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Permalink

https://escholarship.org/uc/item/1tc2m19p

Journal

Academic Emergency Medicine, 24(9)

ISSN

1069-6563

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Publication Date

2017-09-01

DOI

10.1111/acem.13213

Copyright Information

Peer reviewed

Article type   : Original Contribution

Title: Efficacy of intravenous cobinamide versus hydroxocobalamin or saline for treatment of severe hydrogen sulfide toxicity in a swine (Sus Scrofa) model

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Running Title: IV cobinamide for hydrogen sulfide toxicity

Keywords: cobinamide, resuscitation, hydrogen sulfide, toxicity

Abstract

Title: Efficacy of intravenous cobinamide versus hydroxocobalamin or saline for treatment of

severe hydrogen sulfide toxicity in a swine (*Sus Scorfa*) model

Background: Hydrogen sulfide (H2S) is a potentially deadly gas that naturally occurs in

petroleum and natural gas. The Occupational Health and Safety Administration cites H2S as a

leading cause of workplace gas inhalation deaths. Mass casualties of H2S toxicity may be caused

by exposure from industrial accidents or release from oil field sites. H2S is also an attractive

terrorism tool because of its high toxicity and ease with which it can be produced. Several

potential antidotes have been proposed for hydrogen sulfide poisoning but none have been completely successful.

Objective: To compare treatment response assessed by the time to spontaneous ventilation among groups of swine with acute H2S induced apnea treated with intravenous (IV) cobinamide (4mg/kg in 0.8 ml of 225mM solution), IV hydroxocobalamin (4mg/kg in 5 ml saline), or saline alone.

Methods: Twenty-four swine (45-55 kg) were anesthetized, intubated, and instrumented with continuous femoral and pulmonary artery pressure monitoring. After stabilization, anesthesia was adjusted such that animals would spontaneous ventilate with an FIO2 of 0.21. Sodium hydrosulfide (NaHS; concentration of 8 mg/ml) was begun at 1 mg/kg/min until apnea was confirmed for 20 seconds by capnography. This infusion rate was sustained for 1.5 minutes post apnea, and then decreased to a maintenance rate for the remainder of the study to replicate sustained clinical exposure. Animals were randomly assigned to receive cobinamide (4 mg/kg), hydroxocobalamin (4 mg/kg) or saline and monitored for 60 minutes beginning one-minute post apnea. G* power analysis using the Z test determined that equal group sizes of 8 animals were needed to achieve a power of 80% in detecting a 50% difference in return to spontaneous ventilations at $\alpha=0.05$.

Results: There were no significant differences in baseline variables. Moreover, there were no significant differences in the mg/kg dose of NaHS (5.6 mg/kg; p=0.45) required to produce apnea. Whereas all of the cobinamide treated animals survived (8/8), none of the control (0/8) or hydroxocobalamin (0/8) treated animals survived. Mean time to spontaneous ventilation in the cobinamide treated animals was 3.2(±1.1) minutes.

Conclusions: Cobinamide successfully rescued the severely NaHS-poisoned swine from apnea in the absence of assisted ventilation.

INTRODUCTION

Hydrogen sulfide (H2S) is a potentially deadly gas that occurs in petroleum and natural gas. Newer technologies of extracting oil and gas that are now widely used in the United States, such as hydraulic fracturing, produce excessive concentrations of H2S, exceeding the exposure limit set by the U.S. Agency for Toxic Substances and Disease Registry.[1] H2S is one of the leading causes of unintentional workplace gas inhalation deaths.[2] Significant exposures (50-400 ppm) may produce difficulty in breathing, agitation, confusion, nausea and vomiting, elevated blood pressure and loss of consciousness. At higher concentrations H2S rapidly causes myocardial infarction, unconsciousness, seizures, acidosis and death. Just 2-3 breaths of H2S at >700 ppm is immediately fatal.[3]

While H2S is considered an uncommon cause of poisoning death, deaths due to H2S appears to be on the rise. Between 2008 and 2010, the reported instances of intentional death by H2S gas inhalation have increased in the U.S. from 2 cases to 18.[4] Furthermore, from 2011-2013 states reporting to the Agency for Toxic Substances and Disease Registry's National Toxic Substance Incidents Program (NTSIP) reported a total of 43 victims of hydrogen sulfide toxicity including 15 successful suicide victims and 21 victims who were first responders or members of the general public.[5] Research suggests that the incidence of H2S suicide is probably underestimated by public health officials.[6] Although the number of suicide reports is relatively small, the mortality rate remains extraordinarily high.

