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Intramuscular Cobinamide as an Antidote to Methyl Mercaptan Poisoning

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Disclosure Statement

A patent for cobinamide has been issued, but no license or royalties.

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Abstract

Background: Methyl mercaptan occurs naturally in the environment, and is found in a variety of occupational settings, including the oil, paper, plastics, and pesticides industries. It is a toxic gas, and deaths from methyl mercaptan exposure have occurred. The Department of Homeland Security considers it a high threat chemical agent that could be used by terrorists. Unfortunately, no specific treatment exists for methyl mercaptan poisoning.

Methods: We conducted a randomized trial in 12 swine comparing no treatment to intramuscular injection of the vitamin B_{12} analog cobinamide (2.0 ml, 12.5 mg/kg) following acute inhalation of methyl mercaptan gas. Physiological and laboratory parameters were similar in the control and cobinamide-treated groups at baseline and at the time of treatment.

Results: All six cobinamide-treated animals survived, whereas only one of six control animals lived (17% survival) (p=0.0043). The cobinamide-treated animals returned to a normal breathing pattern by 3.8 ±1.1 min after treatment (mean ± SD), while all but one animal in the control group had intermittent gasping, never regaining a normal breathing pattern. Blood pressure and arterial oxygen saturation returned to baseline values within 15 minutes of cobinamide-treatment. Plasma lactate concentration increased progressively until death (10.93±6.02 mmol [mean±SD]) in control animals, and decreased towards baseline (3.79±2.93 mmol [mean±SD]) by the end of the experiment in cobinamide-treated animals.

Conclusion: We conclude that intramuscular administration of cobinamide improves survival and clinical outcomes in a large animal model of acute, high dose methyl mercaptan poisoning.

Introduction

Methyl mercaptan, also known as methanethiol, is produced endogenously in animals and humans during the metabolism of methionine and cysteine, and is released by oral and intestinal microflora during anaerobic metabolism.[1] It is present in a variety of occupational settings, most notably the petroleum, paper and plastic industries, and during the manufacturing of pesticides.[1] It has a noxious rotten cabbage odor, and is added to natural gas to aide in leak detection.[1] Exposure to high concentrations of methyl mercaptan can lead to nasopharyngeal irritation, headache, vomiting, dizziness, muscle fatigue, hypotension, seizures, coma, cardiac arrhythmias, bronchospasm, pulmonary edema, hepatic and renal damage, and death. [1–8] In 2014 four employees died after ~24,000 pounds of methyl mercaptan were released from a pesticide production facility in La Porte, Texas.[9] Mass casualty exposures from industrial accidents remain an ongoing risk. [10]

In 2007 the Department of Homeland Security (DHS) established the Chemical Facility Anti-Terrorism Standards (CFATS) to implement safety and security practices at facilities

where toxic chemicals such as methyl mercaptan are manufactured or used.[11] Moreover, the United States Department of Labor, Occupational Safety and Health Administration (OSHA), developed an emergency preparedness guide for industries that use toxic industrial chemicals (TICS) such as methyl mercaptan.[12] The purpose of the standards and guide are to reduce the risk of worker exposure, as well as to prevent the chemicals from coming into the hands of terrorists who might try to use the chemicals for nefarious purposes.

Methyl mercaptan has several mechanisms of toxicity. First, like cyanide and hydrogen sulfide, methyl mercaptan inhibits mitochondrial cyctochrome c oxidase, thereby disrupting the electron transport chain, reducing ATP production, and increasing production of reactive oxygen species.[13–15] Second, methyl mercaptan can bind to proteins and erythrocytes and can reduce blood oxygen carrying capacity.[15,16] And third, methyl mercaptan inhibits multiple enzymes including sodium-potassium adenosine triphosphatase (Na^+/K^+-ATPase), affecting bioelectrical activities, resulting in lethargy, muscle weakness, seizures, and even paralysis.[17]

The current management of methyl mercaptan poisoning is supportive care, because no FDA-approved antidote exits.[18] Removing victims from the source of exposure and providing respiratory and cardiovascular support is essential. Due to the risk for a mass casualty exposure, developing a safe, easy to administer medical countermeasure is important.

Cobinamide is the penultimate precursor in hydroxocobalamin/vitamin B_{12} biosynthesis, and is a potent antidote for cyanide and hydrogen sulfide poisoning.[14,19–25] Under the FDA Animal Rule, drug efficacy studies should ideally be performed in both large and small animals.[26] Swine have similar cardiovascular and respiratory systems as humans, and are an excellent large animal species for toxicology studies.[27,28] Few inhalation models of methyl mercaptan have been reported and to our knowledge no large animal models of methyl mercaptan inhalation exist. In this study we evaluate the efficacy of cobinamide against inhaled methyl mercaptan in a large swine model.

