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

PRINCIPAL INVESTIGATOR: Robert Crawford

CONTRACTING ORGANIZATION: University of Maryland
Baltimore, MD 21201

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Fort Detrick, Maryland 21702-5012

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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.					
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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?

Major Task 1: Animal use approval and facility set-up. (0-6 months from February 2016).

Subtask 1a: Local Institutional Animal Care and Use Committee (IACUC) Animal use protocol (AUP) review (Expected by June 2016). *Approved June 7, 2016.*

Subtask 1b: DoD Animal Care and Use Review Office (ACURO) protocol review (Expected by August 2016). *Approved August 4, 2016.*

Subtask 1c: Model validation experiments. 6 *Sus Scrofa* swine for model validation and set-up at UMB (Expected by November 2016, re-baselined to March 2017) – *In process, surgical operations complete, delayed by challenges with species, departure of PI from institution, and training of new surgical trainees. Experiments started February 2017, 3 surgeries completed per protocol, but progress with full approval delayed by death of two animals due to development of malignant hyperthermia (MH) in three animals, two of which were euthanized. Issue was attributed to small size and developmental immaturity of the animals, increasing risk of MH. No mortality was due to the experimental procedure or agents. 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 are obtaining animals from a different vendor and have had success with this change. During this time, we transitioned to a new PI for the study and trained additional surgical staff in the procedure. This required local IACUC approval, which further delayed progress. Expected completion in the first half of November 2017.*

Subtask 1d: Review of model validation experiments by local IACUC and formal approval for complete protocol compliment (8-9 months). *Expected completion in the first half of November 2017.*

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

Major Task 2: Determine the optimal dose of EP at 4.5 and 6 hours after induction of ischemia. Endpoints will be biochemical and histological. (Expected August 2016 through January 2018).

Subtask 2a: Completion of Positive (no-reflow) and Negative (sham operation) Control group procedures. *Initiated Spring 2017.*

Subtask 2b: Drug dosage experiments. Two different doses of EP and a control group will be tested, (1) 75 mg/kg, (2) 150 mg/kg, (3) 0 mg/kg (control). *Initiated Spring 2017.*

Subtask 2c: 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. *Pending completion of subtask 2a.*

Subtask 2d: Statistical analysis of subtasks 2a, 2b, and 2c.

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.

Major Task 3: Evaluate the effectiveness of EP as a combination therapy with controlled reperfusion. *Expected January through May 2018. Initiation pending completion of Task 2.*

Subtask 3a: Controlled reperfusion experiments, administration of optimal EP dose (established in **Specific Aim 1**) with controlled reperfusion at two time points, (1) 4.5 hours of ischemia, (2) 6.0 hours of ischemia.

Subtask 3b: Statistical analysis of Subtask 3a, data from subtask 2b and 2c will constitute control groups.

Major Task 4: Preparation and delivery of final report of results. *Expected May through July 2018. Initiation pending completion of Tasks 2 & 3.*

Subtask 4a: Draft Final Report.

Subtask 4b: File for Scientific and Technical Information (STINFO) clearance.

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. Receive approval for preliminary series from UMB IACUC on 6/7/2016.

2. Receive approval from ACURO on 8/4/2016.
3. Apply for approval for full series from UMB IACUC after completion of the first 6 animals as model validation.
 - a. Completion of first animals was originally expected in November 2016, but veterinary resources had to reassign personnel and order specialized equipment for the proposed surgical procedure. They expected to be able to accommodate our first animal in January 2017 and we expected to complete the series by mid-March 2017. This was delayed due to scheduling issues as well as unexpected development of complications within the animal model due to anesthetic administration causing malignant hyperthermia.
 - b. 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.
 - c. The aforementioned issues were resolved and additional animals were added to the preliminary series to demonstrate resolution of the issue to the local IACUC.
 - d. Given that completion of the first several animals was delayed, the report to the UMB IACUC regarding our model development/validation series has been delayed, as has the approval for our full series.
4. Demonstrate proof of concept execution of surgical procedures.
5. Transition to a new PI for the study.
6. Train additional surgical staff in the surgical procedure being performed in 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 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

The following changes were made to our surgical protocol based on our lessons learned from the first experimental animals and approved by our local IACUC.