Hydrogen sulfide is also an attractive weapon for terrorists given its high toxicity, difficulty in diagnosing exposure to high concentrations, and the ease with which it can be produced. The New York State Office of Homeland Security specifically identified H2S as a possible terrorist chemical weapon because of the ease with which it can be made by combining common household items such as toilet bowl cleaner and dandruff shampoo.[7] Moreover, damage to a hydrogen sulfide-bearing pipeline could disperse a H2S cloud causing multiple casualties and pose a significant public safety concern.[8]

The toxic effects of H2S are due, in part, to inhibition of cytochrome c oxidase, similar to the effects of cyanide toxicity. There are currently several intravenous antidotes that exist for both poisons, but many antidotes have severe adverse effects, and none have been definitively successful in reversing H2S poisoning. In 2006, hydroxocobalamin became available in the U.S. for treatment of cyanide toxicity, but because of its limited solubility, a large volume of drug is necessary to treat cyanide toxicity and it cannot be administered intramuscularly. Hydroxocobalamin, therefore, is not an ideal drug for first responders. We have evaluated intraosseous and intramuscular administration of cobinamide as an alternative to hydroxocobalamin in an animal model of cyanide toxicity and found cobinamide to be as effective as hydroxocobalamin.[9] However, while there have been some reports of hydroxocobalamin use for hydrogen sulfide, there are no studies evaluating the efficacy of cobinamide for the treatment of severe H2S toxicity in a large animal clinically relevant model.

Goal of This Investigation

The primary hypothesis of our study is that intravenous cobinamide(4mg/kg in 0.8 ml of 225mM solution), is more effective than either intravenous hydroxocobalamin (4mg/kg in 5ml

saline) or saline alone in reversing the effects of potentially survivable H2S toxicity, i.e.

cobinamide will prolong survival. The model we developed produces apnea, significant

hypotension, and biomarkers that closely parallel exposure of > 500 parts per million (ppm).[2, 10]

Specifically, we compared survival time and evaluated time to spontaneous ventilation, defined

as 6 consecutive breaths, among groups of swine with acute H2S-induced apnea treated with

intravenous (IV) cobinamide (tetranitrocobinamide, 225 molar solution), IV hydroxocobalamin

or saline.

METHODS

Materials

Sodium hydrogen sulfide (NaSH) and hydroxocobalamin were purchased from Sigma

Aldrich and Meridian Medical Technologies respectively. Cobinamide was generated from

hydroxocobalamin by base hydrolysis using cerium hydroxide; it was >96% pure as determined

by high performance liquid chromatography.[11]

Study Design and Setting

We conducted a randomized comparative laboratory investigation. The study was

approved by our Institutional Animal Care and Use Committee at the Wilford Hall Ambulatory

Surgical Center Clinical Research Division. All procedures involving animals complied with the

regulations and guidelines of the Animal Welfare Act, the National Institutes of Health Guide for

the Care and Use of Laboratory Animals, and the American Association for Accreditation of

Laboratory Animal Care. The housing of animals and the performance of the study took place in

the Animal Care Facility at our institution.

Animal Subjects

Female Yorkshire swine (*Sus scrofa*) (N = 24, weighing 45-55 kg) were premedicated with intramuscular ketamine 10 mg/kg. General anesthesia was induced with isoflurane via nose cone. Following endotracheal intubation, the animals were mechanically ventilated with a volume-limited, time-cycled ventilator (Drager-Siemens, Fabius GS anesthesia machine, New York City, NY), and maintained with inhaled isoflurane (1-2.5%) and oxygen (FiO2 of 0.4-0.45). The tidal volume was initially 10 mL/kg and respiratory rate was 10 breaths/min. The minute ventilation was adjusted to maintain an end tidal CO_2 value between 38-42 mm Hg as measured by inline capnography. Lead II of the surface electrocardiogram was monitored continuously. Temperature was maintained at 37.5-39.0 °C.

Animal Preparation

Invasive hemodynamic variables were measured with an eight-French Swan-Ganz CCOmbo V pulmonary artery catheter (Model 777F8) and the Edwards Vigilance II monitor (Edwards Lifesciences, Irvine, CA). Measurements included continuous cardiac output (CO), systemic vascular resistance (SVR), mixed venous oxygen saturation (SVO2), mean arterial pressure (MAP), heart rate (HR), and core temperature. The catheter ports were flushed with saline and the catheter was placed via cutdown in the right external jugular. Arterial pressure was measured continuously through the femoral artery. An 8.5 French introducer (Arrow,

Reading, PA) was placed in the carotid artery for laboratory sampling and another was placed in the internal jugular for medication administration. The animals received a warmed saline intravenous bolus (20 mL/kg) during procedure setup. The Fabius GS anesthesia data collection software embedded in the ventilator's computer was used for data acquisition at one-minute intervals.

