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14. ABSTRACT

The goals of this set experiments was to determine whether different concentrations of melatonin and DMSO along with 4M BHB would be as effective as our standard dosing regimen of 4M BHB/43mM Melatonin 20% DMSO. Based on the data presented data, our standard dosing regimen is the most effective treatment when compared to lower concentrations of melatonin DMSO in 4M BHB.

15. SUBJECT TERMS

Beta-hydroxybutyrate, melatonin

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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:

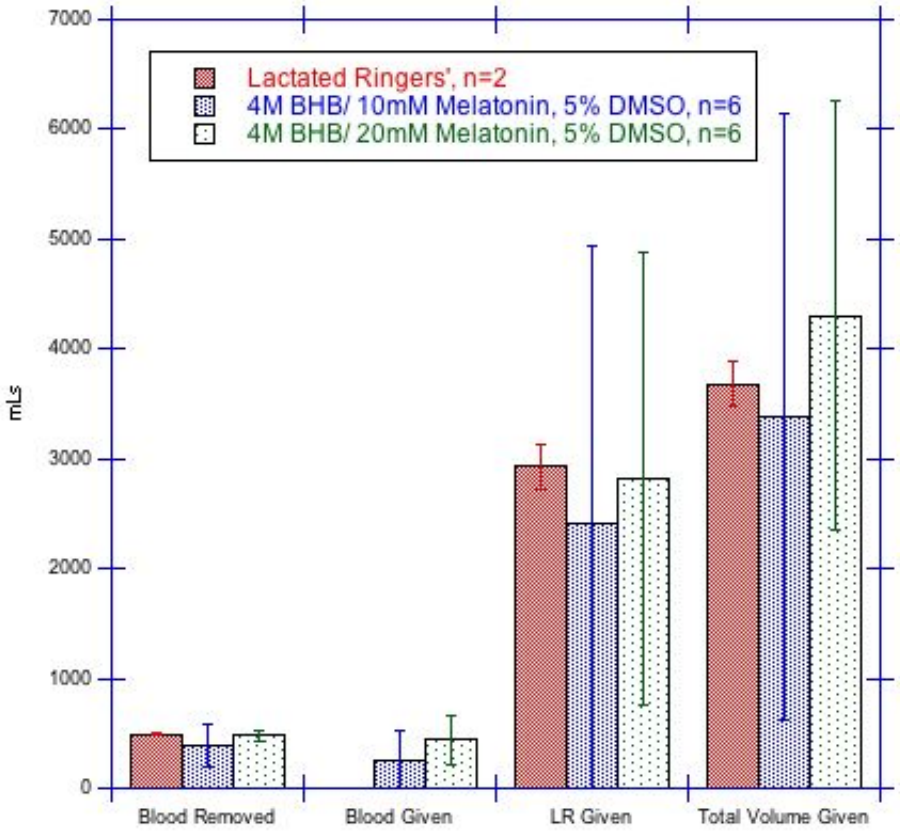
We were able to complete the experiments outlined in Table 1.

Table 1. Randomization table of lower concentration of melatonin/DMSO and standard concentration BHB

Drug Components	Animal Sex
LR	Male
4M BHB, 20mM Melatonin, 5% DMSO	Male
4M BHB, 10mM Melatonin, 5%DMSO	Female
LR	Male
4M BHB, 20mM Melatonin, 5%DMSO	Female
4M BHB, 10mM Melatonin, 5%DMSO	Male
4M BHB, 10mM Melatonin, 5%DMSO	Male
4M BHB, 20mM Melatonin, 5%DMSO	Male
4M BHB, 20mM Melatonin, 5%DMSO	Female
4M BHB, 10mM Melatonin, 5%DMSO	Female
4M BHB, 20mM Melatonin, 5%DMSO	Male
4M BHB, 10mM Melatonin, 5%DMSO	Female
4M BHB, 10mM Melatonin, 5%DMSO	Male
4M BHB, 20mM Melatonin, 5%DMSO	Female

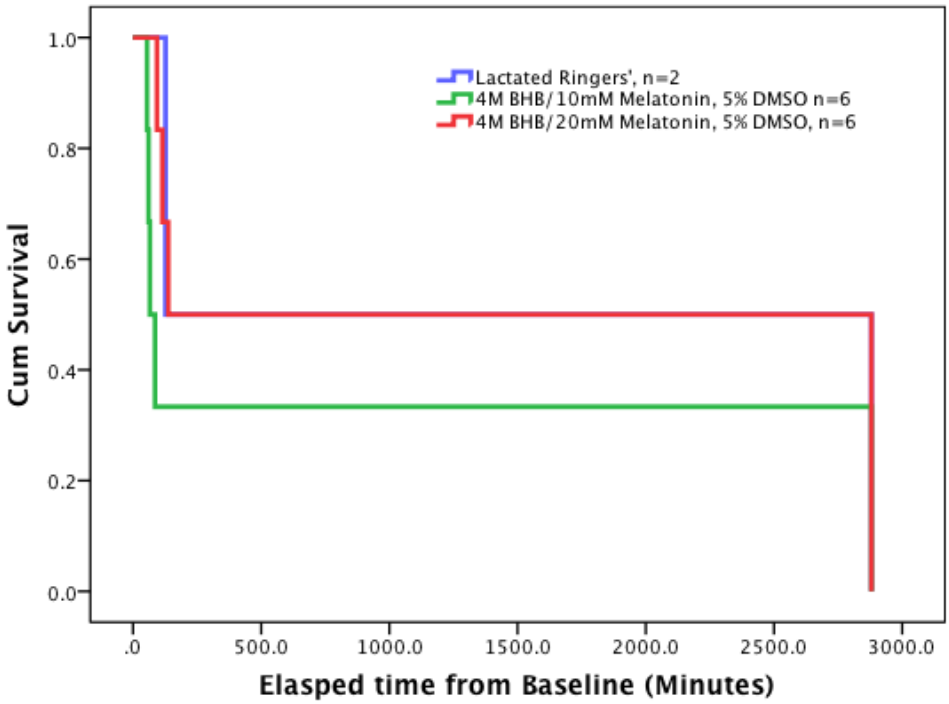
All animals in this set of experiments had the same amount of blood removed and received the same amount of resuscitative fluids (Figure 1). Animals receiving lower concentrations of Melatonin and DMSO had slightly less fluid requirements. However, this trend was not statistically significant.