1. Based on discussions with Dr. Kriel and Dr. Coksaygan, we will be adding Children's chewable aspirin (81mg), as an allowable substitute for enteric coated 325mg Aspirin. 4 of these tabs (324mg) may be substituted for 1 tab (325mg) of EC aspirin if the animal is refusing to consume that tab. This adjustment was made as some animals can refuse to take EC aspirin due to taste, which would prevent proper antiplatelet dosing ahead of the surgery and may impair our ability to restore blood flow following the ischemic interval.
2. Based on Adjustment 1, we will omit the concurrent therapy with famotidine (Pepcid). This is because the chewable aspirin, in Dr. Kriel's experience, dissolves completely enough during ingestion so as to avoid causing the ulceration that can be seen with pills that remain more intact within the stomach and small bowel.
3. Based on actual blood draw needs, the total volume drawn at any time point will now be less than 30mL. Any excess blood drawn will be returned immediately to the animal. The total blood volume required is clarified based on actual laboratory need and to allow for an additional blood draw without theoretically exceeding the total allowed blood draw. The new total volume better reflects actual needs and uses.
4. A time-point for blood draws that was omitted in the prior draft was added, so that a sample of blood is drawn during ischemia approximately 15 minutes prior to reperfusion (prior to initial dosing of study agent). This blood draw time point was intended to demonstrate effective induction of ischemia in the affected limb, which may be difficult to establish based on 2hr blood draws alone. It will effectively demonstrate that equivalent ischemia has been induced and will provide a baseline for analysis of the effects of study agents. Its omission from the original study protocol was an error.
5. The 12-hour post-reperfusion time-point for blood draws was shifted to 18 hours to avoid disturbing normal animal sleep rhythms and facilitate safe animal handling practices. We were concerned that accessing the animal at 2-4am would disrupt normal sleep behavior following surgery and might expose personnel to additional risks due to the timing of the access to the animal facility.
6. As the animals have not had time to recover by 2 hours following reperfusion, we will not attempt to encourage ambulation will be made at that time and no gait observation will be made. Animals will still be visited and observed for wellness, blood draw, and study agent dosing. As animals were still in immediate recovery from anesthesia at this

time point, we felt that encouraging ambulation was futile and unnecessary. The remaining time points will be sufficient to demonstrate recovery of limb and normalization of gait.

7. According with the changes listed above (3-5), the gait observation times are now 4, 6, 18, and 24 hours. The blood draws will now be at sedation or at jugular access, 15min prior to reperfusion, and at 2, 6, 18, and 24 hours post-reperfusion. The timing of administration of the study agent will remain unchanged, at 15 minutes before reperfusion, at reperfusion, and at +30 min, +60 min, +2 hr, +4 hr, and +6 hr from reperfusion. This point is simply to clarify the adjustment in the schedule incurred by adjustments 3-5.
8. A secondary intravenous access (an ear peripheral IV) will be placed following induction of sedation and secured as a secondary means of access so that additional sedation will not be required to obtain secondary access (for blood draws and to administer the study agent) if the central venous line malfunctions. The central line available to us was noted to be less reliable than desired and could not always be depended upon. Adjustments 7-9 are proposed in order to ensure access and dosing is possible, to avoid the necessity for additional animal use if primary access is not functional.
9. The central line used may be a previously sterilized central line that was intended for human use and which will be re-sterilized with Ethylene Oxide if out of date prior to use in any animals.
10. The central venous line may be instilled with a “heparin lock” consisting of heparin-saline or heparin-LR to maintain patency overnight. Concentration of heparin will be 1:1000 to 1:5000 of a volume not to exceed 10mL. This will be administered into the line at the conclusion of any medication dosing, blood draw, or line access if continuous infusion is not being used.
11. As no EMG technicians are currently available and trained for pig handling, we are making EMG measurements optional at all time-points based on future availability. Instead, non-invasive Doppler ultrasonography will be used to assess return of pulsatile arterial flow to the extremity. Our literature search and additional input from Dr. Watson regarding experiences with this technique in prior series means that this technique may not be worth the additional time and efforts associated with its use.
12. We added the use of semi-rigid 5-8 french polypropylene urinary catheters (as used in K-9 urinary catheterization) to our parent protocol. This was added as an alternative to currently approved use of Foley catheters. Placement is the same as for the Foley but securing the catheter would utilize placement of adhesive tape wings and either suturing or stapling the tap to the peri-vaginal skin to prevent slippage intra-operatively. The suture or staple placement locations would be prepped as for surgery described in the parent protocol. Skin staples or sutures and Foley will be removed prior to return to the animal's assigned husbandry run. We found that urethral openings may be small in some pigs related to planned Foley use. The walls to the polypropylene catheters are less thick so a smaller catheter diameter may be placed without reduction in catheter lumen size. The red rubber is another alternative that will be attempted. This addition was based on consultation with Dr. Ned Kriel as a functional alternative when needed.
13. The application of the tether system was made optional as a continuous infusion to maintain patency of the central venous catheter will not be required and will be replaced by use of a heparin-locked central line (modification 10, above). Given the good