Baseline biochemical measurements included oxygen saturation, PaO2, PaCO2, hemoglobin (Hb), pH, bicarbonate, lactate (ABL 800 Flex blood gas analyzer, Radiometer America, Westlake, OH), prothrombin time (PT), partial thromboplastin time (PTT), (STA-R Evolution, Diagnostic Stago Inc., Parsippany, NJ), platelet count (Advia 120, Siemens, Norwood, MA), cytokines (Gen5 software, Winooski, VT), and urine thiosulfate [Waters HPLC 2695 Separations Module (fluorescence detection), Milford, MA].

Following instrumentation, isoflurane was reduced to 0.5-1.5% after which animals were acclimated and stabilized for 10 minutes. After stabilization, anesthesia was adjusted such that animals would spontaneous ventilate with an FIO2 of 0.21, without mechanical ventilator assistance. Successful weaning from ventilator, defined as spontaneous ventilation with minute volume of ≥7.0 L/min, etCO2 50-53 mmHg, oxygen saturation ≥95% and respiratory rate 25-35 breaths per minute (BPM) was confirmed prior to the start of the sodium hydrosulfide (NaHS) infusion.

Sodium hydrosulfide (Sigma Aldrich, St. Louis, MO), dissolved in sterile saline at a concentration of 8 mg/ml, was prepared minutes before each experiment. The infusion rate was determined previously on the basis of pilot experiments not included in the data presented here. The solution was infused using an infusion pump (Plum A+ Infusion System, Hospira, Lake

Forest, IL) at 1 mg/kg/min and continued at that rate until apnea occurred, confirmed for 20

seconds by capnography. The NaHS infusion rate was sustained for 1.5 minutes post apnea,

then decreased to 0.7 mg/kg/min for 3 minutes, then decreased again to 0.1 mg/kg per minute

for the remainder of the study. Animals were randomly assigned, using a random numbers

generator (https://www.randomizer.org) to receive saline, cobinamide (4mg/kg), or

hydroxocobalamin (4 mg/kg) one minute post apnea, and then monitored for 60 minutes.

Methods of Measurement and Outcome Measures

Because this model was, as intended, a lethal but potentially survivable model of H2S

toxicity, survival time and time to spontaneous ventilation were primary outcomes. We also

compared cardiac output, mean arterial pressure, systemic vascular resistance, mixed venous

oxygen saturation, and heart rate. Hemodynamic measurements were recorded at 1-minute

intervals and analyzed at 1-minute intervals from the start of the NaHS infusion until ≈ 10

minutes post apnea. Serum blood sampling for arterial blood gases was taken at baseline, 5

minutes after NaHS infusion start (unless apnea occurred prior to the 5 minute mark), 5 minute

post apnea and for survivors, 10, 20, 40, 50 and 60 minutes after treatment. Urine sampling for

urine thiosulfate was drawn at baseline, 5 minutes after NaHS infusion start, 1, 2, 4, 6, 8, 10

minutes post apnea and for survivors, 20, 30, 40, 50 and 60 minutes post apnea. Cytokine

sampling was taken at baseline, 5 minutes after NaHS infusion start (unless apnea occurred

prior to the 5 minute mark), at apnea and for survivors, 20, 30, 40, 50 and 60 minutes post

apnea.

Quantitation of Serum Cytokine Concentrations

Serum was collected with the appropriate vacutainer blood collection tube and frozen at -80. After completion of the experiment, cytokines were analyzed in duplicate via ELISA according to manufacturer's instructions. TNF-a, IL-1B, IL-6, IL-10 were analyzed using pig specific kits (R&D Systems, Minneapolis, MN). ELISA plates were analyzed using BIO-TEK Synergy H4 Microplate reader with Gen5 software (Winooski, VT).

High Performance Liquid Chromatography (HPLC) Analysis

We measured urine thiosulfate concentrations to confirm that all animals were exposed to NaHS. Elevated urine thiosulfate concentrations have been used to demonstrate HS exposure incidents in the workplace.[12] Thiosulfate (TS) was determined by a modified and validated HPLC method as described.[13, 14] To prevent premature HPLC column plugging by particulates, centrifugation as well as precolumn guards were utilized.

Eighty-five microliters of urine was aliquoted to a polypropylene microcentrifuge tube and derivatized with 102 µl of 7.66 monobromobimane (mBrBm) in acetonitrile, followed by 85 µl of 160 mM / 16 mM in water of HEPES / EDTA at pH of 8.0 with vortexing. The reaction was stopped after 30 minutes in the dark by 170 µl of 65 mM methanesulfonic acid. Particulates were removed by centrifugation, and supernatant transferred to autosampler vials for HPLC fluorescence analysis.

