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13. SUPPLEMENTARY NOTES					
14. ABSTRACT Abstract: Objective: To compare the return to baseline of mean arterial blood pressure (MAP) between 2 groups of swine in acute cyanide toxicity and treated with IV HOC or IO HOC. We also compared blood cyanide, lactate, pH, nitrotyrosine levels, cerebral oxygenation, and inflammatory markers. Method: 24 swine (48-52kg) were intubated, anesthetized, and instrumented (MAP and cardiac output (CO) monitoring). Cyanide was continuously infused until severe hypotension (50% of baseline MAP). Animals were randomly assigned to IV HOC 150mg/kg or IO HOC 150mg/kg and monitored for 60 min after start of antidote. Results: Baseline mean weight, time to hypotension, and cyanide dose at hypotension were similar. At hypotension, mean MAP, blood cyanide, and lactate levels were similar. Both groups had similar return to baseline MAP. Conclusions: Intraosseous hydroxocobalamin led to similar return to baseline of MAP as intravenous. Mortality, heart rate, cardiac output, lactate, nitrotyrosine, cerebral NIRS, and inflammatory markers were also similar. IO HOC may be as effective as IV HOC in acute, severe cyanide toxicity.					
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WHMC – Animal Final Report

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1. Protocol Number: FWH2009170A

2. Type of Research:

Animal Research

3. Title: Intraosseous hydroxocobalamin in the treatment of acute, severe cyanide induced cardiotoxicity in a swine (Sus Scrofa) model- an alternate administration route for chemical mass casualties.

4. Principal Investigator (PI):

Name	Rank	Date of IACUC Training	Branch of Service / Corps	Staff Resident Fellow Civilian	Department / Office Symbol	Email (if other than WHMC Outlook)	Phone	Pager
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5. Purpose: The purpose of this study is to directly evaluate intraosseous hydroxocobalamin for cyanide toxicity using an animal model of cyanide-induced hypotension. We will also compare IO hydroxocobalamin to another non-IV antidote, hydroxylamine given intramuscularly. An non-intravenous routes of administration would be preferred for disasters and for military medic use. Other aims are the following

We will also measure serum nitric oxide levels. One report suggests cyanide causes hypotension by releasing nitric oxide and hydroxocobalamin may be effective by scavenging it..

We will also measure inflammatory mediators. We will measure TNF-alpha, IL-1beta, IL-6, and IL-10. A reduction in inflammation early could reduce late cyanide induced adverse neurologic effects. If a difference in inflammation is found in this short study, longer studies could prove clear benefit. Previous studies have found inflammatory marker changes in as early as one hour in non-toxicology models. Inflammatory markers had not been previously measured in cyanide toxicity.

We will measure hydroxocobalamin levels in first experiment to more precisely compare the intraosseous and intravenous routes with liquid chromatography tandem mass spectrometry (LC MS/MS).

Finally, we will measure neurotoxic effects of cyanide by three methods – LICOX (PbO₂), near infrared spectrophotometry (NIRS), and microdialysis. We will use three devices to assess neurologic function after antidotal treatment. These measures are near infrared spectroscopy, brain oxygen tension, and brain microdialysis.

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Hypothesis

We hypothesize that there will be no difference between the return to baseline of mean arterial blood pressure between the two treatment groups hydroxocobalamin IV vs IO in the first experiment. The second experiment will compare hydroxocobalamin IO vs hydroxylamine.

To test this hypothesis we will test the following research question:

- 1) Is there a statistically significant difference in the rate of return to baseline of mean arterial blood pressure between intraosseous and intravenous hydroxocobalamin as measured by repeated measures analysis of variance and/or Cox proportional hazards model?
- 2) Is there a statistically significant difference in the rate of return to baseline of mean arterial blood pressure between intraosseous hydroxocobalamin and intramuscular hydroxylamine as measured by repeated measures analysis of variance and/or Cox proportional hazards model?
- 3) Is there a difference in the proportion of pigs surviving at the end of the experiment?
- 4) Is nitric oxide elevated during cyanide-induced hypotension and do the antidotes reduce the serum nitric oxide?
- 5) Do the antidotes reduce inflammatory mediators and improve early neurological effects in acute cyanide toxicity?

An additional objective of the study will be a GME tool to advance the education of three residents (Capt Tuepker, Capt Maddry and Capt Muncy). I will teach the residents the fundamentals of research, toxicology, and resuscitative science. It will fulfill their GME research requirement and allow them opportunity for scientific presentation and publication.