tolerance of the strain to handling in preliminary animals, we will intermittently access the line and administrating personnel will stand in run, following the freely-moving animal, to complete the dosing. The animals proved to not require this for regular access to the central line and it may add to animal distress and prevent free movement in animals that otherwise readily tolerate handling. If the animal does not tolerate handling, a tether may still be used.

14. A method of emergency euthanasia was added at the suggestion of Dr. Kriel: In the case that emergency euthanasia is required overnight when veterinary resources staff are not present, the central line will be used to administer a dose of Propofol (8-14mg/kg) and, following sedation, KCl (2Meq/kg) to induce cardiac arrest. Dr. Kriel noted that the surgical team may find the animal in distress at a time point when VR staff are not available to administer the primary method of euthanasia. In this case, the suggested regimen can be administered to safely and rapidly induce sedation and euthanasia by surgical staff, who do not currently have access to a functional lock box and who will not have access to the VR medications.
15. Buprenorphine will be administered every 6-12 hours, with the opportunity to administer a half-strength dose at night in order to bridge the analgesia until morning. This amendment was added to ensure more complete and lasting pain control for the animals in the postoperative period.
16. Rocephin (Ceftriaxone) and Midazolam administration were modified to include the possibility for IV administration. This amendment was added to allow for more complete coverage and to ease administration of medication dosing if IV access is available.
17. "IV bottles" have been modified to "IV bottles/bags" to improve flexibility in drug administration. This amendment was intended to better represent the method of sterile medication administration.
18. The final layer of skin closure may be performed with sterile surgical staples of veterinary grade. The use of sterile staples is consistent with regular clinical practice and will reduce the amount of time spent under anesthesia following the restoration of blood flow to the hind limb.
19. The central venous line will be tunneled in the first phase of the operation (following femoral clamping and, if operating staff are available, even before femoral clamping). Our experience also indicates that the catheter can be safely tunneled laterally and posteriorly on the neck and then can be run up into the collar of the infusion vest. Immediately posterior tunneling creates challenges to the sterility of the final stage of the operation and adds unnecessary complexity. A lateral tunneling position is more readily accomplished by wide preparation of the neck and allows direct visualization of potential vascular and nervous structures. The performance of the tunneling process at the conclusion of the case adds time under anesthesia following reperfusion and can be performed earlier in the case.
20. Moist laparotomy pads will be placed within the neck and midline incision when those areas are not being actively operated upon, to avoid tissue dessication and destruction. These pads will be covered with an operating towel to further aid in the maintenance of an appropriately moist environment. This is a formalization of standard clinical practice for open surgical wounds.
21. Noninvasive study of limb perfusion may be performed during the ischemic period (following vessel clamping) and following reperfusion to establish restoration of

significant arterial flow past the induced lesion. Handheld Doppler ultrasound is a painless, non-invasive, and highly sensitive diagnostic modality that can be used to evaluate the presence and, to a limited extent, the quality of arterial flow. The use in this role is a formalization of standard clinical practice for revascularization procedures.