Materials and Methods

Materials

Methyl mercaptan sodium salt (CH_3NaS) was purchased from TCI America (Portland, OR) and hydrochloric acid (HCl) was purchased from Sigma Aldrich (St. Louis, MO).

The generic term cobinamide is used in the text without reference to the axial ligands. It was synthesized as described previously by base hydrolysis of hydroxocobalamin with purification over reversed-phase resins.[29] It was converted to bis-acetyltetrazole-cobinamide, the form used in these studies, by adding eight molar equivalents of 5-acetyltetrazolate to an aqueous solution of cobinamide. The acetyltetrazole ligand was added to increase cobinamide absorption after intramuscular injection.

Animal Subjects

Experiments were approved by the University of Colorado's Institutional Animal Care and Use Committee (IACUC), and complied with the regulations and guidelines of the Animal Welfare Act and the American Association for Accreditation of Laboratory Animal Care. Animals were housed and experimentation took place in the animal care facility.

General Experimental Procedures

Male and female Yorkshire swine (*Sus scrofa)* weighing 30–40 kg (Midwest Research Swine, Gibbon MN) were anestheized with 10–20 mg/kg ketamine (MWI, Boise, ID) and 3–5% isoflurane (MWI, Boise, ID) via nosecone. They were intubated with a cuffed 7.5–8.0 mm endotracheal tube (Teleflex, Morrisville, NC), a peripheral catheter was placed in the lateral ear vein, and a one-time bolus (7.5 ml/kg) of warm saline (B. Braun, Bethlehem, PA) was administered. During instrumentation, sedation was maintained using a Drager Apollo anesthesia machine (Drager, Houston, TX) with 1–3% isoflurane and 0.4 FiO_2. The animal's tidal volume was set at 8 ml/kg with a respiratory rate 16–20 breaths per minute. The minute volume was adjusted to maintain an end tidal CO_2 of 38–42 mm Hg.

The femoral vein and artery were visualized using an M9 ultrasound system (Mindray, Mahwah, NJ), and central venous and arterial catheters were placed. The animals then received a one time bolus of intravenous propofol and fentanyl, and sedation was maintained with a continuous intravenous infusion of propofol and fentanyl, adjusting doses until ventilator support was no longer required and animals were breathing spontaneously with an FiO_2 of 0.21. During this time, the animals were weaned off isoflurane. Vital signs including pulse rate, arterial blood pressure, pulse oximetry, body temperature, and ECG were recorded every minute using a Drager Infinity Delta Monitor and Patient Watch Software (Drager, Houston, TX). Respiratory parameters including tidal volume, respiratory rate, minute volume, end tidal CO_2, and peak expiratory and inspiratory flow were recorded using a Phillips Respironics NM3 monitor (Andover, MA). To ensure the safety of laboratory personnel, experiments were conducted in a Class II, Type B2 biosafety cabinet (Labconoco, Kansas City, MO).

Study Design

Animals were randomized to either control no treatment (6 animals, 3 male, 3 female), or to treatment with 12.5 mg/kg bis-acetyltetrazole-cobinamide administered intramuscularly as ~ 2 ml of a 200 mM solution (6 animals, 3 male, 3 female).

Exposure to Methyl Mercaptan Gas

Methyl mercaptan gas was produced by slowly adding CH_3NaS to briskly-stirred HCl in a stoppered vessel, which was connected to a closed breathing circuit system. The concentration of methyl mercaptan gas in the circuit was measured continuously using a GX6000 (RKI Instruments, Union City, CA) photoionization detector (PID). The GX6000 gas detector was configured by the manufacturer with a PID specific to methyl mercaptan and capable of measuring concentrations up to 6000 ppm. It is professionally calibrated every 3 months (Mallory Instruments, Denver, CO). When the gas concentration reached a steady state concentration of 3000 ppm, the circuit was connected to the animal's

endotracheal tube and the animal began inhaling methyl mercaptan gas (Figure 1). Inhalation of the gas was continued until 5 minutes after apnea or onset of agonal respirations (defined as erratic breaths with a tidal volume of > 15 ml/kg for two consecutive minutes) at which time the circuit was disconnected from the animal's endotracheal tube and the animal began breathing room air (Figure 1). The cobinamide was given at 2 minutes after apnea or onset of agonal respirations by injection into the semitendinosus muscle using a 22 guage 1.5 inch long needle. In an effort to reduce the number of animals used we did not include an additional saline IM control group since it seemed unlikely than an injection of 2 ml of saline would have an effect and this would not be done in humans. Animals were observed for 90 minutes post treatment or until death, defined as a mean arterial pressure of < 30 mm Hg for 10 continuous minutes. [14,19–25]

Outcome Measures

The primary outcomes were survival and time to death. Other measured variables were oxygen saturation, respiratory rate, blood pressure, serum pH, and serum lactic acid concentration.