6. Results: We determined the IO Hydroxocobalamin was as effective as IV hydroxocobalamin. Both groups had similar mortality and similar lactate, pH and blood pressure at the end of the study.

In our second experiment we compared intramuscular hydroxylamine and intraosseous hydroxocobalamin. We found that IM hydroxylamine, a methemoglobin inducer, was effective for cyanide but not as effective as IO hydroxocobalamin. We are conducting complete statistical analysis of the data for preparation into a publishable manuscript. We have just received microdialysis results this month and we are waiting on inflammatory markers from Ms. Dixon. When complete we will combine the data and prepare it for publication.

See Appendix for full abstracts.

7. How may your findings benefit the Air Force? This study provides support for administering IO hydroxocobalamin instead of IV for a mass casualty or wartime scenario. In addition we found that IM hydroxylamine was effective. This is important for two reasons. First, IM antidotes will work for cyanide toxicity with hypotension. The blood flow is sufficient to circulate IM administered drugs in the blood. Secondly, further research to investigate hydroxylamine and similar methemoglobin inducers to validate this antidote. IM administered drugs are simpler to deliver and can be administered by non-clinicians. Our findings will also benefit DoD providers who treat cyanide toxicity from chemical exposures or structural fires.

8. Number of Animals

Projected Enrollment of Animals at the Beginning of Study: 50
Experiment animals 42, Technique development animal 6

Amendment #1 Dated 7Oct2010 added 5 additional animals

Actual Number of Animals Enrolled: Number originally approved 50, per amendment #1-5, for a total of 55. All animals were euthanized per protocol.

9. Status of Animals Entered Into the Protocol: We replaced 5 animals per the Oct2010 amendment. We replaced 1 animal in experiment one for illness (acidemia and hypoglycemia). We replaced 3 animals in experiment two. One animal died before the study started. Finally, we requested an additional animal for protocol development to refine the surgical technique. No other unexpected issues with the status of the animals occurred.

10. Status of Funds:

Our study was funded by the AF SGR. We are under budget and all funds have been allocated.

11. Reason for Closure:

Objectives of the study were met

12. Specific Problems: We had a delay in receiving hydroxocobalamin due to manufacturing issues, but we were able to complete our study. No other unexpected problems occurred with this study.

13. Publications and Presentations:

Presentations:

- 1) AFMS-August 2011-poster, oral
- 2) NACCT-September 2011-poster
- 3) ACEP-October 2011-poster

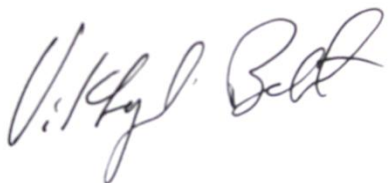
These Presentations and Publications have been cleared by 59 CRD and Public Affairs.

Publications:

None; however we are preparing the manuscripts now. These Presentations and Publications have been cleared by 59 CRD and Public Affairs.

14. Exceptional Achievements: None

15. Signature of Principal Investigator:



Vikhyat Bebart, Lt Col, USAF, MC
Chief, Medical Toxicology

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APPENDIX

Abstract 1

Intravenous hydroxocobalamin versus intraosseous hydroxocobalamin in the treatment of acute, severe cyanide toxicity in a validated swine model

Background

Previously we have demonstrated intravenous (IV) hydroxocobalamin (HOC) alone is effective acute cyanide induced shock. However, simple, non IV administration routes are needed for first responders, military troops, and emergency department staff. Hydroxocobalamin cannot be given intramuscular. Intraosseous infusion is simple and an effective route for other resuscitation drugs.

Objective

To compare the return to baseline of mean arterial blood pressure (MAP) between 2 groups of swine in acute cyanide toxicity and treated with IV HOC or IO HOC. We also compared blood cyanide, lactate, pH, nitrotyrosine (nitric oxide marker) levels, cerebral near infrared spectrometry (NIRS) oxygenation, and inflammatory markers.

Methods

24 swine (48-52 kg) were intubated, anesthetized, and instrumented (continuous MAP and cardiac output (CO) monitoring). Cyanide was continuously infused until severe hypotension (50% of baseline MAP). Animals were randomly assigned to IV HOC (150 mg/kg) or IO HOC (150 mg/kg) and monitored for 60 min after start of antidotal infusion. A sample size of 12 animals per group was determined by group size analysis based on power of 80% to detect an effect size of 0.54 difference (approximately 1 stdev) of the mean MAP between the groups and an alpha of 0.05. RMANOVA was used to determine statistically significant changes between groups over time.

