AWARD NUMBER: W81XWH-10-2-0121

TITLE: Hibernation-Based Therapy to Improve Survival of Severe Blood Loss

PRINCIPAL INVESTIGATOR: Greg Beilman M.D.

CONTRACTING ORGANIZATION: WRIGHT PATTISON AIR FORCE BASE
Minneapolis, MN 55455-2003

REPORT DATE: 15 Jul 2014

TYPE OF REPORT:
Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE October 2014		2. REPORT TYPE Annual		3. DATES COVERED 01 Oct 2013 - 30 Sept 2014	
4. TITLE AND SUBTITLE Hibernation-Based Therapy to Improve Survival of Severe Blood Loss				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-10-2-0121	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Greg Beilman M.D., Kristine Mulier M.B.S., Kristin Colling M.D., Andrea Wolf E-Mail: beilm001@umn.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Minnesota 200 Oak Street S.E., Suite 450 Minneapolis, MN 55455				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT <p>The goal of this set of experiments was to determine whether a higher dose of BHB/M would improve outcomes. Based on the data presented, while animals treated with 2X 4 M BHB/43mM Melatonin did have death as well as other physiologic and hematologic changes associated with experimental outcome, animals treated with 4X 4 M BHB/43mM Melatonin had more severe outcomes and measures. Therefore, improved outcomes are not associated with a higher dose of BHB/M and in fact would appear detrimental.</p> <p>The individual components given I.V. to the injured animals at MTD (4X, determined in the previous set of experiments) resulted in death in 7/9 animals. Non-injured animals that received MTD (4X) of the individual components survived until the end of the experiments (8/8). It is concluded that it is likely that the animals died from a combination of both the injury and the high dosing of drug.</p>					
15. SUBJECT TERMS Beta-hydroxybutyrate, melatonin					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 23	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
1. Introduction.....	1
2. Keywords.....	2
3. Overall Project Summary.....	3
4. Key Research Accomplishments.....	13
5. Conclusion.....	14
6. Publications, Abstracts, and Presentations.....	15
7. Inventions, Patents and Licenses.....	16
8. Reportable Outcomes.....	17
9. Other Achievements.....	18
10. References.....	19
11. Appendices.....	20

Introduction:

Blast injuries have been responsible for the majority of combat deaths in Iraq and Afghanistan, and the likelihood of being exposed to explosives is increasing for military personnel and civilians alike in war zones and other regions of political conflict. The injuries sustained are often accompanied by severe blood loss, and shock from this blood loss is the most common cause of potentially salvageable deaths from combat related injuries. D-beta hydroxybutyrate and melatonin (BHB/M) is a novel therapy designed to prolong survival in patients who are risk for bleeding to death. Our overall strategy in this series of studies is to use physiologic adaptive responses in hibernating mammals to aid in salvage of a patient with a potentially life-threatening blood loss, permitting survival to reach effective medical care. BHB/ M includes both an alternate fuel source for cells (D-beta hydroxybutyrate) and a powerful anti-oxidant, melatonin, to protect cells against damage. Our goal is to evaluate BHB/M in animal models of injury that simulate the battlefield casualty. Our previous work has shown increased survival for both rats and pigs treated with BHB/M. We wish to prove that BHB/M is a safe and effective therapy that can decrease mortality and improve outcomes for injured casualties suffering from polytrauma and blast injuries.

Key Words:

Beta-hydroxybutyrate

Melatonin

Poly-trauma

Resuscitation

Hemorrhage

Shock

Project Summary:

Do higher doses of BHB/M given I.V. result in improve outcomes?

The below randomization was completed in early May 2014.

Drug Components	Concentration of Drug	Dose	Number of Animals
I.V. BHB/M 2X	4M BHB/43mM Melatonin	2 cc/kg bolus 1.32cc/kg/hr	3 Male, 3 Female
I.V. BHB/M 4X	4M BHB/43mM Melatonin	4 cc/kg bolus 2.64 cc/kg/hr	2 Male, 3 Female

All animals (n=5) receiving 4X BHB/M had died about 5 hours after the start of BHB/M infusion. Two of the animals receiving 2X BHB/M (n=6) died within the first 10 hours BHB/M infusion, two of the animals died as they were unable to be extubated and 2 of the animals survived until end of experiment (Figure 1).

Figure 1. Kaplan Meier Curve for observation of Maximum Tolerated Dose (MTD)

Unsurprisingly, BHB concentrations are significantly different ($p < 0.05$) in those animals treated with 2X BHB/M at LR 1 and FR 2 when compared to animals treated with 4X BHB/M at the same time points (Figure 2). The same effect is seen in Melatonin concentrations (Figure 3).