Euthanasia

At the end of the study, all animals were euthanized with an intravenous administration of 100 mg/kg sodium pentobarbital according to the regulations and guidelines of the Animal Welfare Act and the American Association for Accreditation of Laboratory Animal Care.

Data Analysis

A sample size of six animals per group was determined using an alpha of 0.05 and a power of 0.80, estimating a 75% difference in survival between groups. Values are expressed as mean ± standard deviation. An unpaired *t* test with Welch's correction was used to calculate 95% confidence intervals, and a two-tailed *t* test was used for comparison between control and cobinamide-treated animals. A *p* value of less than 0.05 was considered significant. Survival between groups was assessed by a Kaplan-Meier survival curve and analyzed by a log-rank, Mantel-Cox test. Prism 7.0 (GraphPad, La Jolla, CA) was used for statistical analysis.

Results

At baseline and at the time of apnea or onset of agonal respirations, physiological parameters including arterial blood pressure, pulse oximetry, respiratory rate, blood pH, and lactate concentration were similar in the control and cobinamide-treated groups (Tables 1 and 2). Animals in the control group developed apnea or agonal respirations within 46.0±18.6 minutes (mean ± SD), and the cobinamide-treated group 45.9±23.0 minutes (mean ± SD) of starting to inhale methyl mercaptan gas (Table 2).

All but one untreated control animal died within 34.1±13.5 minutes (mean ± SD) of onset of respiratory depression (17% survival) (Figure 2). This is to be contrasted with 100% survival of the cobinamide-treated animals (Figure 2) (p=0.0043).

All but one animal in the control group had intermittent gasping, never regaining a normal breathing pattern, whereas the cobinamide-treated animals returned to a normal breathing rate by 3.8±1.1 minutes after treatment (mean ± SD) (Figure 3a). All animals developed acidosis as indicated by blood pH and hyperlactatemia (Figure 3b, 3c). Animals in the control group showed a progressive increase in the serum lactate concentration to 10.9±6.02 mmol at the time of death. The serum lactate concentration began to decrease in the cobinamide-treated animals at 30 minutes after treatment, decreasing to near baseline by the end of the study (3.8±2.93 mmol) (Table 3, Figure 3b).

Physiological parameters also improved with cobinamide treatment. The mean arterial blood pressure (MAP) of control animals remained low, while the MAP of cobinamide treated aniamals returned to baseline within 15 minutes after treatment (Figure 3d). Similarly, arterial oxygen saturation fell progressively in control animals until death, whereas it returned to baseline in the cobinamide-treated animals within 15 minutes of treatment ($p<0.0062$) (Table 3, Figure 3e).

Discussion

In a lethal, large swine model of methyl mercaptan gas inhalation, we found that intramuscular injection of cobinamide rescued all animals when administered at the time of severe respiratory depression. Our study also demonstrated a reversal of lactic acidosis, as shown by a decrease in plasma lactate concentration. Importantly, cobinamide has also been shown to be efficacious in other models of metabolic poisoning, including hydrogen sulfide and cyanide.[14,19–25]

Despite methyl mercaptan being a high-threat chemical agent, few studies have been published on high dose inhalation, and treatment efficacy. Furthermore, the majority of work has been done in rodents. Fang et al reported on a non-lethal, repeated, low dose exposure to methyl mercaptan in rats, showing bronchiole constriction, alveolar thickening, and pulmonary exudate consisting of fibrin, erythrocytes, and monocytes.[13] Rat studies of acute, high dose inhalation reported 100% lethality following a 20 minute exposure to 2,000 ppm. [30] Case reports of human exposure resulting in death are in the literature, but concentration and duration of exposure data are limited. [30] In an effort to mimic human exposure the concentration of gas used in these studies resulted in physiological responses and death, similar to what has been reported in humans.

In a human case report, sodium nitrite was used to treat a patient following methyl mercaptan exposure. Whether the sodium nitrite was helpful is not clear, but sodium nitrite causes methemogobinemia, which can result in decreased oxygen transport and hypoxia.[7]

Based on this work, cobinamide might be useful to treat methyl mercaptan-poisoned patients in the field. Intramuscular administration would allow for rapid administration, with the potential to administer to victims prior to or quickly after removing them from the source of the gas. Additional studies assessing efficacy following multiple doses of methyl mercaptan exposures, as well as long term survival are needed to evaluate cobinamide as a treatment for methyl mercaptan poisoning.

