Hydroxocobalamin for Neuroprotection in a Hemorrhagic Swine Model Traumatic Brain Ischemia: POI and ERC Care

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HYDROXOCOBALAMIN FOR NEUROPROTECTION IN A HEMORRHAGIC SWINE MODEL OF GLOBAL AND TRAUMATIC BRAIN ISCHEMIA: POI and ERC Care

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14. ABSTRACT-

Traumatic hemorrhage is a leading cause of death in the military and civilian environment. Occurrence of Traumatic Brain Injury (TBI), concurrently with hypotension is associated with poor prognosis. TBI and global brain ischemia (GBI) cause many of the same injures in the brain. We have shown that pre-clinical treatment with Hydroxocobalamin (HOC), is as effective as whole blood and Hextend in maintaining mean arterial pressure (MAP) in a swine model of hemorrhage.

Our intention was to determine any neuroprotective effects that HOC may have provided in models of TBI or GBI with hypotension. Based on previous studies with HOC, we anticipated that it was valuable to pursue research endeavors with HOC. We hypothesized that 1) hemorrhaged swine treated with HOC after cardiac arrest or TBI will show normal behavior during the 72 hour observation period (as determined by a neurologic severity score); 2) that brain injury biomarkers will decrease over time in hemorrhaged swine treated with HOC and; 3) GBI or TBI animals treated with HOC will show less brain damage compared to untreated animals, as determined by a certified veterinary pathologist.

In preparation for the execution of this project, several problems were encountered that caused delays, ultimately leading to study closure. This included 1) a two-year delay with no resolution, on the acquisition of the TBI cortical impact device (PCI3000, Hatteras Instruments). As a result, a new TBI model/device was proposed, supplies were ordered and bench tests on swine carcasses began in December 2019. 2) For model refinement of the new TBI device, those bench tests required leveraging swine carcasses left from other protocols at the CIRS laboratory. A few bench tests were conducted; however, during the testing phase the COVID-19 pandemic occurred resulting in a slow-down of activity at the CIRS laboratory, making it difficult to obtain the remaining carcasses to refine the model. 3) For the GBI portion of the project, the drug epinephrine was required but a National shortage delayed its acquisition. All this culminated in the cancelation of the project since it was not possible to complete it before the end of the current fiscal year.

15. SUBJECT TERMS- Swine, Hydroxocobalamin, HOC, global brain ischemia, GBI, traumatic brain ischemia, TBI

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1.0 EXECUTIVE SUMMARY

Traumatic hemorrhage has been a leading cause of death in the military and civilian environments. Occurrence of Traumatic Brain Injury (TBI), concurrently with hypotension is associated with a poor prognosis. TBI and global brain ischemia (GBI) cause many of the same injures in the brain. We have previously shown that pre-clinical treatment with Hydroxocobalamin (HOC), a U.S. FDA approved drug, is as effective as whole blood and Hextend in maintaining mean arterial pressure (MAP) in a swine model of class III hemorrhage.

With this project, it was our intention to determine any neuroprotective effects that HOC may provide in combined models of TBI or GBI with hypotension. Based on the results from previous studies with HOC, we anticipated that it was valuable to pursue research endeavors with HOC in both GBI and TBI. We hypothesized that 1) hemorrhaged swine treated with HOC after cardiac arrest or TBI will show normal behavior during the 72 hour observation period (as determined by a neurologic severity score), 2) that brain injury biomarkers (S100b, neuron-specific Enolase (NSE) and glial fibrillary acidic protein (GFAP) will decrease over time in hemorrhaged swine treated with HOC, and 3) GBI or TBI animals treated with HOC will show less brain damage compared to untreated animals, as determined by a certified veterinary pathologist.

To evaluate those hypotheses, we formulated the following Aims: 1) To determine whether hemorrhaged swine with induced GBI from cardiac arrest and treated with HOC show greater neurological function compared to untreated animals. 2). To determine whether hemorrhaged swine with TBI induced by a commercially available powder actuated piston and treated with hydroxocobalamin show greater neurological function compared to untreated animals.