22. To avoid excess blood loss during attempted access and repair of the jugular and carotid, the vessel can be ligated distally prior to access and proximally, to secure the central venous and arterial lines at the end of the first phase of the operation and to prevent bleeding from the carotid artery at the conclusion of the final phase. The alternative method risks considerable blood loss that, while not appreciated in our animals to date, could conceivably result in the unnecessary loss of animals and generate the need for additional animals without achieving intended experimental end-points.
23. An amendment was added to photographically document the steps of the procedure and the visible changes effected in the hind limbs during the procedure to demonstrate the onset of ischemia as well as recovery and reperfusion. Photographic documentation will facilitate future training of research staff and will provide a means of confirming the effective onset of ischemia and demonstrating recovery from ischemia.
24. Given limitations on the veterinary intravenous flow control pumps, the intravenous infusions around the time of reperfusion will be delivered as a continuous infusion (due to confluence of the boluses). The adjustment in study agent bolus dosing is a factor of the limitations of the existing pump systems and the need to simplify the burden on the veterinarian performing anesthetic support during the critical and precarious time of reperfusion.
25. We considered added the application of dantrolene as a rescue agent, but instead added an alternative end-point for the operative procedure that reads as follows: "If an animal is observed on preparation for surgery to have an elevated exhaled end-tidal carbon dioxide level (EtCO₂) in combination with persistent hyperthermia (T>103) or other signs or symptoms of malignant hyperthermia, the case will be cancelled and the animal will be euthanized per the protocol." Dantrolene is a well-established therapeutic modality for malignant hyperthermia in both humans and swine, however the use of dantrolene is likely to interfere significantly with the interpretation of results as the drug acts on the calcium channels that are also part of the expected pathway to cell death following ischemia and reperfusion. We thus cannot use any animals treated with dantrolene to develop experimental results. Of not, malignant hyperthermia was not a major problem in the original series of more than 100 animals as performed by Dr. Watson in Texas and appears to be largely a supplier issue.
26. We increased the target size for the animals from the current 30-40kg to 40-60kg. Based on our review of the literature, we expect that the increase in animal size will provide more mature animals that may have a reduced risk of MH.
27. We amended the means of anesthesia induction and maintenance to include either isoflurane mask 3-5% or sevoflurane 4.5-7% in 100% O₂ via precision vaporizer with a charcoal scavenge for waste gases. Sevoflurane is a commonly used alternative inhaled anesthetic generally considered to be equivalent to isoflurane. Sevoflurane may have reduced rates of arrhythmias compared to isoflurane (Swindle, Swine in the Laboratory, 2008) and may be less irritating or damaging to pulmonary alveoli (Takala et al. Alveolar integrity and ultrastructure in pigs remain undamaged after exposure to sevoflurane. *Acta Anaesthesiologica Scandinavica*. 2002. 46(9): 1137-43). Compared to continuous

infusion alternatives, specifically propofol, sevoflurane does not result in as severe hemodynamic compromise, which might result in need for pressor support (Annecke, Effects of sevoflurane and propofol on ischaemia-reperfusion injury after thoracic-aortic occlusion in pigs, Br J Anaesth. 2007 May;98(5):581-90. PMID: 17371775). Propofol may also have an undesirable anti-inflammatory effect within the swine model (Rodriguez Lopez et al. Laboratory investigation: effects of propofol on the systemic inflammatory response during aortic surgery. Canadian Journal of Anaesthesia. 2006. 53(7): 701-10.)

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

Delay: A couple of pauses in the surgical series were required due to scheduling of the surgical team.

Action: Additional personnel were added to the local IACUC protocol and were trained in the performance of the procedure.

Delay: Unexpected animal deaths due to MH and illness caused pauses while new animals were acquired and acclimated.

Action: The protocol was adjusted to reduce the risk of MH. We have tested our revised protocol and are confident we can reduce the risk of perioperative MH in our animals.

Delay: Former-PI, Dr. Robert Crawford, left the institution, requiring transfer of all protocols to a new PI.

Action: We have transitioned all protocols to the new PI, Dr. Rajabrata Sarkar, Chief of the Division of Vascular Surgery and an accomplished researcher in the field of Vascular Surgery.

Anticipated Delay: Given the recent delays in starting initial animal model development surgeries to meet the requirements of the UMB IACUC, we anticipate a delay in the completion of this requirement and, accordingly, a delay in the final approval for the full complement of animals outlined in the ACURO protocol. We have also needed to repeat some surgeries in order to complete the preliminary surgical series, which will incur additional delays as these animals must be scheduled and integrated into our surgical schedule.