There are several limitations to our study. First, no animal model mimics human exposures exactly. However, swine have a similar size, cardiovascular system, and physiology to humans and make an excellent choice to model human toxicity.[27,28] Second, the animals in this study were anesthetized, as required by our IACUC, which could have affected the animals' response to the methyl mercaptan gas. However, we used intravenous rather than inhaled anesthesia to reduce pulmonary effects and interactions with the inhaled methyl mercaptan gas, and we used the same anesthesia protocol in control and treated animals. Third, although the animals did not receive ventilatory support during the exposure and treatment periods, the animals were intubated. An intubated model was needed to achieve a high dose exposure and maintain the safety of laboratory personnel, and intubation itself is unlikely to have a substantial effect on response to cobinamide compared to similary intubated control animals. Fourth, in an effort to minimize the number of animals used we compared cobinamide treatment to animals receiving no treatment. It seemed unlikely than an injection of 2 ml of saline would have an effect, and it did not seem necessary, since it would not happen in humans. Fifth, pathology was not included in this study; however, we plan to evaluate it in future studies. And sixth, the observation period following cobinamide treatment was short, but for our primary outcome of survival, the observation period was adequate.

Conclusion

Intramuscular administration of cobinamide improves survival and clinical outcome in a large swine inhalation model of acute, high dose methyl mercaptan poisoning.

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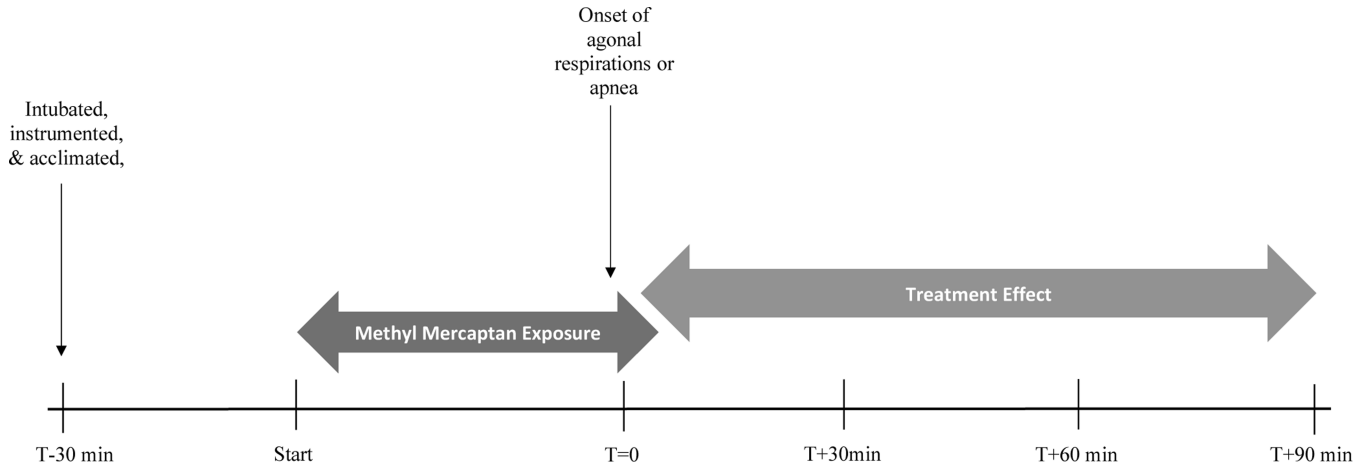


Figure 1.

Timeline of inhaled methyl mercaptan model

Yorkshire pigs were allowed to acclimate for 30 min following instrumentation (T-30) prior to the start of methyl mercaptan exposure (Start). Treatment with intramuscular bis-acetyltetrazole-cobinamide occurred at 2 min after onset of agonal breathing or apnea (T = 0) and the treatment effect was monitored for 90 minutes.