HPLC was performed using a Waters-2695 module, coupled to a 474 (Waters) fluorescence detector (excitation 380 nm, emission 480 nm) and a Waters Symmetry C18 Cartridge, 100Å, 5 µm, 4.6 mm X 250 mm, (WAT054215) reverse-phase column. PIPES (10 mM,

pH 6.6) and methanol (isocratic 50:50) were used at a flow rate of 0.75 mL/min. Area under the curves (AUC) of the TS peaks was integrated by the software of the Waters-2695 device.

Linearity was obtained from 10 to 400 µM thiosulfate and a maximal intraday and interday variability of 7.3% and 10.0 % respectively. The lower level of detection was approximately 5.0 uM.

Urinary creatinine concentrations were measured using a Sirrus Chemistry Analyzer. Thiosulfate concentrations were normalized to these concentrations for expression of µg sodium thiosulfate (STS) per mg urine creatinine (uCr).

DATA ANALYSIS

Power Analysis

Power was assessed using G*Power Version 3.1. Return to spontaneous ventilations among groups in our cyanide studies[9] was used to estimate a priori power as it is a critical measure of outcome. G power analysis using the Z test, difference between two independent proportions, determined that equal group sizes of 14 animals were needed to achieve a power of 80% in detecting a 50% difference in the primary outcome of return to spontaneous ventilations at a significance level of $\alpha=0.05$. However, data collection terminated after data was collected on 8 animals per group as it was clear that only the cobinamide treated animals survived.

Descriptive Statistics

Means, standard deviations and standard errors of measurement were calculated for variables of interest. Frequency distributions were calculated for discrete variables of interest. Descriptive statistics were analyzed using multivariate analysis of variance (MANOVA).

Primary Outcome Parameters

Mean values for time to spontaneous ventilation after apnea were compared among groups using repeated measures analysis of variance. Survival time was analyzed by developing Kaplan-Meier curves for each treatment and the survival distributions compared using a 3-sample log-rank test using Kaplan-Meier analysis. A priori death was defined as a MAP less than 20 mmHg for 10 minutes.

Secondary Outcome Parameters

Secondary outcome variables were modeled using repeated measures multivariate analysis of variance (RMANOVA) with adjustment for treatment, time, and the interaction of treatment by time with an auto-regressive covariance structure assumed. Post hoc analysis was performed on all variables that showed a significant treatment by time interaction, for which treatment contrasts were measured at each post treatment time-point with a Bonferroni adjustment for multiple testing applied.

All statistical testing was two sided with a significant level of alpha of 0.05 and completed using SAS version 9.3 (Cary, NC, USA) and IBM SPSS Statistics version 22 (Armonk, NY, USA). All graphical presentations were made using R version 2.15.1. Mean values for the following variables were compared among groups using repeated measures multivariate analysis of variance (RMANOVA): MAP, HR, CO, SVO2. Because of the different sampling times,

PaO2, Pco2, SaO2, oxyhemoglobin percent, pH and serum lactate values were analyzed with a second RMANOVA. Urine thiosulfate, and inflammatory markers – TNF alpha, IL-1B, IL-6, IL-10 were analyzed separately using repeated measures ANOVA. Data met the assumptions for repeated measures MANOV and ANOVA such as the following assumptions: (1) random samples, (2) normal distribution, and (3) group sizes equal. No group comparisons were analyzed after NaHS had been infused for 10 minutes as time points after 10 minutes did not contain sufficient data i.e. animals in the hydroxocobalamin and control groups' were not surviving.

Post Hoc Tests

In the event of significant multivariate effects, multiple univariate repeated measures analysis of variance and contrasts were used to investigate effects for vital signs and biomarkers using the Hochberg or Bonferroni-Holm procedure to correct the level of significance for multiple comparisons.

RESULTS

Characteristics of Study Subjects

At baseline, the groups had similar vital signs and biochemical variables (MANOVA p>0.05; Table 1). Results are reported as mean +/- standard deviation. There were no significant difference in mean end tidal isoflurane percent by group during the period of spontaneous ventilation [mean isoflurane cobinamide, 1.18±0.2; hydroxocobalamin, 1.07±0.07; control 1.05±0.2; p >0.3]. There were no significant differences in the total dose (p=0.3) or the mg/kg dose (p=0.45) of NaHS to produce apnea among the groups, nor was there a difference in the

time required for NaHS to produce apnea (p=0.42; Table 2). Although urine thiosulfate concentrations increased over time for all groups (within-in group difference; p<0.05), suggesting that NaHS was producing toxicity, there were no between group differences over time from start of infusion to 8 minutes post apnea (Table 3). Concentrations of urine thiosulfate are expressed as µg sodium thiosulfate (STS) per mg urine creatinine (uCr). Low concentrations of thiosulfate can be expected in baseline urine due to metabolism of dietary proteins as well as other compounds containing sulfur groups as a result of enzyme pathways involving sulfur metabolism,[15] therefore baseline urine thiosulfate concentrations may have high variability. The large variability of thiosulfate concentrations at each time point likely contributed to our not finding any significant differences between the groups at any time.