In preparation for the execution of this project, several problems were encountered that caused delays, ultimately leading to study non-completion and closure. This included:

1) A two-year delay with no resolution, on the acquisition of the computer-controlled cortical impact device (PCI3000, Hatteras Instruments) as the instrument to induce TBI. As a result, a new TBI model/device was proposed based on literature reviews with a similar model presented by Earle S, de Moya M, et.al. titled: "Cerebrovascular Resuscitation after Polytrauma and Fluid Restriction." published in the *J. American College of Surgeons* in 2007. An amendment to the IACUC was submitted to change the model, supplies were ordered, and bench tests on swine carcasses began in December 2019.

2) For model refinement of the new TBI device, those bench tests required leveraging swine carcasses left from other protocols at the CIRS laboratory. A few bench tests were conducted; however, during the testing phase, the COVID-19 pandemic occurred, resulting in a slow-down of activity at the CIRS laboratory, making it difficult to obtain the remaining carcasses to refine the model.

3) For the GBI portion of the project, the drug epinephrine was required but a National shortage delayed its acquisition. All this culminated in the cancelation of the project since it was not possible to complete it before the end of the current fiscal year.

2.0 INTRODUCTION

There is little doubt that combat-related traumatic brain injury is a leading cause of morbidity and mortality. As of July 2016, moderate-to-severe traumatic brain injuries (TBI) sustained by members of the Department of Defense worldwide since 2000 totaled 34,855 cases.[1] Traumatic brain injuries from explosive blasts are often associated with traumatic amputations and hemorrhage. A review of the Joint Theater Trauma

Registry indicated that 72% of casualties from 2001 to 2009 were due to explosives causing TBI and hemorrhagic injures.[2]

Trauma related brain injury on the battlefield may also be a consequence of hemorrhage-induced cardiac arrest. The most common cause of cardiac arrest on the battlefield is hemorrhagic trauma. Eighty percent of battlefield injuries in which hemorrhage was the cause of death were potentially survivable.[2] For patients who initially achieve return of spontaneous circulation (ROSC) after cardiac arrest, subsequent morbidity and mortality is due, in large part, to cerebral dysfunction caused by global anoxia. From January 2007 to January 2014, 582 casualties were administered cardiopulmonary resuscitation (CPR) and 56% of those were administered CPR on the battlefield or forward operating medical facilities. In fact, of the 52 documented hemorrhage-induced cardiac arrests which occurred over seven (7) months at one Role of Care 3 hospital, 14 (27%) achieved ROSC.[3] Although CPR is not currently recommended by the Tactical Combat Casualty Care Committee for military personnel found on the battlefield pulseless with penetrating trauma, research aimed at better treatments for hemorrhagic trauma could potentially change guidelines. For example, research continues in the use of resuscitative endovascular balloon occlusion of the aorta (REBOA) as an alternative to resuscitative thoracotomy for treatment of hemorrhagic cardiac arrest and subsequent cardiopulmonary resuscitation.[4] Studies to evaluate its use in the prehospital setting are ongoing. And as CPR techniques improve, survival from hemorrhagic cardiac arrest may increase. Treatment to mitigate effects of global brain ischemia (GBI) post cardiac arrest will be essential.

The pathology of global brain ischemia (GBI) and traumatic brain injury (TBI) have many elements in common. Both are characterized by cerebral edema, ischemia and neuronal death. Neuronal cell death is characterized by decreased ATP, increased cellular calcium, release of glutamate, increased arachidonic acid, and gene activation leading to cytokine synthesis, free radical and nitric oxide production, and ultimately the activation of the apoptotic pathway.[5] Many of these processes are calcium driven. One of the neuronal cell populations most sensitive are pyramidal cells in the CA1 region of the hippocampus,[6] a brain region important in learning and memory. Accordingly, the cognitive deficits after GBI and TBI specifically include dysfunctions in memory acquisition and retention. Other cell populations that are sensitive include neurons in the striatum and purkinje cells in the cerebellum. Severity of outcome is correlated with the duration of brain ischemia and with the extent of the resulting neuronal cell death.