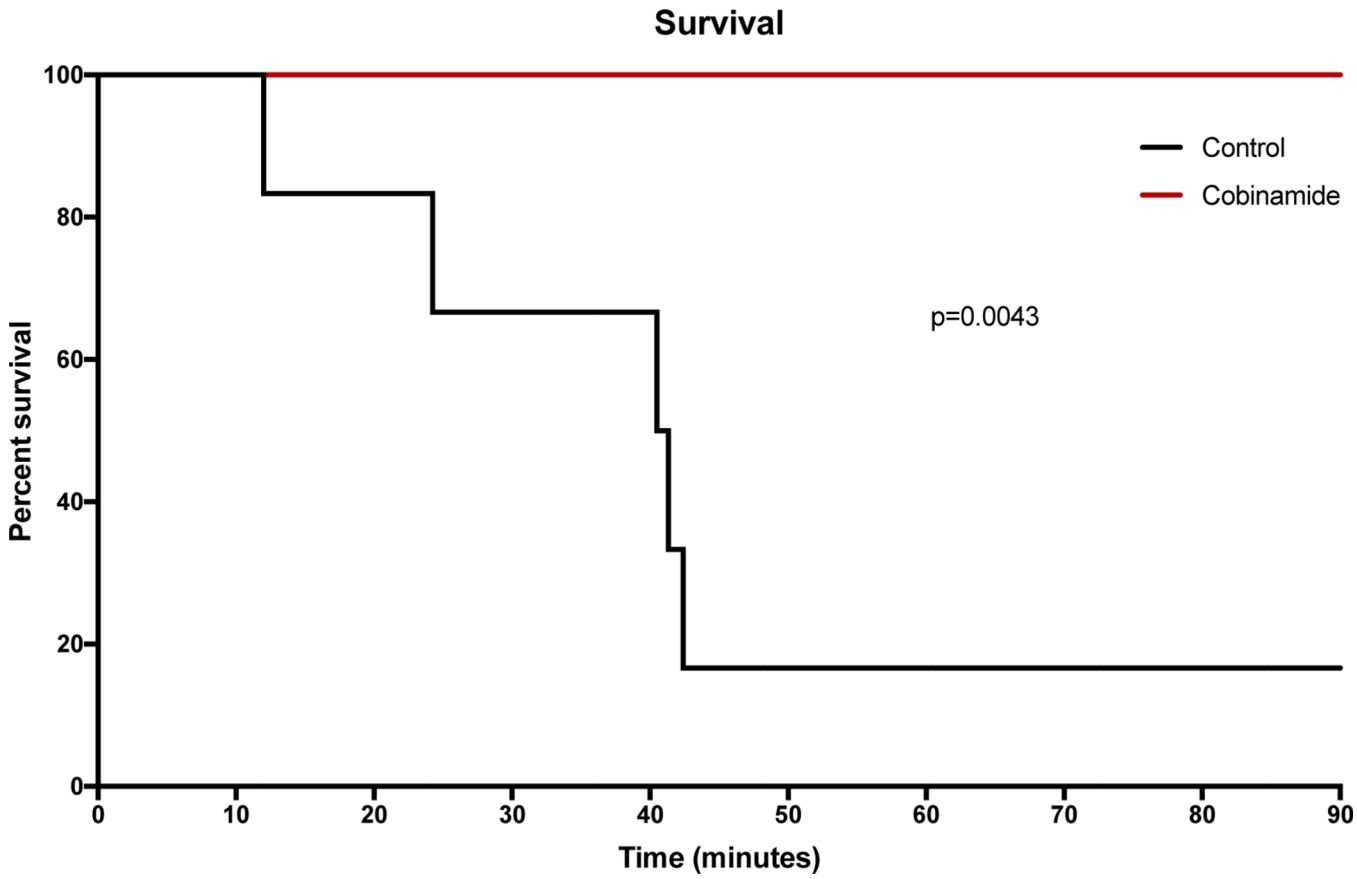
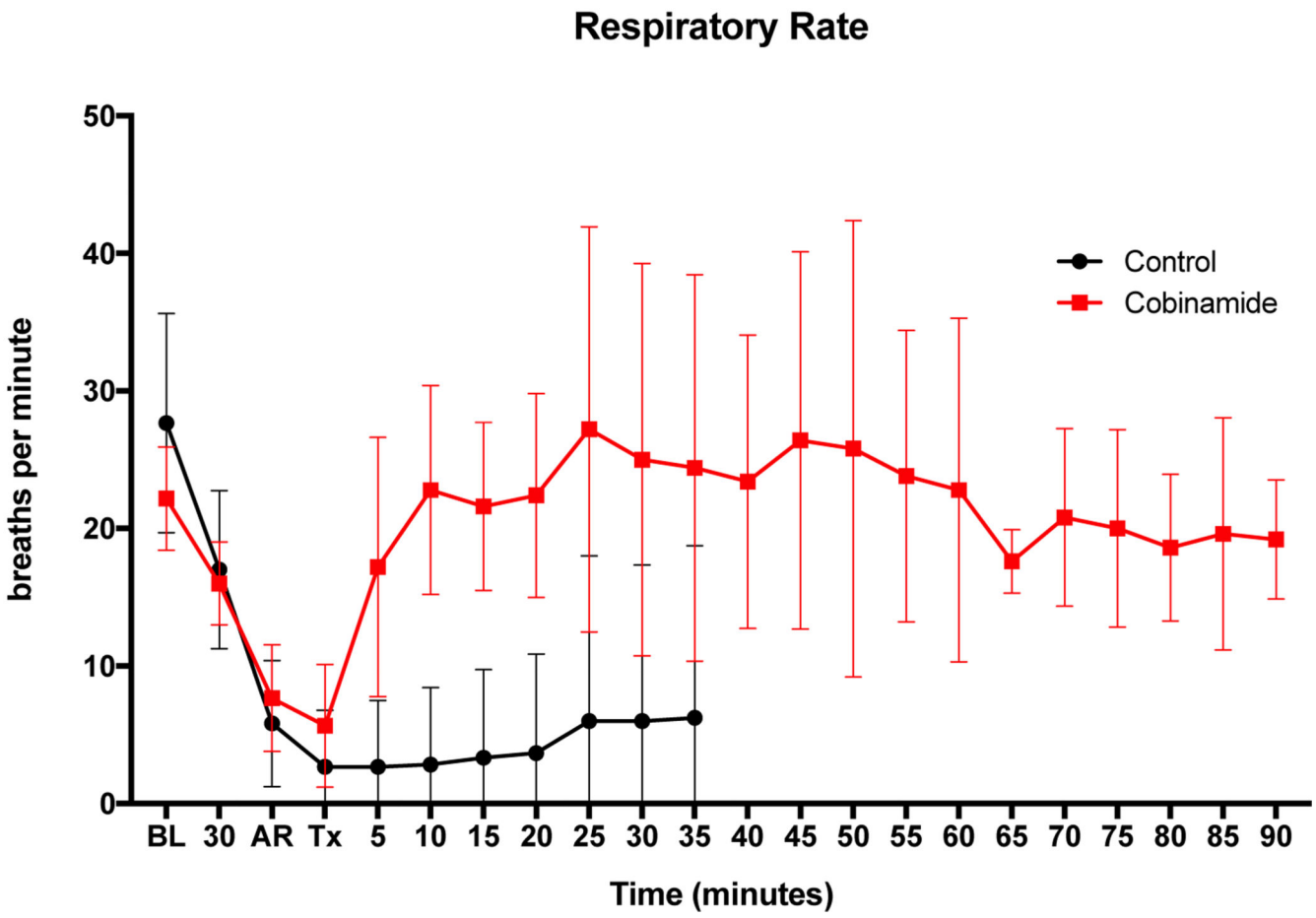