Results

Baseline mean weights (48, 50 kg), time to hypotension (31, 35 min), and cyanide dose at hypotension (4.9, 5.8 mg/kg) were similar. At hypotension mean MAP (45, 45 mg Hg), blood cyanide (3.5, 3.1 mcg/ml) and lactate levels (8.9, 8.8 mmol/L) were similar. 10/12 animals in the IV group and 10/12 in IO group survived ($p=1.0$). Both groups has a similar return to baseline MAP and had similar MAP until end of the study ($p=0.997$). CO, SVO₂ and SVR were also similar between groups ($p>0.4$). Bicarbonate, pH, and lactate, levels were similar ($p>0.8$). Cyanide levels were undetectable after HOC infusion through study end in both groups ($p=1.0$). Cerebral and renal NIRS oxygenation were also similar ($p>0.9$). They decreased in parallel to MAP during CN infusion and increased after antidote infusion. Serum nitrotyrosine rose during CN infusion in all animals, and decreased similarly in both arms after HOC infusion ($p>0.5$). TNF- α , IL-1 β , IL-6, and IL-10 were similar between groups.

Conclusions

Intraosseous hydroxocobalamin led to similar return to baseline of mean arterial pressure as intravenous. Mortality, heart rate, cardiac output, lactate, nitrotyrosine, cerebral NIRS, and inflammatory markers were also similar. Intraosseous hydroxocobalamin may be as effective as intravenous hydroxocobalamin in acute, severe cyanide toxicity.

Abstract 2

Intraosseous hydroxocobalamin versus intramuscular hydroxylamine in a validated swine model of acute cyanide toxicity and shock

Background

Non-intravenous routes of cyanide (CN) antidotes are needed as an easily administered antidote for first responders and military troops. Our group has previously demonstrated intravenous (IV) hydroxocobalamin (HOC) is effective for acute CN induced shock, and intraosseous (IO) HOC is as effective as IV HOC. Intramuscular (IM) hydroxylamine (HAM) has been effective for CN induced respiratory failure, but its effectiveness for cyanide induced shock has not been reported.

Objective

To compare the return to baseline of mean arterial blood pressure (MAP) between 2 groups of swine in acute CN toxicity and treated with IO HOC or IM HAM. Secondary outcomes included blood cyanide, lactate, pH, nitrotyrosine (nitric oxide marker) levels, and cerebral near infrared spectrometry (NIRS) oxygenation.

Methods

24 swine (48-52 kg) were intubated, anesthetized, and instrumented (continuous MAP and cardiac output (CO) monitoring). CN was continuously infused until severe hypotension (50% of baseline MAP). Animals were randomly assigned to IO HOC (150 mg/kg) or IM HAM (50 mg/kg) and monitored for 60 min after start of antidotal infusion. A sample size of 12 animals per group was determined by group size analysis based on power of 80% to detect an effect size of 0.54 difference (approx 1 stdev) of the mean MAP between the groups and an alpha of 0.05. RMANCOVA was used to determine statistically significant changes between groups over time.

Results

Baseline mean weights (49, 50 kg), time to hypotension (29, 31 min), and CN dose at hypotension (5, 5 mg/kg) were similar. At hypotension mean MAP (42, 42 mm Hg), blood CN (3.2, 2.9 mcg/ml) and lactate levels (7.4, 7.8 mmol/L) were similar. 12/12 animals in the HOC group and 9/12 in HAM group survived ($p=0.11$). IO HOC resulted in a faster return to baseline of MAP ($p < 0.001$). SVO₂ and SVR were greater in the IO HOC group ($p < 0.002$). CO was greater in the IM HAM group ($p < 0.003$). Bicarbonate, pH, and lactate, levels were similar. Methemoglobin (12.8% HAM, 1.2% HOC) and CN (15.5 mcg/ml in HAM, 0 in HOC) levels were greater in the HAM group at 60 min ($p < 0.001$). Cerebral NIRS oxygenation decreased in parallel to MAP during CN infusion and was similar in both groups after antidote ($p=0.78$). Serum nitrotyrosine rose during CN infusion in all animals, but was lower in the IO HOC group at 60 min ($p=0.03$). TNF- α , IL-1 β , IL-6, and IL-10 were similar.

Conclusions

Intraosseous hydroxocobalamin led to a faster return to baseline mean arterial blood pressure compared to intramuscular hydroxylamine. Methemoglobin and serum cyanide levels were significantly greater in the hydroxylamine group; however, mortality, serum acidosis, and lactate were similar.