Current treatment for cardiac arrest associated GBI and TBI are similar and limited to therapeutic hypothermia, sedation, seizure control, mechanical ventilation to maintain oxygenation > 94%, circulatory support and prevention of hyperglycemia. Studies evaluating therapeutic hypothermia suggest that it is more effective than normothermia in patients whose initial cardiac arrhythmia is ventricular fibrillation (VF) and may be beneficial for TBI patients.[7] The data are not as conclusive for patients whose initial arrhythmia is asystole or pulseless electric activity.

Because GBI and TBI are characterized by calcium-driven processes, much of the experimental treatments have included calcium modulating treatments such as N-methyl-aspartate receptor (NMDAR) antagonists and calcium channel blockers. While a variety of different targets have been tested, many efforts have focused on inhibitors of NMDAR.[8] Over-activation of the NMDAR is thought to be a lead cause for "excitotoxic" neuronal cell death in several conditions, including GBI, stroke, and TBI. However, these have not shown benefit compared to no treatment.[9] Modulation of calcium's influence in apoptosis and inhibition of nitric oxide synthase continue to be a robust area of interest for treatment of GBI and TBI.[10]

Hydroxocobalamin targets the Calcium/calmodulin (CaM)-dependent nitric oxide pathway by tissue specific regulation of endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS), and neuronal nitric oxide synthase (nNOS).[11] CaM-dependent enzymes continue to remain active beyond the relatively brief NMDAR over-activation. This makes hydroxocobalamin a potentially more

attractive drug than the NMDAR antagonists, and nitric oxide regulators have shown promise in animal models.[12] Whereas nitric oxide production by eNOS may play a protective role in brain ischemia, nitric oxide produced by iNOS and nNOS is damaging.[13] Although there are few studies elucidating the tissue specific effects of NOS regulation by hydroxocobalamin, based on the efficacy of other NOS regulators it is reasonable to conjecture that it will have a beneficial effect on brain ischemia. Our laboratory has extensive experience in evaluating the efficacy of hydroxocobalamin in animal models of mitochondrial poisoning[14], hemorrhage[15] and sepsis (manuscript in progress). Hydroxocobalamin has shown benefit in all of these models.

There are advantages to using large animals in these brain injury studies. Although rodents are often used as models for ischemic brain injury, swine more closely proximate the neuroanatomy and physiology of human brains. First, the swine brain is larger (≈ 200 grams) compared to a rodent brain (≈ 0.5 grams), it is gyrencephalic, and has a similar white to grey matter ratio within the brain as compared to humans (60:40 white to grey matter). Second, with large animals it is easier to gain arterial and venous access providing the ability to obtain serial blood gases, brain injury biomarkers, cytokines, and chemistries. These combined characteristics may produce a more easily translatable model.

3.0 METHODS, ASSUMPTIONS AND PROCEDURES

Below is the summary of the original procedure plan for each experimental arm within this protocol -

Experiment 1:

Cardiac arrest models of brain ischemia using domestic swine such as Yorkshire Landrace cross, have been used in many studies to evaluate neuroprotective measures. The cardiac arrest model that we attempted to use and refine in our laboratory to produce global brain ischemia, was one that would ensure survival in a significant number of animals. During the CPR phase, we proposed to use left ventricular compressions rather than standard compressions. Survival data from previous studies (FWH20150042A: " Protection from neuronal cell death caused by global cerebral ischemia induced by cardiac arrest followed by cardiopulmonary resuscitation in swine (Sus scrofa)", and FWH20150086A: "Hydroxocobalamin with cardiopulmonary resuscitation compared to cardiopulmonary resuscitation alone in protection of neuronal cell death produced by global cerebral ischemia induced by cardiac arrest in swine (Sus scrofa)"), indicate that greater than 80% of study animals survive when apnea is limited to 5-8 minutes followed by CPR for 2-4 minutes. Our model of GBI, based on unpublished data has been shown to produce measurable and identifiable neurodegeneration via histopathology.

Experiment 1 Specific Aim: To determine whether hemorrhaged swine with induced global brain injury from cardiac arrest and treated with hydroxocobalamin show greater neurological function compared to untreated animals.