Figure 2.

Percent survival in swine treated with intramuscular cobinamide compared to controls displayed as a Kaplan Meier analysis.

Survival is significantly improved with intramuscular cobinamide administration following inhaled methyl mercaptan exposure compared to controls. *p* value determined by log rank (Mantel-Cox) test for comparison, *p* value ≤ to 0.05 was considered significant. Zero time was defined as shown in Figure 1.

3a



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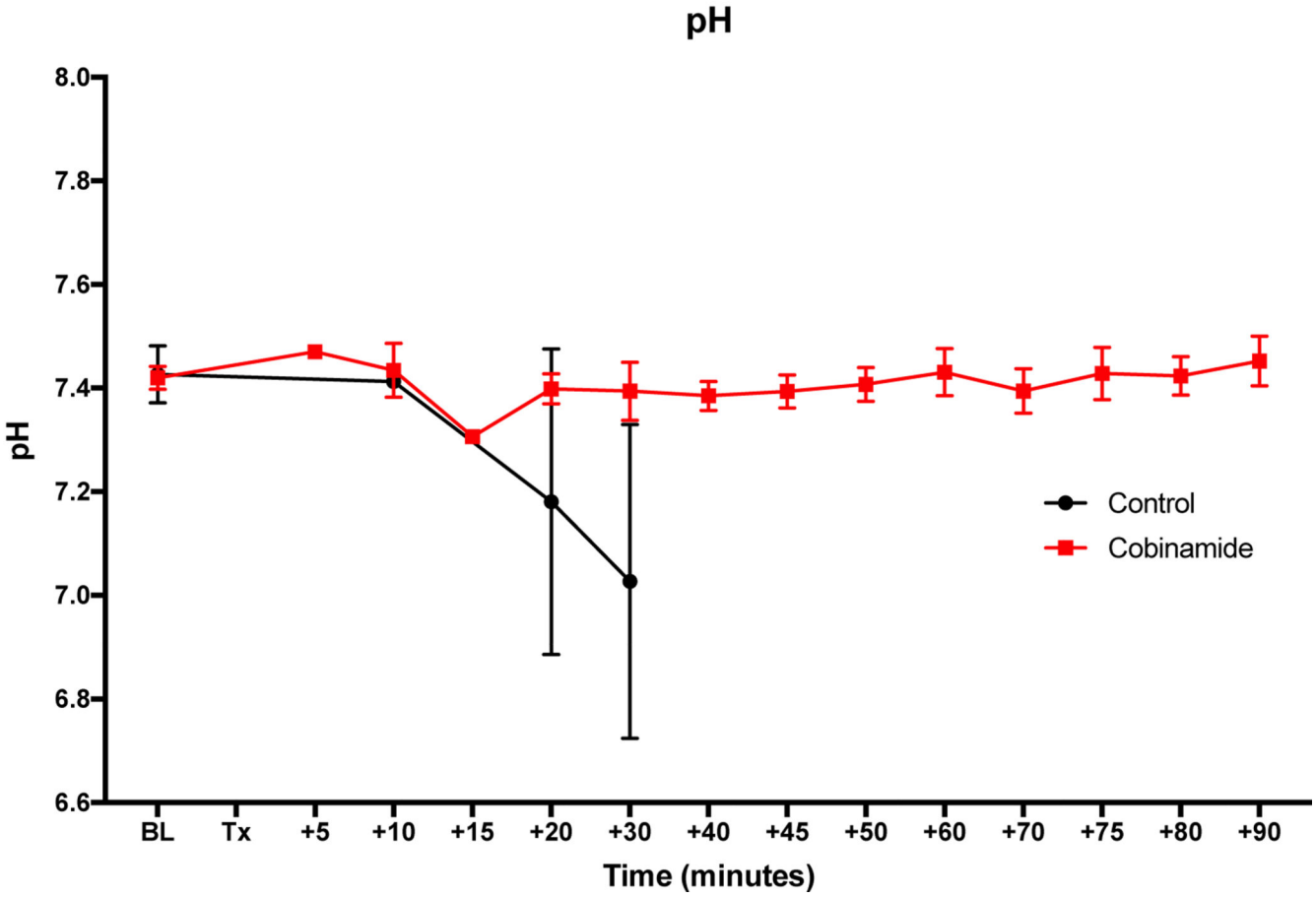