Figure 1. Hemorrhage and resuscitation requirements.



All animals receiving standard of care (n=2) or 4M BHB/20mM Melatonin, 5% DMSO (n=6) survived longer than those that were treated with 4M BHB/10mM Melatonin, 5% DMSO (Figure 2).

Figure 2. Kaplan Meier Curve for the observation of lower concentrations of Melatonin and DMSO.



Unsurprisingly, BHB concentrations were not statistically different between animals treated with 4M BHB and either 10mM Melatonin, 5% DMSO or 20mM Melatonin, 5% DMSO (Figure 3). Melatonin concentrations were not statistically significant between animals treated with 4M BHB and either 10mM Melatonin, 5% DMSO or 20mM Melatonin, 5% DMSO until FR160 and were back to baseline levels at FR 20 (Figure 4).

Figure 3. BHB concentrations in animals treated with lactated Ringers', 4M BHB with either 10mM Melatonin, 5% DMSO or 20mM Melatonin, 5% DMSO.

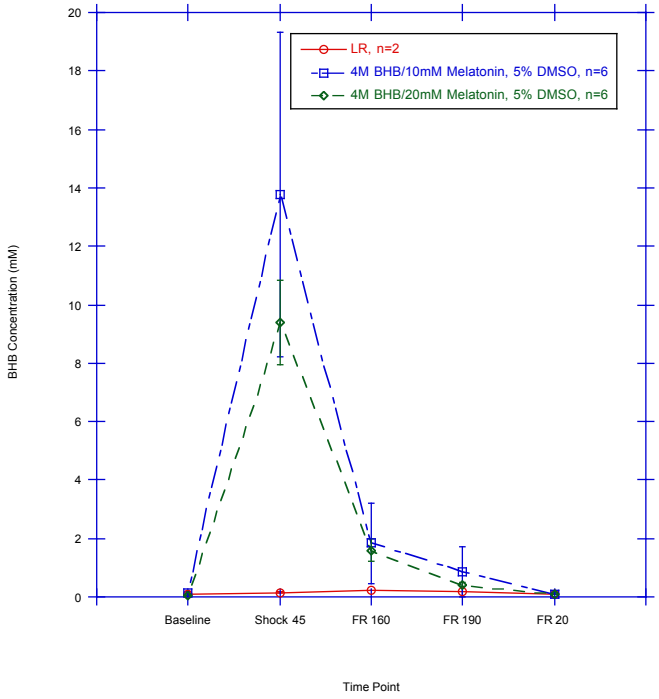
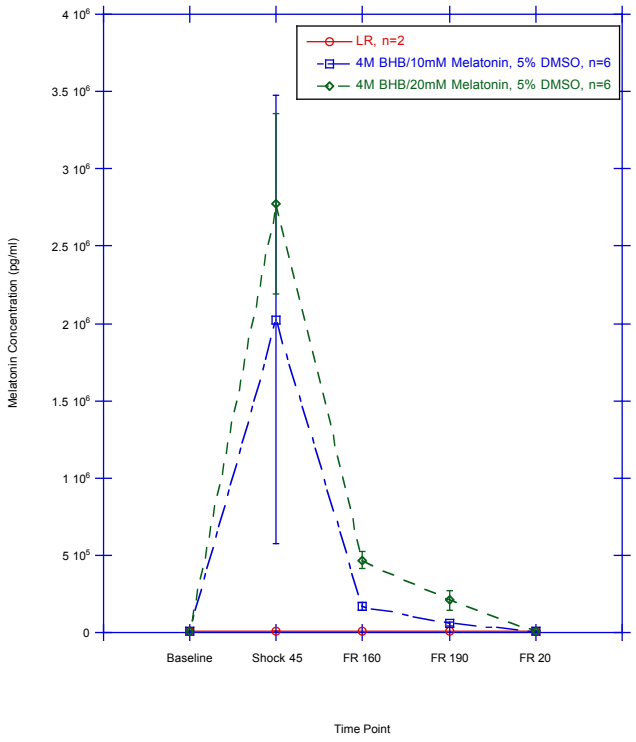


Figure 4. Melatonin concentrations in animals treated with lactated Ringers', 4M BHB with either 10mM Melatonin, 5% DMSO or 20mM Melatonin, 5% DMSO.



Key Research Accomplishments:

- Completed lower concentration melatonin/DMSO work
- Completed outlined grant work

Conclusion:

The goal of this set of experiments was to determine whether lower concentrations of melatonin and DMSO would be more effective as standard of care. Based on the data presented, animals treated with 4M BHB and either 10mM Melatonin/5% DMSO or 20mM Melatonin/5% DMSO showed no survival benefit when compared to standard of care (lactated Ringers'treatment).

Publications, Abstracts, and Presentations:

- A manuscript entitled "Safety of D-ß-Hydroxybutyrate and Melatonin for the Treatment of Hemorrhagic Shock with Polytrauma" has been published at Shock.

Inventions, Patents and Licenses

- None

Reportable Outcomes:

- Working on obtaining NCE to complete final data analysis.

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