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TITLE: Alkaline Phosphatase for the Prevention of Intestinal and Renal Injury in a Rat Model of Cardiopulmonary Bypass with Deep Hypothermic Circulatory Arrest

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Alkaline Phosphatase for the Prevention of Intestinal and Renal Injury in a Rat Model of Cardiopulmonary Bypass with Deep Hypothermic Circulatory Arrest

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**6. AUTHOR(S)**

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**5f. WORK UNIT NUMBER**

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

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**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

Purpose: To determine if alkaline phosphatase decreases intestinal and renal injury in a piglet model of cardiopulmonary bypass with DHCA, mediated in part through adenosine signaling pathways.
Scope: Evaluate histologic (primary outcome), physiologic, and biomarker evidence of intestinal and kidney injury in this model with administration of escalating doses of bovine intestinal alkaline phosphatase or specific inhibitors of native alkaline phosphatase. Assess the role of downstream adenosine as a mediator of alkaline phosphatase effect.
Major Findings: For the first reporting period, we had successfully established the model, demonstrating appropriate levels of physiologic disturbance (hemodynamic instability and lactic acidosis). Systemic interventions are nearly complete, at which time the initial analysis of the primary outcome will be performed for these groups.

**15. SUBJECT TERMS**

Nothing listed

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1. **Introduction**

   Cardiopulmonary bypass and deep hypothermic circulatory arrest are frequently required for the repair of complex congenital heart disease in neonates and infants. While use of these techniques is necessary for surgical repair, the associated ischemia-reperfusion injury to the intestines and kidneys can lead to substantial post-operative morbidity. The purpose of this project is to provide preliminary evaluation of the potential role of alkaline phosphatase for the prevention of intestinal and kidney injury after pediatric cardiopulmonary bypass with deep hypothermic circulatory arrest. In this model, we place 5-10kg infant pigs on cardiopulmonary bypass, cool them to 22 degrees C rectal temperature, stop blood flow for 75 minutes, rewarm using the bypass circuit, then separate from bypass for 4 hours prior to euthanasia. Injury from this basic model is being compared to systemic or intestinal treatment with bovine intestinal alkaline phosphatase as well as inhibition of native alkaline phosphatase. The primary outcomes are changes in acute intestinal and kidney injury histology scores; secondary outcomes include physiology changes and biomarkers of organ injury.

2. **Keywords:** Alkaline phosphatase, adenosine, cardiopulmonary bypass, deep hypothermic circulatory arrest, cardiac surgery, acute kidney injury, intestinal barrier function

3. **Accomplishments**

   a. **What were the major goals of the project?**

      i. Protocol Development and Approval: 4 months
      ii. Initial Model Completion: 2 months
      iii. Testing Alkaline Phosphatase Interventions: 10 months
      iv. Mechanistic Assays (Adenosine receptor stimulation/blockade): 2.5 months
      v. Data Analysis, Abstracts, Publication: 5 months

   b. **What has been accomplished under these goals?**

      i. Protocol Development and Approval: Complete. This task was delayed by ~5 months due to the need to transition to a piglet model from an adult rodent model (please see section 5a). We had IACUC approval for the rodent model originally, and while awaiting final funding approval we began working on the basic model development using internal funds. We quickly found that the variability of the surgery was too great in our hands and requested transition to the technically easier (and more scientifically applicable) infant pig model. The transition required initial approval by USAMRMC, resubmission to IACUC, and final approval by ACURO resulting in the initial delay. Following this transition, the timeline we have had a remarkably smooth experience with the project and encountered no other substantial delays.
      ii. Initial Model Completion: Complete
      iii. Testing Alkaline Phosphatase Interventions: 50% complete. As of the first week in October, all standard bypass model animals as well as all systemic supplementation/inhibition animals will be complete, allowing for the first blinded analysis for the primary outcomes. We will then proceed with the intestinal interventions.
      iv. Mechanistic Assays: Pending in the next reporting period
      v. Data analysis, Abstracts, Publication: Pending in the next reporting period

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

Nothing to report

**d. How were the results disseminated to the communities of interest?**

Nothing to report

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

i. Testing Alkaline Phosphatase Interventions: As stated in the prior section, we are close to completing the systemic interventions with the remaining animals in this group scheduled for surgery over the next two weeks. Following completion of the systemic group we will turn to the intestinal group. Surgeries are scheduled through December to allow us to complete the majority of this group by the end of 2017. The remaining 3-4 surgeries will be performed in January, allowing for blinded analysis for the primary outcomes in February.

ii. Mechanistic Assays: The six surgeries needed for the preliminary mechanistic assays will be performed between January and February 2017.

iii. Data analysis, Abstracts, Publication: Analysis of the primary outcomes for the systemic group (blinded histology grading of intestinal and kidney samples) as well as physiologic parameter analysis and initial biomarker analysis will begin immediately upon completion of the systemic animal groups in October. We anticipate the first abstract submissions to either Pediatric Academic Society or American Thoracic Society meetings by November. Secondary analysis of serum biomarkers, immunohistochemistry, and tissue RNA/protein expression as well as metabolomics profiling of serum, urine, and tissue will be ongoing. Primary analysis of animals in the intestinal intervention group will begin in January following completion of the final surgeries.

