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

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13. SUPPLEMENTARY NOTES				
14. ABSTRACT				
<p>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.</p>				
15. SUBJECT TERMS				
Ischemia-reperfusion injury, ethyl pyruvate, animal models of human disease				
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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. Our initial experiments with the positive control group animal resulted in death of the animal as it failed to recover fully from the severe ischemia. Due to this, after consultation with the UMB veterinarian, the animal had to be euthanized.
2. We then performed one more experiment but due to technical complications in the anesthesia machine, the animal under surgery suffered severe hypoxia resulting in death.
3. Our original IACUC protocol 0416003 expired in April, 2019 and we submitted a new animal protocol to the UMB IACUC for review and approval. We obtained the final approval of this protocol 0419017 on 06/03/2019.
4. This protocol was subsequently submitted to ACURO for review and approval on 06/04/2019. We received final approval from ACURO on 09/13/2019.
5. We trained new surgical staff in the surgical procedure needed for this study.

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 on completing work that has been described in the **Task 1 of the modified SOW** for the second year of no cost extension period.

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 new animal surgery protocol. 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 Task 1 of the modified SOW.

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: 2

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: Brittany O. Aicher, MD

Project Role: Surgical Resident/Post-doctoral fellow

Researcher Identifier (ORCID ID):

Nearest person month worked: 4

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: 4

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.

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.