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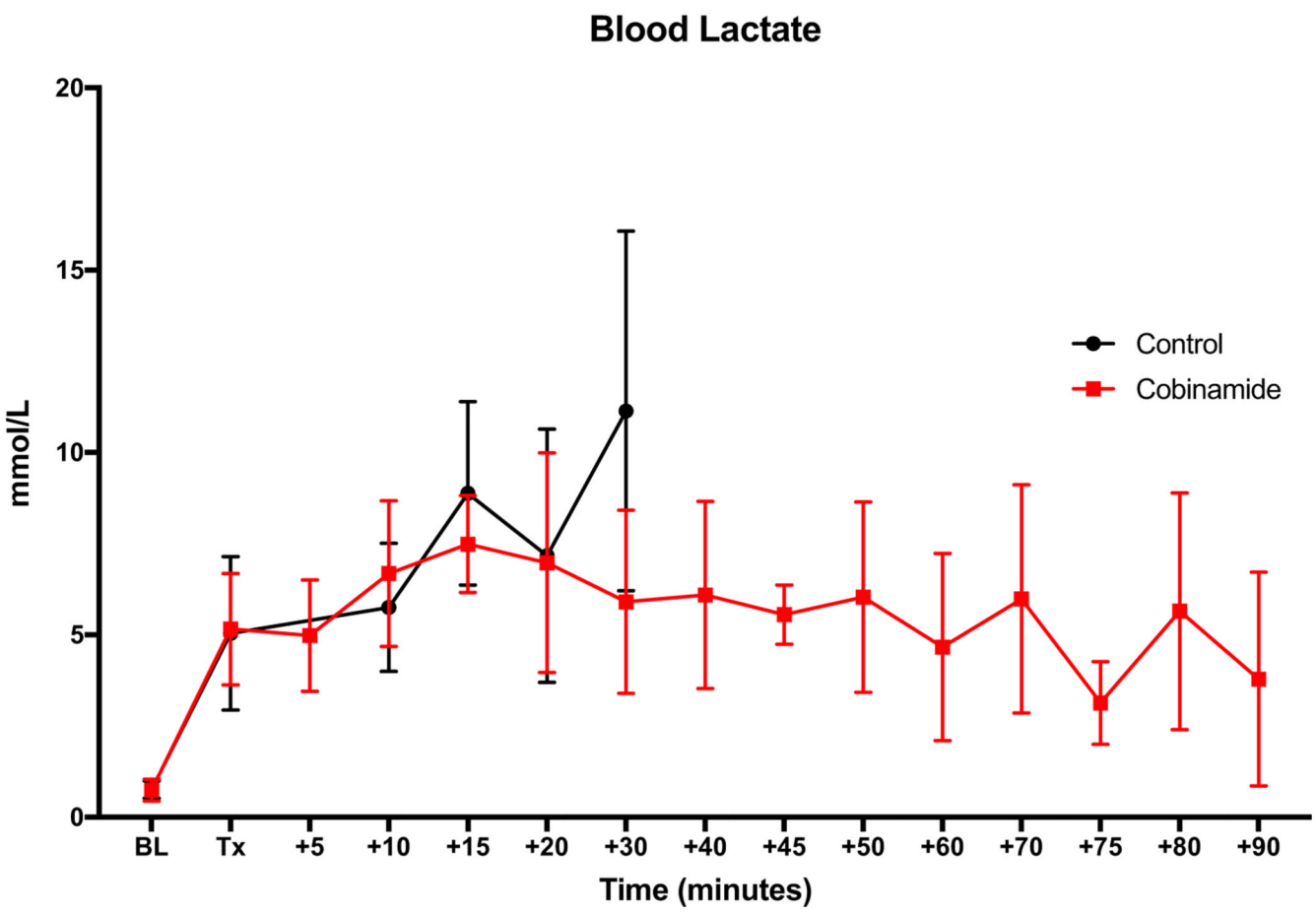

