then we will start on the Experiment 1 series of animals (**Specific Aim 1**). We plan to complete **Major Task 1** and being **Major Task 2**.

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?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. **CHANGES/PROBLEMS**

Changes in approach and reasons for change

Nothing to report.

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

We faced significant delays in receiving the final approval for our modified animal surgery protocol from ACURO. This was subsequently received and we do not anticipate any further delays in next year.

Changes that had a significant impact on expenditures

Delays in beginning our surgical series resulted in reductions in spending over the past years from our initial estimates. The remaining funds will be used in the upcoming year as we continue to work toward completing **Major Goals 1 & 2**.

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

Nothing to report.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS: 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**
  Nothing to report.

  Journal publications.

  Nothing to report.

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

  Nothing to report.

  Other publications, conference papers and presentations.

  Nothing to report.

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

  Nothing to report.

- **Technologies or techniques**

  Nothing to report.

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

  Nothing to report.

- **Other Products**

  Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Rajabrata Sarkar, MD, PhD
Project Role: PI/Surgical Attending
Researcher Identifier (ORCID ID):
Nearest person month worked: 2
Contribution to Project: Supervision of laboratory analysis, protocol revisions, and equipment purchases.

Name: Charles Drucker, MD
Project Role: Surgical Resident/Research Coordinator
Researcher Identifier (ORCID ID): 0000-0002-5846-2027
Nearest person month worked: 4
Contribution to Project: Prepared and revised animal protocols, prepared animal protocol amendments and documentation, obtained instruments and prepared equipment sets, established instrument sterilization plan and protocol, completed surgical procedure, completed animal pre-operative and post-operative care and medication administrations, managed acquisition of tissue and blood samples, coordinated with veterinary resources staff, and arranged for equipment purchases.

Name: John D. Watson, MD
Project Role: Surgical Fellow/Research Associate
Researcher Identifier (ORCID ID):
Nearest person month worked: 1
Contribution to Project: Provided assistance with preparation of animal protocols, guidance of development of procedure, and assisted with equipment selection and acquisition.

Name: Brittany O. Aicher, MD
Project Role: Surgical Resident/Post-doctoral fellow
Researcher Identifier (ORCID ID):
Nearest person month worked: 1
Contribution to Project: Prepared and revised animal protocols, prepared animal protocol amendments and documentation, completed surgical procedure, and coordinated with veterinary resources staff.

Name: Laura DiChiacchio, MD, PhD
Project Role: Surgical Resident/Post-doctoral fellow
Researcher Identifier (ORCID ID):
Nearest person month worked: 1
Contribution to Project: Prepared and revised animal protocols, prepared animal protocol amendments and documentation, completed surgical procedure, and coordinated with veterinary resources staff.

Name: Subhradip Mukhopadhyay, PhD
Project Role: Post-Doctoral Research Fellow
Researcher Identifier (ORCID ID):
Nearest person month worked: 3
Contribution to Project: Provided assistance with preparation of animal data analytic protocols, development of therapeutic agent preparation protocol and prepared agents for blinded administration, supported preparation and sterilization of operative instruments, arranged for tissue and blood sample acquisition/storage/analysis, and assisted with equipment selection and acquisition.

Name: Theresa Nolan
Project Role: UMB Veterinary resources technician
Researcher Identifier (ORCID ID):
Nearest person month worked: 2
Contribution to Project: Assisted with veterinary resources review of animal protocol revisions, assisted with equipment selection and acquisition, provided VR support for the surgery, monitoring, and testing, and assisted in developing veterinary resources personnel plans.

Name: Jennifer Hunt
Project Role: UMB Veterinary resources coordinator
Researcher Identifier (ORCID ID):
Nearest person month worked: 2
Contribution to Project: Assisted with veterinary resources review of animal protocol revisions, assisted with animal and equipment acquisition; provided VR support for the surgery, postoperative monitoring, and laboratory testing; and assisted in developing veterinary resources personnel plans.

Name: Ned Kriel, VMD
Project Role: Veterinary Resources Veterinarian/Chief, Clinical Veterinary Medicine

Researcher Identifier (ORCID ID):
Nearest person month worked: 1
Contribution to Project: Provided veterinary resources review of animal protocols and their revisions; arranged for VR support of protocols; provided veterinary care to swine; provided intraoperative support to the operative procedure and necropsy.

Name: Rigoberto Sanchez, PhD
Project Role: Veterinary Resources Coordinator
Researcher Identifier (ORCID ID):
Nearest person month worked: 1
Contribution to Project: Supervised veterinary resources personnel and VR scheduling.

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?

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.

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.