Primary Outcome

Whereas all of the cobinamide treated animals survived through the 60 minute observation period, none of the control or hydroxocobalamin treated animals survived past 15.4±2.3 minutes. None of the control or hydroxocobalamin met our definition of return to spontaneous ventilation, defined as 6 consecutive breaths. Therefore time to spontaneous ventilations was not compared among the groups. The mean time to spontaneous ventilations for the cobinamide group was 3.23±1.1 minutes. Survival was calculated from the start of the study to death, defined as a MAP below 20 mmHg for 10 minutes. Kaplan-Meier method of survival analysis clearly showed a significant difference by group (log rank p<0.001; figure 1). Mean survival times for the control group was 15.4±2.3 minutes and 14.9±2.4 minutes for the hydroxocobalamin treated group.

Secondary Outcomes

There were no significant differences in vital signs (table 4) or arterial blood gas values (table 5) among the groups from baseline to 1 minute post apnea which was designated treatment time, with the exception of the cobinamide group which demonstrated a lower oxygen partial pressure at 1 minute post apnea compared to the other groups ($p < 0.05$). At 10 minutes post apnea, whereas the cobinamide animals were recovering, hydroxocobalamin treated and control animals continued to deteriorate which was reflected in vital signs and arterial blood gas values (RMANOVA $p < 0.01$). Vital signs are graphically depicted in figures 2a-c.

Aggregate data analysis of proinflammatory cytokines IL-1β, TNF-α, and IL-6 demonstrated an increase in all groups from baseline to apnea [$p < 0.05$; figure 3 (data shown in aggregate. Data after 10 minutes reflects cobinamide group only)]. When proinflammatory cytokines were compared by group, the control group had significantly greater increase in TNF-α (figure 4) compared to the IV cobinamide group ($p < 0.03$). No other pairwise comparisons were significant. Values for IL-10 did not significantly change over the observation period. There were no significant differences in coagulation biomarkers.

DISCUSSION

Intravenous cobinamide (4.2 mg/kg; 0.8 mL) successfully resuscitated all NaHS toxic animals, whereas neither hydroxocobalamin (4 mg/kg; 5 mL) nor control (saline) provided effective resuscitation. Indeed the mean time to spontaneous ventilation, 6 consecutive breaths, after treatment with cobinamide was 3.23 minutes with no animal in that group taking

longer than 5.3 minutes (range 1.5-5.3 minutes) to spontaneously ventilate. No animal in either of the other two groups had sufficient respiratory effort post treatment to reach our definition of return to spontaneous ventilation. Treating animals at a time when they were apneic would likely be similar to clinical scenarios, since apnea occurs quickly after H2S exposure, and lowering the NaSH infusion rate post apnea would also likely be similar to a clinical scenario of removing a person from the sulfide-contaminated area. Vital signs and arterial blood gases continued to deteriorate in the hydroxocobalamin and control groups post treatment, but improved in the cobinamide-treated group, supporting the premise that cobinamide successfully resuscitates swine with apnea induced NaHS toxicity. Urine sodium thiosulfate levels demonstrate the initial significant ($p<0.05$) increase in the biological marker for toxicity[16] with subsequent decrease in those animals that survived (table 3).

Cytokine data supports the model as a toxic but survivable model. Similar to cyanide-induced cytokine upregulation,[17] a NaHS infusion induced TNF α, IL-1β and IL-6. Expression of TNF α increased within minutes following the NaHS infusion and in the case of the cobinamide treated animals began to decrease after treatement.[18] IL-1β and IL-6 also increased, but had more sustained increases in expression such that there were no difference among the groups. Because most of the animals in the hydroxocobalamin and control groups did not survive past ≈ 10 minutes, finding an increase in the expression of IL-10 would be unlikely. Interestingly, the expression of TNF-α decreased after treatment with hydroxocobalamin as well as cobinamide suggesting that hydroxocobalamin may have provided some benefit (though time of survival did not change). It is not clear if the cytokines were induced due to apnea/hypoxia or some other mechanism. Hypoxia is known to affect cytokine production such that IL-β, IL-6, IL-8, and TNF-α

are upregulated.[19] However, physiologic concentrations of NaHS have been shown to attenuate secretion of proinflammatory cytokines.[20, 21] Whether the effect of NaHS on cytokine production is a dose dependent function is unclear.