Figure 2. BHB concentrations in animals treated with either 2X or 4X BHB/M

Figure 3. Melatonin concentrations in animals treated with either 2X or 4X BHB/M

Both wedge and bladder pressures were significantly different in animals receiving 4X BHB/M when compared to those receiving 2X BHB/M ($p<0.05$) (Figure 4 a and b). Arterial pH, lactate and sodium concentrations were significantly different between the groups ($p<0.05$) (Figure 5 a, b and c). All of these measures, both physiologic and hematologic would indicate that there is an issue with fluid flow that is dependent upon the dosing of BHB/M.

Figure 4 a) Wedge pressure and b) bladder pressure in animals treated with either 2X or 4X BHB/M.

a.

b.

Figure 5 a) Arterial pH b) serum lactate and c) serum sodium concentrations in animals treated with either 2X or 4X BHB/M.

a.

b.

C.

Pathology reports noted some kidney necrosis in a few animals treated with either 2X or 4X 4 M BHB/43mM Melatonin. All other pathologic abnormalities were as a result of the poly-trauma.

The maximum tolerated dose (MTD) of the individual components of BHB/M had to be tested. As it became evident that increased doses (4X) of the individual components were not associated with improved outcome, that set of experiments stopped and additional studies, with no injury, were added to determine whether the animals were dying from injury, drug or a combination of both (Table 1).

Table 1. Summary of MTD with Injury and MTD with No Injury Experiments.

Drug Components	Concentration of Drug	Animal Sex
I.V. BHB 4X	4M BHB	Male
I.V. Melatonin 4X	43mM Melatonin	Female
I.V. DMSO 4X	20% DMSO	Male
Lactated Ringers' 4X	Lactated Ringers'	Female
I.V. BHB 4X	4M BHB	Male
I.V. DMSO 4X	20% DMSO	Female
I.V. BHB 4X	4M BHB	Female
I.V. Melatonin 4X	43mM Melatonin	Female
I.V. DMSO 4X	20% DMSO	Female
I.V. BHB 4X No Injury	4M BHB	Male
I.V. DMSO 4X No Injury	43mM Melatonin	Male
I.V. Melatonin 2X No Injury	43mM Melatonin	Female
I.V. BHB 2X No Injury	4M BHB	Male
I.V. Melatonin 4X No Injury	43mM Melatonin	Female
I.V. BHB 2X No Injury	4M BHB	Female
I.V. Melatonin 2X No Injury	43mM Melatonin	Male
I.V. BHB 4X No Injury	4M BHB	Female

3 animals received injury and 4x 4M BHB
➤ 1 died after Shock 35, 2 died just prior to FR 7

2 animals received injury and 4x 43mM Melatonin
➤ 1 survived until FR 7, 1 died after Shock 35

1 animal received injury and 4x Lactated Ringers'
➤ 1 survived until FR 7

3 animals received injury and 4x 20% DMSO
➤ 1 animal died after LR 1 and 2 animals died after Shock 35

2 animals received no injury 2x 4M BHB
➤ 2 animals survived until FR 7

2 animals received no injury 2x 43mM Melatonin
➤ 2 animals survived until FR 7

2 animals received no injury 4x 4M BHB
➤ 2 animals survived until FR 7

2 animals received no injury 4x 43mM Melatonin
➤ 2 animals survived until FR 7

The animals that had injury and received a 4x dose of 4M BHB had consistently high serum concentrations from Shock 35 and started to decrease after FR 2 (roughly when the BHB infusion is complete). Animals that had no injury and a 4x dose of 4M BHB had increasing serum concentrations of BHB which peaked at FR 2 and decreased until levels returned to near Baseline at FR 7. Animals having no injury and receiving a 2x dose of 4M BHB had fairly consistent serum concentrations of BHB until FR 2 at which time they started to decrease reaching near Baseline levels at FR 7 (Figure 6).

Figure 6. BHB concentrations in animals receiving injury and no injury at various dosing concentrations

The animals that received both injury and a 4x dose of 43mM Melatonin exhibited higher serum concentrations of melatonin. The concentrations of melatonin peaked at Shock 35 and continued to go down throughout the experiment. Animals receiving 4x dose of 43mM Melatonin and had no injury exhibited increasing serum concentrations of melatonin that peaked at FR 2 and continued to decrease until the end of the experiment. Animals that had no injury and received a 2x dose of 43mM Melatonin had consistent levels of melatonin that decreased after FR 190 (Figure 7).

Figure 7. Melatonin concentrations in animals receiving injury and no injury at various dosing concentrations

Pathology, vital, lab and blood gas data continue to be analyzed.

