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TITLE: Amelioration of Ischemia/Reperfusion Injury During Resuscitation from Hemorrhage by Induction of Heme Oxygenase-1 (HO-1) in a Conscious Mouse Model of Uncontrolled Hemorrhage

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<b>14. ABSTRACT</b>  Ischemia occurs whenever there is interruption of the flow of blood to tissues or organs. It is the most common cause of death in heart disease and stroke as well as traumatic injury. Survival of the initial insult is followed by further injury that occurs during the reintroduction of oxygen with the restoration of blood flow. This injury occurs following hemorrhage because some tissues are deprived of blood to protect others as part of the fight or flight response. Heme oxygenase-1 (HO-1) induction is correlated with a significant reduction in ischemic injury and 1-[2cyano-3, 12-dioxoleana-1, 9(11)-dien 28-oy]imidazole (CDDO-lm) a new synthetic triterpenoid that has been shown to possess potent anti-inflammatory and antioxidant properties, and is a potent inducer of HO-1. The hypothesis to be tested is that a significant reduction in indices of I/R injury will be obtained by induction of HO-1 during resuscitation with CDDO-IM following hemorrhage.						
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## **INTRODUCTION:**

Ischemia occurs whenever there is interruption of the flow of blood to tissues or organs. It is the most common cause of death in heart disease and stroke as well as traumatic injury. Survival of the initial insult is followed by further injury that occurs during the reintroduction of oxygen with the restoration of blood flow. This injury occurs following hemorrhage because some tissues are deprived of blood to protect others as part of the fight or flight response. One of the few treatments thus far reported to reduce ischemia/reperfusion (I/R) injury following hemorrhage is introduction of the steroid hormone 17 $\beta$  estradiol (17 $\beta$ E) during resuscitation. Recent investigations into the mechanism of action of 17 $\beta$ E suggests that its effect is mediated via induction of heme oxygenase-1 (HO-1). However, 17 $\beta$ E is a weak HO-1 inducer and much more potent inducers have been discovered. One of these, 1[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im) a new synthetic triterpenoid that has been shown to possess potent anti-inflammatory and antioxidant properties, and is a potent inducer of HO-1. The hypothesis to be tested is that a significant reduction in indices of I/R injury will be obtained by induction of HO-1 during resuscitation with CDDO-Im.

## **BODY:**

Heme oxygenase-1 is highly inducible and its induction is correlated with significant protection from the deleterious effects of ischemia. 1[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im) a new synthetic triterpenoid has been shown to possess potent anti-inflammatory and antioxidant properties, and is a potent inducer of HO-1 and is being investigated as an additive to a new resuscitation fluid that might be counteracting the deleterious effects of the ischemia of hemorrhage shortly after injury during the initial resuscitation. One of the most critical components of developing a new drug of treatment of a specific disease state is determination of an appropriate dose of the drug with maximum benefit and minimum off-target effects. We are employing a new technique 1) termed snapshot pharmacokinetics to hone in on an appropriate dose of CDDO-Im for use in the mouse model of hemorrhage. Following determination of an appropriate dose we will determine the timing of resuscitation for maximum benefit of the drug.

## KEY RESEARCH ACCOMPLISHMENTS:

Task 1 & 2 has been substantially completed. This work was performed at the animal facility at Brook City Base, San Antonio, TX due to closure of the US Army Institute of Surgical Research facility due to Base Realignment and Closing (BRAC) remodeling. However, in vivo imaging was still performed at the USAISR where the Xenogen in vivo imaging system resided. This required transporting the animals from Brooks City Base to USAISR (about 15 miles away). Under BRAC, all small animal work was to be transferred to the new research building in March of 2010 when it was scheduled for completion. As of 1 July 2011 we have been able use the new small animal vivarium and work has been initiated. Due to almost 1.5 years of unavailability of the vivarium we need to get core funding research completed. We have requested and been granted an one year extension in order to complete Task 3 of this project which will be done by August 30 2011.

1. Jan-Mar 2010: Established role for hypoxia in some organs of the mouse following hemorrhage of the FVB.1 29S6-Gt(ROSA)26Sortml (HIF1 $\alpha$ Luc)Kael/J (HIF1 $\alpha$ Luc) inbred strain. The intestine spleen and liver were effected organ while brain, lung, skeletal muscle and heart are not much effected.
2. Jan-June 2010: Determined that 100 nm CDDO was optimal in vitro for induction of HO-1 in the skin fibroblasts of the HIF1 $\alpha$ Luc strain of mouse.
3. Mar-Aug 2010: Determined that 100 nM CDDO was also effective in inducing HO-1 in human umbilical vein endothelial cells indicating a cross species benefit.
4. Feb. 2010- July 2011: Awaiting availability of rodent vivarium at the US Army Institute of Surgical Research

## REPORTABLE OUTCOMES:

1. P. Bowman, J. Bynum, J.G. Kiang, D. Baer, J. Johnson, M. Dubick. Noninvasive imaging of hemorrhage-induced global ischemia with a transgenic mouse expressing luciferase coupled to hypoxia-inducible factor (HIF1 $\alpha$ ). Experimental Biology 2009; Annual Meeting of the Federation of American Societies for Experimental Biology, New Orleans, LO.

2. X. Wang, S. Stavchansky, P. Bowman. Cytoprotective effect of a synthetic triterpenoid against oxidative stress in human umbilical vein endothelial cells. *Experimental Biology 2009*; Annual Meeting of the Federation of American Societies for Experimental Biology, New Orleans, LO.
3. Wang X, Bynum J, Stavchansky S, Bowman PD. Network Analysis of the Cytoprotective Effect of CDDO-IM against Oxidant Stress in Human Umbilical Vein Endothelial Cells (HUVEC), *Experimental Biology Annual Meeting*, Anaheim, California, April 2010.
4. Hypoxia Inducible Factor 1 Alpha (HIF1a) by Caffeic Acid Phenethyl Ester (CAPE) and Caffeic Acid Phenethyl Amide (CAPA) in Mouse Skin Fibroblasts, *Experimental Biology Annual Meeting*, Anaheim, California, April 2010.
5. Ashish Rastogi, James Bynum, Salomon Stavchansky and Phillip Bowman. The Relationship between In Vivo Bioluminescent Imaging and Production of Light by Organs in Transgenic Mouse Engineered to Express Luciferase in Response to Hypoxia. *Experimental Biology Annual Meeting*, Washington DC. April 2011
6. Ashish Rastogi, James Bynum, Salomon Stavchansky, and Phillip Bowman. Bioluminescent Imaging and Detection of Luciferase Activity in Organs of Transgenic Mice Engineered to Express Luciferase in Response to Hypoxia. Submitted to *Journal of Bioluminescence*.

## **CONCLUSION:**

No drug is currently used to treat hemorrhagic shock and while hemorrhagic shock is complex, in principal, treatable component is the subsequent ischemia produced by shunting of blood away from certain organs. The Defense Advanced Research Projects Agency (DARPA) through their persistence in combat program, has funded research into use of  $17\beta$  estradiol for ameliorating hemorrhagic shock but this therapy has not proven beneficial. CDDO-Im is proving to be protective of a variety of injuries (2-7) and is the best candidate drug for protecting the injured from the ischemia/reperfusion injury of hemorrhage.

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APPENDICES: N/A