Serum concentrations of sulfide decreased after administration of hydroxocobalamin for H2S toxicity suggesting that hydroxocobalamin may be efficacious in other doses.[22] Our intent in this experiment was to demonstrate that cobinamide is more efficacious compared to hydroxocobalamin on a mg/mg basis. We have previously shown that hydroxocobalamin is as effective as cobinamide in reversing cyanide toxicity but five times more hydroxocobalamin than cobinamide was required.[9]

Acute H2S toxicity (> 500 PPM) causes central neurotoxicity, apnea and pulmonary edema.[23] Because our model is not an inhalation model, alveolar injury with subsequent pulmonary edema was not evident. However central nervous system effects were apparent. It has been suggested that the central nervous system effects of H2S are due to inhibition of cytochrome c oxidase, similar to cyanide toxicity. Inhibition of cytochrome c oxidase and thus inhibition of oxidative phosphorylation causes decrease adenosine triphosphate production, a shift to anaerobic metabolism, and subsequent metabolic acidosis.[24] Although we cannot directly attribute the metabolic acidosis seen in our animals to inhibition of cytochrome c oxidase, it likely contributed to lactic acidosis together with apnea.

Because all animals had equivalent treatment until 1 minute after apnea, we analyzed grouped data from baseline to apnea to further evaluate our model. We found that SVO2, SaO2, oxyhemoglobin, peripheral oxygen saturation (SPO2), mean arterial pressure, (MAP) and cardiac output (CO) decreased over that time, with the most significant decrease occurring at

apnea (tables 4 and 5). If NaHS inhibits cytochrome c oxidase in a manner similar to H2S or cyanide poisoning, it might be expected that neither SaO2 nor oxyhemoglobin would decrease. However, it has been postulated that poisoning from H2S produces a significant decrease in cardiac output and cardiac contractility from blockade of L calcium channels in cardiomyocytes and that the effects of this blockade happen more rapidly than the effects of inhibition of cytochrome c oxidase.[24] Because time from the start of the NaHS infusion to the apneic event was short (5.28 ± 4.2 min) and because we did not measure molecular events, it is difficult to say with certainty which factors contributed to the decreases in oxygenation and cardiac output. What is clear is that cobinamide was 100% successful in rescuing the NaHS toxic animals whereas hydroxocobalamin or no treatment did not.

Cobinamide, a water soluble cobalamin analog, has a high binding affinity for cyanide and sulfide. Because, the major toxic effect of H2S is similar to cyanide, i.e. inhibition of cytochrome c oxidase, we speculated that cobinamide would reverse the effects of H2S toxicity. Administration of cobinamide produced a return to normal respirations and oxygenation, an increase in heart rate above baseline, a moderate increase in mean arterial pressure, and an increase in cardiac output. These trends are congruent with our experiments examining the efficacy of cobinamide to treat cyanide toxicity.[9]

Although hydroxocobalamin has been shown to bind sulfide and thiosulfate,[22] it was not clinically effective in this model at the same mg/kg dose as cobinamide. This result is consistent with our previous work with cyanide where the effective hydroxocobalamin dose was about 5 times greater than the cobinamide dose required to treat severe cyanide poisoning in a swine model.[9] Cobinamide's greater potency against cyanide and sulfide compared to

hydroxocobalamin is because cobinamide: (i) has a greater affinity for the CN⁻ and HS⁻ ions, (ii) has a higher rate of complex formation with these ions, and (iii) can neutralize two CN⁻ and HS⁻ ions per mole instead of only one.[25, 26]

The volume of hydroxocobalamin necessary to deliver 4 mg/kg was 3 times greater than that of cobinamide, because hydroxocobalamin is less water soluble. Hence hydroxocobalamin cannot be administered intramuscularly, making it less attractive as an antidote for mass casualty situations.

LIMITATIONS

There were a number of limitations to this study. First, we infused NaHS rather than use a H2S inhalation model. The intravenous infusion model allowed us to tightly control the level of toxicity and prevented exposure of laboratory personnel to potential leaks of H2S gas. However, given that the swine developed symptoms and findings similar to those described in patients who inhale H2S and given that H2S is primarily a mitochondrial inhibitor, the route of exposure may not be essential. Other models have used intravenous infusion to validate antidotes. Nevertheless, the results may have been different had we used an inhalation model where inhaled hydrogen sulfide has different kinetics. Second, we used an equal dose of hydroxocobalamin and cobinamide. A larger dose of hydroxocobalamin may have proved efficacious. However, our intent in this study was to compare equal doses of hydroxocobalamin and cobinamide for the treatment of H2S poisoning. Finally, the study was not blinded; however we have meticulously reported the objective criteria, i.e. time to death, vital signs, and urine thiosulfate concentration, to limit subjective interpretations.