Delay to start for this specific aim was two-fold. The first delay was due to a back order of epinephrine in 2019 (National Shortage). Additionally, the team continued to work with Hatteras Instruments and Logistics to order the computer-controlled cortical impact device (CCI) for Experiment 2 to keep study progress moving.

Experiment 2:

To evaluate the efficacy of hydroxocobalamin in TBI, we wanted to use the controlled cortical impact (CCI) model. Compared to the fluid percussion injury model, this model allows for better control over mechanical factors, such as velocity of impact and depth of resulting deformation. The device we wanted to use would accommodate swine up to 40 kg in weight. Although this model does not perfectly simulate TBI secondary

to blast injury, it replicates clinical brain injury with skull deformation and it is used to analyze neuronal cell death and resulting neurological deficits following TBI.

Experiment 2 Aim: To determine whether hemorrhaged swine with traumatic brain injury induced by a CCI device and treated with hydroxocobalamin show greater neurological function compared to untreated animals.

Continued delay to start this specific aim was due to the difficulty in obtaining the CCI device from Hatteras Instruments with ultimately no resolution. To thwart this delay, a new TBI model/device was researched/discussed and selected. The protocol was amended and bench tests were started using swine carcasses from other protocols that became available at the CIRS laboratory. However, ultimately the study still came to a halt due to the COVID-19 pandemic.

Additional amendments to the study protocol included:

Amendment #1, dated 06 March 2019 – Addition of Dr. Jennifer Rebeles as an associate investigator (AI).

Amendment #2, dated 18 March 2019 - Addition of Dr. Melissa Clemons as an AI.

Amendment #3, dated 01 July 2019 – Addition of Dr. R. Madelaine Paredes as an AI who further helped to amend the TBI model due to not being able to purchase the original CCI device.

Amendment #4, dated 27 August 2019 – Request to collect cerebral spinal fluid (CSF) at three different time points and to measure the biomarker UCHL-1 from blood samples and CSF.

Amendment #5, dated 06 December 2019 – Add "*Sus scrofa*" to the protocol title.

4.0 MAJOR EVENTS/MILESTONES/SUCCESS

In preparation for the execution of this project, several problems were encountered that caused delays, ultimately leading to study non-completion and closure. See Technical Challenges (below) for additional details.

- Kick Off Meeting – Date: N/A
- IACUC Approval – Date: 11 December 2018
- All experimental procedures completed – date: N/A
- Data Analysis – date: N/A
- Poster presentation – provide location and date: N/A
- Manuscript submitted to – name of journal and date: N/A
- Dissemination of Results –N/A

5.0 RISK ASSESSMENT

5.1 Risk Analysis:

Medium and High Risk for non-completion has been highlighted in the quarterly reports. The 3 risks to study completion identified were:

1. National shortage of epinephrine
2. Computer-controlled cortical impact device: 2 year delay with no resolution. Change in TBI device required.
3. COVID-related facility closure to R&D lab activity.

5.2 Technical Challenges

In preparation for the execution of this project, several problems were encountered that caused delays, ultimately leading to study closure. This included 1) a two-year delay with no resolution,

on the acquisition of the computer controlled cortical impact device (PCI3000, Hatteras Instruments) as the instrument to induce TBI. As a result, a new TBI model/device was proposed, based on literature reviews, were a similar model presented by Earle S, de Moya M, et.al. was selected: "Cerebrovascular Resuscitation after Polytrauma and Fluid Restriction". Published in the J. American College of Surgeons, 2007. An amendment to the IACUC was submitted to change the model, supplies were ordered, and bench tests on swine carcasses began in December 2019. 2) For model refinement of the new TBI device, those bench tests required leveraging swine carcasses left from other protocols at the CIRS laboratory. A few bench tests were conducted; however, during the testing phase, the COVID-19 pandemic occurred, resulting in a significant slow-down of activity at the CIRS laboratory making it difficult to obtain the remaining carcasses to refine the model. 3) For the GBI portion of the project, the drug epinephrine was required but a National Shortage delayed its acquisition. These challenges culminated in the cancelation of the project since it was not possible to complete it before the end of the current fiscal year.