The consultants that are aiding in set-up of a Phase I clinical trail recommended that additional concentration work be completed to see if a lower concentration of melatonin and thusly the concentration of DMSO could be utilized without risking the benefits observed previously using the drug. A six-month no cost extension was recently granted and the following work has begun (Table 2).

Table 2. Outline of experiments to be completed in the next 6 months.

Drug Components	Animal Sex
LR	Male
4M BHB, 20mM Melatonin, 10% DMSO	Male
4M BHB, 10mM Melatonin, 10% DMSO	Female
4M BHB, 20mM Melatonin, 10% DMSO	Female
4M BHB, 10mM Melatonin, 10% DMSO	Male
LR	Female
4M BHB, 10mM Melatonin, 10% DMSO	Male
4M BHB, 20mM Melatonin, 10% DMSO	Male
4M BHB, 20mM Melatonin, 10% DMSO	Female
4M BHB, 10mM Melatonin, 10% DMSO	Female
4M BHB, 20mM Melatonin, 10% DMSO	Male
4M BHB, 10mM Melatonin, 10% DMSO	Female
4M BHB, 10mM Melatonin, 10% DMSO	Male
4M BHB, 20mM Melatonin, 10% DMSO	Female

Key Research Accomplishments:

- Completed dosing experiments of BHB/M given I.V.
- Completed MTD work of individual components

Conclusion:

The goal of this set of experiments was to determine whether a higher dose of BHB/M would improve outcomes. Based on the data presented, while animals treated with 2X 4 M BHB/43mM Melatonin did have death as well as other physiologic and hematologic changes associated with experimental outcome, animals treated with 4X 4 M BHB/43mM Melatonin had more severe outcomes and measures. Therefore, improved outcomes are not associated with a higher dose of BHB/M and in fact would appear detrimental.

The individual components given I.V. to the injured animals at MTD (4X, determined in the previous set of experiments) resulted in death in 7/9 animals. Non-injured animals that received MTD (4X) of the individual components survived until the end of the experiments (8/8). It is concluded that it is likely that the animals died from a combination of both the injury and the high dosing of drug.

Publications, Abstracts, and Presentations:

- A manuscript entitled “Safety of D-β-Hydroxybutyrate and Melatonin for the Treatment of Hemorrhagic Shock with Polytrauma” has been submitted and is in Press at Shock.

Inventions, Patents and Licenses

- None

- **Reportable Outcomes:**
- Obtained NCE for additional concentration work with melatonin and DMSO

Other Achievements:

- None

References:

- None

Appendices:

Time points defined, Limited Resuscitation (LR)=maintenance of SBP above 80 mmHg, Full Resuscitation (FR)=maintenance of SBP above 90 mmHg, Hgb above 6 and Urine output > 1 cc/kg/hr.

Time point	Elapsed time from Baseline
Baseline	0
Shock 15	15 minutes
Shock 35	35 minutes
Shock 45	45 minutes
LR 30	30 minutes from the start of Limited Resuscitation phase, ~1.5 hours from baseline
LR 1	60 minutes from the start of Limited Resuscitation phase, ~2 hours from baseline
FR 1	1 hour from the start of Full Resuscitation, 2 hours from the start of Limited Resuscitation, ~3 hours from Baseline
FR 2	2 hour from the start of Full Resuscitation, 3 hours from the start of Limited Resuscitation, ~4 hours from Baseline
FR 160	160 minutes from the start of Full Resuscitation, 3 hours 40 minutes from the start of Limited Resuscitation, ~4.7 hours from Baseline
FR 170	170 minutes from the start of Full Resuscitation, 3 hours 50 minutes from the start of Limited Resuscitation, ~4.83 hours from Baseline
FR 3	3 hour from the start of Full Resuscitation, 4 hours from the start of Limited Resuscitation, ~5 hours from Baseline
FR 190	190 minutes from the start of Full Resuscitation, 4 hours 10 minutes from the start of Limited Resuscitation, ~5.2 hours from Baseline
FR 4	4 hour from the start of Full Resuscitation, 5 hours from the start of Limited Resuscitation, ~6 hours from Baseline
FR 5	5 hour from the start of Full Resuscitation, 6 hours from the start of Limited Resuscitation, ~7 hours from Baseline
FR 6	6 hour from the start of Full Resuscitation, 7 hours from the start of Limited Resuscitation, ~8 hours from Baseline
FR 7	7 hour from the start of Full Resuscitation, 8 hours from the start of Limited Resuscitation, ~9 hours from Baseline
FR 20	20 hour from the start of Full Resuscitation, 21 hours from the start of Limited Resuscitation, ~22 hours from Baseline