CONCLUSION

We have shown that cobinamide was 100% effective in this fatal model of NaHS toxicity, and that cobinamide was significantly more efficacious than hydroxocobalamin when compared at equal mg/kg dosages. Moreover, this model appears to be robust and potentially may be used to evaluate other antidotes to H2S. Further research is necessary to determine if cobinamide may serve as an effective antidote, particularly in areas with a high risk of exposure such as fracking areas.

Declaration of interest

No author had any financial or personal relationships with other people or organizations that inappropriately influenced the study.

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Table 1. Baseline characteristics 30 minutes before apnea

Characteristics	IV COB (n=8)	IV HOC (n=8)	Control (n=8)	p value group differences
Weight, kg	50.1(3.1)	47.8(3.7)	51.0(4.2)	NS
Heart rate, beats/min	92(16.7)	102(15.4)	95(21.7)	NS
Mean arterial pressure, mm Hg	100(17.7)	113(12.3)	107(12.3)	NS
Cardiac output, L/min	5.5(1.3)	5.9(1.6)	5.7(1.6)	NS
Systemic vascular resistance, Dynes-sec/cm^5	1495(240)	1588(249)	1520(433)	NS
Lactate, mmol/L	1.0(0.3)	1.0(0.4)	1.1(0.2)	NS
pH	7.46(0.02)	7.47(0.02)	7.47(0.04)	NS
Hemoglobin, gm/dl	8.4(0.6)	8.7(1.0)	8.0(1.0)	NS
Mixed venous oxygen saturation, %	60(10.1)	64(4.2)	60(4.9)	NS
Peripheral oxygen saturation, %	92.8(2.8)	96.2(3.0)	93.8(2.4)	NS
Urine thiosulfate, µg sodium thiosulfate/mg creatinine	3.7(3.2)	4.8(5.4)	4.5(3.0)	NS

COB, cobinamide; **HOC**, hydroxocobalamin; **Kg**, kilograms; **mm Hg**, millimeters of mercury; **L/min**, liter per minute; **Dynes-sec/cm^5**, dynes-seconds per centimeter^5; **mmol/L**, millimoles per liter; **gm/dl**, grams per deciliter. **µg**, micrograms; **mg**, milligrams. Data presented as means (standard deviation). There were no significant differences.

Table 2. Dose and duration of NaHS infusion to produce apnea

Parameters at apnea	IV COB (n=8)	IV HOC (n=8)	Control (n=8)	p value group differences
Total dose of NaHS, mg	275(205)	210(109)	362(283)	NS
Dose of NaHS, mg/kg	5.2(4.3)	4.2(2.4)	6.9(5.3)	NS
Duration of infusion, minutes	5.2(4.3)	3.9(5.4)	6.6(5.3)	NS

NaHS, sodium hydrosulfide; **COB**, cobinamide; **HOC**, hydroxocobalamin; **mg/kg**, milligrams per kilogram. Data presented as means (standard deviation). There were no significant differences.

Table 3. Urine thiosulfate (μg sodium thiosulfate/mg creatinine) levels from start of infusion to 10 minutes post apnea

Time of urine specimen	IV COB (n=8)	IV HOC (n=8)	Control (n=8)	p value group differences
Start of infusion	43.9(25.1)	15.3(29.0)	9.92(26.9)	NS
1 minute post apnea	476.7(270)	231.6(312)	458.2(289)	NS
2 minutes post apnea*	856.5(686)	416.0(792)	1455.6(733)	NS
4 minutes post apnea*	1065.7(803)	557.9(928)	1724.6(859)	NS
6 minutes post apnea*	1305.5(635)	710.9(733)	1420.6(678)	NS
8 minutes post apnea*	1417.7(1241)	885.6(622)	1674.2(3165)	NS

μg, microgram**; mg**, milligram; **COB**, cobinamide; **HOC**, hydroxocobalamin; **mg/kg**, milligrams per kilogram. Data presented as means (standard deviation). There were no significant differences between the groups. There was a significant within-group difference such that by two minutes post infusion all groups had significantly higher levels of urine thiosulfate compared to baseline (p<0.05). Asterisk indicates significant difference.