**4. Impact**

**a. What was the impact on the development of the principal discipline of the project?**

Nothing to report

**b. What was the impact on other disciplines?**

Nothing to report

**c. What was the impact on technology transfer?**

Nothing to report

**d. What was the impact on society beyond science and technology?**

Nothing to report

**5. Changes/Problems**

**a. Changes in approach and reasons for change**

The primary change in approach for the study was the transition from a rodent model to the infant piglet model. This change occurred immediately following the project award and was approved by USAMRMC. The reason for the change was inconsistency in the ability to cannulate the rats for cardiopulmonary bypass, leading to unacceptable variation in the physiologic condition of the rats. Transition to the piglet model also had multiple benefits beyond greater consistency of surgical approach. We now have a true pediatric model and the piglet physiology more consistently mirrors human infant physiology leading to the potential for easier translation of our ultimate findings. The

transition did result in a delay in model development as previously discussed, as we needed first overall approval from the sponsor, followed by approval of the full new protocol by the local regulatory agency as well as the Department of Defense. Model development thus did not begin until late January 2017; however, since that time we have made excellent progress and fully anticipate completion of all animal experiments in the protocol by the end of the full funding period.

**b. Actual or anticipated problems or delays and actions or plans to resolve them?**

Other than the initial delay, problems with the protocol have been relatively minor:

i. Donor blood: During model set up we realized that the donor blood for the bypass circuit contained multiple biomarkers of inflammation and injury that could affect our assessment of native production of these biomarkers by the experimental animal. Therefore, we have limited exposure to the donor blood to that needed to prime the pump (300ml in all cases) and use only blood from the circuit or crystalloid for volume resuscitation after separation from bypass.

ii. Urine Sampling: Bladder catheterization proved to be much more challenging than anticipated, with ~70% success rate typically involving some uretheral trauma (bleeding) that worsened with heparinzation for bypass cannulation. This led to both physiologic instability for the piglet as well as blood contamination of the urine samples. As a best option available, we decided to forgo catheterization in the final model and instead perform direct bladder puncture at the time of euthanasia for collection of all pooled urine. This technique provides a non-contaminated sample for accurate analysis and a full sampling of all urine produced during the case. Negative impacts include primarily the inability to test differences in urine composition in pre versus post bypass samples.

iii. Duration of Circulatory Arrest: We originally planned on a 60 minute deep hypothermic circulatory arrest period, although acknowledged in the original proposal that this may need to be extended up to 90 minutes if there was evidence of insufficient organ injury. We trialed 60 and 90 minutes with 60 producing too little injury and 90 minutes producing too much injury. Ultimately we settled on 75 minutes, which has produced physiology very similar to our children undergoing moderate to high risk surgery (elevated lactate, cardiovascular instability requiring inotropic support, and consistent acute kidney injury, intestinal mucosal injury, and acute lung injury at 4 hours post bypass). We have also adjusted the target rectal temperature from 18 degrees C to 22 degrees C in the final model. Originally we did not have access to esophageal temperature monitoring (standard of care in our operations in children) and were using rectal temperature monitoring. We became concerned that the large amount of stool in our piglets was insulating the rectal

probe and underestimating the overall degree of cooling. We were able to borrow an esophageal probe to perform simultaneous recordings and indeed found that a rectal temperature of 22 degrees C best represented an esophageal temperature of ~18 degrees C.

iv. Anticipated problem: Controls for tissue analysis, biomarkers, and PCR. Our one significant anticipated problem as we developed the model was the lack of relatively healthy control tissue for comparison with the different experimental interventions. Originally we felt that the standard model would be sufficient to serve as the control for the alkaline phosphatase interventions, but we now recognize that both true anesthesia-only controls as well as control animals with alk phos infusion but no bypass/circulatory arrest would be valuable additions to help us better understand the tissue level changes in our experimental animals. Currently we are in the process of submitting an amendment to IACUC to allow 5 animals in each of these categories. Once the amendment is approved we will forward to ACURO for final approval. Of note, the extra expenses incurred by these additional animals will be covered by internal funding.

v. Anticipated problem: Assessment of intestinal barrier function. After further calculations we decided that measurement of intestinal barrier function with FD-4 was difficult and very expensive to perform even in a subset of the larger animals (initially proposed for the rodent model). Instead we are planning to perform lactulose/mannitol gavage to monitor lactulose to mannitol ratio in the blood and urine. This change is also included in the upcoming amendment and will be forwarded to ACURO following IACUC approval.

**c. Changes that had a significant impact on expenditures**

None to date. As stated in the prior section, the addition of non-bypass control groups would create additional expense that we would cover with available internal funding.

**d. Significant changes in use or care of vertebrate animals**

See prior sections.

**6. Products**

Nothing to report

**7. Participants and Other Collaborating Organizations**

a. Jesse Davidson: No change
b. Suzanne Osorio: No change
c. James Jaggers: No change
d. Scott Lawson: No change
e. Suhong Tong: No change
f. Christine Baird: Ms. Baird did not ultimately work on the project beyond the initial planning phase. Her role has been transitioned to Ludmilla Khailova

g. Ludmilla Khailova
    i. Role: PRA
    ii. Research Identifier: None
    iii. Nearest person month worked: 6
    iv. Contributions to the project: Ms. Khailova is the primary research assistant on this project. Her role covers a multitude of tasks including surgical first assist in the OR, lab processing in the OR, data entry, specimen processing, histology, ELISA, and PCR.
    v. Funding support: Ms. Khailova is partially funded by the grant. Additional funding is provided by AHA17IRG33410724 (PI Davidson) and NIH/NHLBI 1K23HL123634 (PI Davidson).

**8. Special Reporting Requirements**

Nothing to report

**9. Appendices**

None