6.0 TRANSITION PLAN

6.1 Military Relevance

Since 2000, 34,855 military personnel have sustained moderate to severe traumatic brain injury with many of these injuries associated with penetrating injuries that cause significant blood loss. In fact, traumatic hemorrhage has been a leading cause of death in military environments. Traumatic brain injury concurrently with hypotension is associated with a poor prognosis. We have previously shown that Hydroxocobalamin (HOC), a U.S. Food and Drug Administration-approved drug, is as effective as whole blood and Hextend in maintaining mean arterial pressure in a swine model of class III hemorrhage. Without the completion of this study, a continued gap exists in determining neuroprotective effects that HOC may provide for models of TBI or GBI with hypotension.

6.2 Transition Strategy

If Hydroxocobalamin could provide neuroprotection and reverse hypotension, it could prove to be a valuable pharmacologic agent for battlefield ischemic brain injuries. There is still valuable information needed to understand HOC and its potential therapeutic benefits for brain injuries.

7.0 RESULTS

No protocol animals were completed. Based on preliminary bench tests, the weight range for a future survivor TBI model should be 35kg -45kg when the powder-actuated piston is used to induce TBI.

8.0 CONCLUSION/DISCUSSION

Occurrence of Traumatic Brain Injury (TBI), concurrently with hypotension is associated with a poor prognosis. TBI and global brain ischemia (GBI) cause many of the same injures in the brain. We have previously shown that pre-clinical treatment with Hydroxocobalamin (HOC), a U.S. FDA-approved drug, is as effective as whole blood and Hextend in maintaining mean arterial pressure (MAP) in a swine model of class III hemorrhage.

With this project, it was our intention to determine any neuroprotective effects that HOC may provide in combined models of TBI or GBI with hypotension. Based on the results from previous studies with HOC, we anticipated that it was valuable to pursue research endeavors with HOC in both GBI and TBI. We hypothesized that 1) hemorrhaged swine treated with HOC after cardiac arrest or TBI will show normal behavior during the 72 hour observation period (as determined by a neurologic severity score), 2) that brain injury biomarkers (S100b, neuron-specific Enolase (NSE) and glial fibrillary acidic protein (GFAP) will decrease over time in hemorrhaged swine treated with HOC, and 3) GBI or TBI animals treated with HOC

will show less brain damage compared to untreated animals, as determined by a certified veterinary pathologist.

In preparation for the execution of this project, several problems were encountered that caused delays, ultimately leading to study closure. These challenges included a national shortage of epinephrine (required for the study), inability to obtain computer-controlled cortical impact device, change of device required, and COVID-related facility closure. There is still valuable information needed to understand HOC and its potential therapeutic benefits for brain injuries. Future steps needed to complete this study would include completion of bench tests for the powder-actuated piston to determine appropriate animal weight range, model development, and completion of study methods. If Hydroxocobalamin could provide neuroprotection and reverse hypotension, it could prove to be a valuable pharmacologic agent for battlefield ischemic brain injuries.

9.0 DELIVERABLES

9.1 Publications: None

9.2 Presentations: None

10.0 COST

This work was funded by a J9 award. Total funding awarded for this Project Code Number, J917EC04, in the amount of $460,000.00 were expended by September 29, 2019. No funds are remaining to assist with the completion of this study.

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FIGURES AND TABLES: None

12.0 LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

1. TBI – traumatic brain injury
2. GBI – global brain ischemia
3. HOC – Hydroxocobalamin
4. MAP – mean arterial pressure
5. NSE – neuron specific enolase
6. GFAP – glial fibrillary acidic protein
7. ROSC – return of spontaneous circulation
8. CPR – cardiopulmonary resuscitation
9. REBOA – resuscitative endovascular balloon occlusion of the aorta
10. VF – ventricular fibrillation
11. NMDAR – N-methyl-aspartate receptor
12. CaM – Calcium/calmodulin
13. eNOS – endothelial nitric oxide synthase
14. iNOS – inducible nitric oxide synthase
15. nNOS – neuronal nitric oxide synthase
16. CCI – controlled cortical impact