Actions: We anticipate an unavoidable delay in this area.

Changes that had a significant impact on expenditures

Delays in beginning our surgical series resulted in reductions in spending over the past year from our initial estimates. These 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: Robert Crawford, MD

Project Role: Former-PI/Surgical Attending

Researcher Identifier (ORCID ID):

Nearest person month worked: 2

Contribution to Project: Supervision of preparation of all animal protocols, protocol revisions, and direction for 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?

Former-PI, Dr. Robert Crawford, left the University of Maryland Medical Center, requiring transfer of all protocols and grants to a new PI. We have transitioned all protocols and grants to the new PI, Dr. Rajabrata Sarkar, Chief of the Division of Vascular Surgery and an accomplished researcher in the field of Vascular Surgery. Dr. Sarkar was involved in the original design of the experiment and has extensive experience with the surgical procedure being performed in both his clinical practice and prior research experience.

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.

Adjunctive Therapy to Improve Functional Recovery after Limb Ischemia Reperfusion Injury

BA150585

PI: Rajabrata Sarkar, MD, PhD

Org: University of Maryland Medical Center

Award Amount: \$620,000



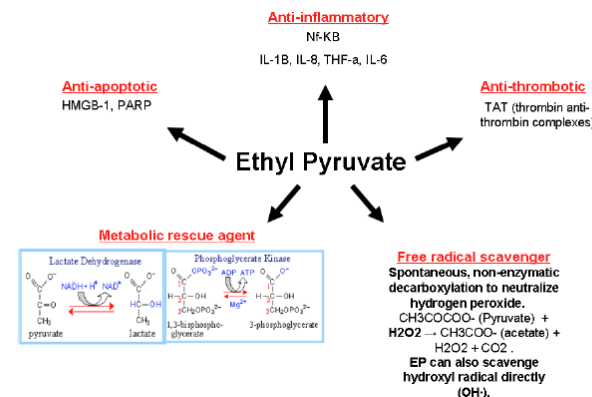
Study/Product Aim(s)

- To evaluate the effects and dose-response curves of EP used as a pharmacologic adjunct in the treatment of severe ischemia reperfusion injury in a clinically relevant swine model.
- Determine the optimal dose of ethyl pyruvate at 4.5 and 6 hours after induction of ischemia.
- Determine the effects of combination therapy with controlled reperfusion.

Approach

Using a swine model we will induce ischemia to the lower extremity to evaluate the effect of ethyl pyruvate and controlled reperfusion on the acute neuromuscular, functional, biochemical, and histological outcome.

Accomplishment: Successful completion of model validation experiments.



Goals/Milestones

CY17 Goal – Model validation experiments

- ☒ Preliminary experiments with 6 *Sus Scrofa* swine.
- ☐ Review of model validation experiments by local IACUC and formal approval for complete protocol compliment.

CY18 Goal – Determine optimal dose of EP after induction of ischemia

- ☐ Positive (no-reflow) and Negative (sham operation) control group procedures.
- ☐ Evaluate dose of EP, (1) 75 mg/kg, (2) 150 mg/kg, (3) 0 mg/kg (control).
- ☐ Evaluate timing of EP, (1) 4.5 hours, (2) 6.0 hours of ischemia.
- ☐ Evaluate the effects EP at optimum dose with controlled reperfusion at, (1) 4.5 hours of ischemia or (2) 6.0 hours of ischemia

Comments/Challenges/Issues/Concerns

- There were unforeseen delays in establishing surgical facilities, limiting our ability to commence the study.
- Model validation and full approval delayed by malignant hyperthermia in 3 animals and consequential procedural and animal care adjustments requiring local IACUC approval. No mortality was due to the experimental procedure or agents.
- During CY17 we transitioned to a new PI for the study and trained additional surgical staff.

Budget Expenditure to Date

Projected Expenditure: \$200,000

Actual Expenditure: \$88,000

Timeline and Cost

Activities	CY	16	17	18
Animal use approval and facility set-up				
Determine optimal dose of EP				
Evaluate effect of EP in combination with controlled reperfusion				
Estimated Budget (\$K)		\$50K	\$150	\$200K

Updated: 9/22/2017