Table 4. Serial vital sign values

Serial vital signs	IV COB (n=8)	IV HOC (n=8)	Control (n=8)	p value group differences
Heart rate; BPM				
baseline	92(16)	102(15)	95(21)	NS
5 min pre infusion	88(13)	88(18)	88(19)	NS
apnea + 1 min	96(13)	78(35)	78(33)	NS
apnea + 5 min	150(47)*	29(31)	9(14)	P<0.01
apnea + 10 min	157(42)*	2(2)	2(3)	P<0.01
Cardiac output; L/min				
baseline	5.5(1.2)	5.9(1.6)	5.7(1.6)	NS
5 min pre infusion	5.7(1.4)	6.2(1.4)	5.9(1.2)	NS
apnea + 1 min	5.8(1.4)	5.3(2.4)	5.1(2.3)	NS
apnea + 5 min	5.8(1.3)	5.4(2.4)	3.6(2.9)	NS
apnea + 10 min	6.6(0.7)*	0	0	P<0.01
Mean Arterial Pressure; Dynes-sec/cm5				
baseline	100(17.7)	113(12.3)	107(22.5)	NS
5 min pre infusion	63.2(25.8)	87(34.5)	73(11.6)	NS
apnea + 1 min	66(12.5)	87(37.2)	79(31.8)	NS
apnea + 5 min	75(16.6)	94(37.4)	20(8.8)*	P<0.01
apnea + 10 min	70.8(7.8)*	24.3(17.2)	13(9.3)	P<0.01
SVO2; %				
baseline	60(10.1)	64(4.2)	60(4.9)	NS
5 min pre infusion	63(5.1)	65(9.8)	54(12.8)	NS
apnea + 1 min	52.2(12.4)	62.7(16.6)	48(24.5)	NS
apnea + 5 min	50.3(20.9)	44.3(14.9)	35.3 (25.2)	NS
apnea + 10 min	61.3(11.8)*	28(14.0)	33(29.1)	P<0.01

COB, cobinamide; **HOC**, hydroxocobalamin; **BPM**, beats per minute; **L/min**, liters per minute; **Dynes-sec/cm^5**, dynes-seconds per centimeter^5; **%**, percent. Data presented as means (standard deviation). Significant differences are annotated with an asterisk

Table 5. Serial arterial blood gas values

Serial arterial blood gases	IV COB (n=8)	IV HOC (n=8)	Control (n=8)	p value group differences
pH				
baseline	7.46(0.02)	7.47(0.04	7.47(0.04)	NS
5 min post infusion	7.49(0.07)	7.46(0.04)	7.46(0.06)	NS
apnea + 1 min	7.33(0.03)	7.34(0.05)	7.32(0.03)	NS
apnea + 10 min	7.43(0.07)*	7.25(0.04)	7.22(0.05)	P<0.01
Pco2, mmHg				
baseline	41.8(7.6)	41.2(6.1)	42.5(7.5)	NS
5 min post infusion	42.9(7.5)	46.2(6.1)	44.9(7.5)	NS
apnea + 1 min	63.1(5.8)	65.2(8.7)	66.7(5.8)	NS
apnea + 10 min	43.3(10.9)*	69.5(8.5)	75(9.9)	P<0.01
Po2, mmHg				
baseline	96.1(11.5)	93.4(10.8)	100(15.3)	NS
5 min post infusion	76.1(11.2)	75.3(14.8)	62.5(11.6)	NS
apnea + 1 min	34.7(5.8)	58.2(16.9)	47.7(17.6)	NS
apnea + 10 min	76.7(17.6)*	47.4(13.8)	44.1(19.3)	P<0.01
Lactate, mmol/L				
baseline	1.0(0.3)	1.0(0.4)	1.1(0.2)	NS
5 min post infusion	1.2(0.2)	2.2(1.9)	1.4(0.4)	NS
apnea + 1 min	2.7(0.6)	4.1(1.5)	3.9(1.2)	NS
apnea + 10 min	2.6(1.3)*	6.3(0.7)	6.7(1.6)	P<0.01
SaO2 %				
baseline	96.8(2.8)	96.7(1.9)	96.7(1.9)	NS
5 min post infusion	94.1(1.2)	92.8(5.7)	87.6(6.9)	NS
apnea + 1 min	45.8(14)	71.9(21)	61.5(22)	NS
apnea + 10 min	91.0(7.7)*	39.8	40.1(12.8)	P<0.01
Oxyhemoglobin %				
baseline	95.0(1.8)	90.5(11.8)	94.9(3.1)	NS
5 min post infusion	92.6(0.7)	90.0(4.7)	86.5(7.1)	NS
apnea + 1 min	44.7(14.9)	69.8(20.6)	60.1(21.0)	NS
apnea + 10 min	85.8(7.7)*	30.6	38.9(11)	P<0.01

COB, cobinamide; **HOC**, hydroxocobalamin; **Pco2**, partial pressure of carbon dioxide; **mm Hg**, millimeters of mercury, **Po2,** partial pressure of oxygen; **mmol/L**, millimoles per liter; **SaO2,** oxygen saturation. Data presented as means (standard deviation). Significant differences are annotated with an asterisk.

60-minute Survival by Treatment Groups

Log-rank p=0.001

HR

CO

MAP

Change in cytokines

Legend: TNF-a, IL-1b, IL-6

Treatment at apnea+1

Non-survival point for IV-HOC and control groups

Change from Baseline to 10min Post Tx

Legend: Control, IV-COB, IV-HOC