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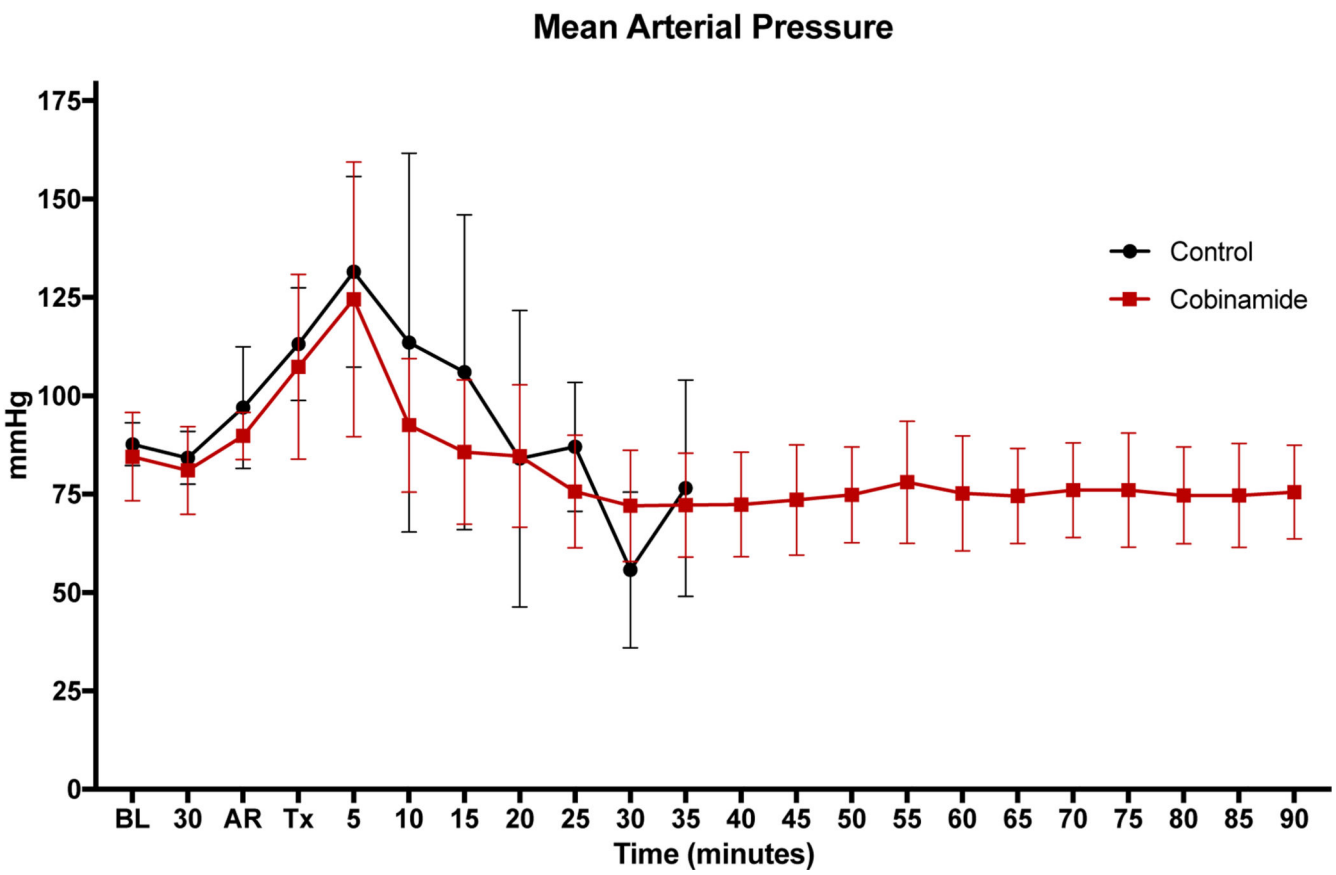

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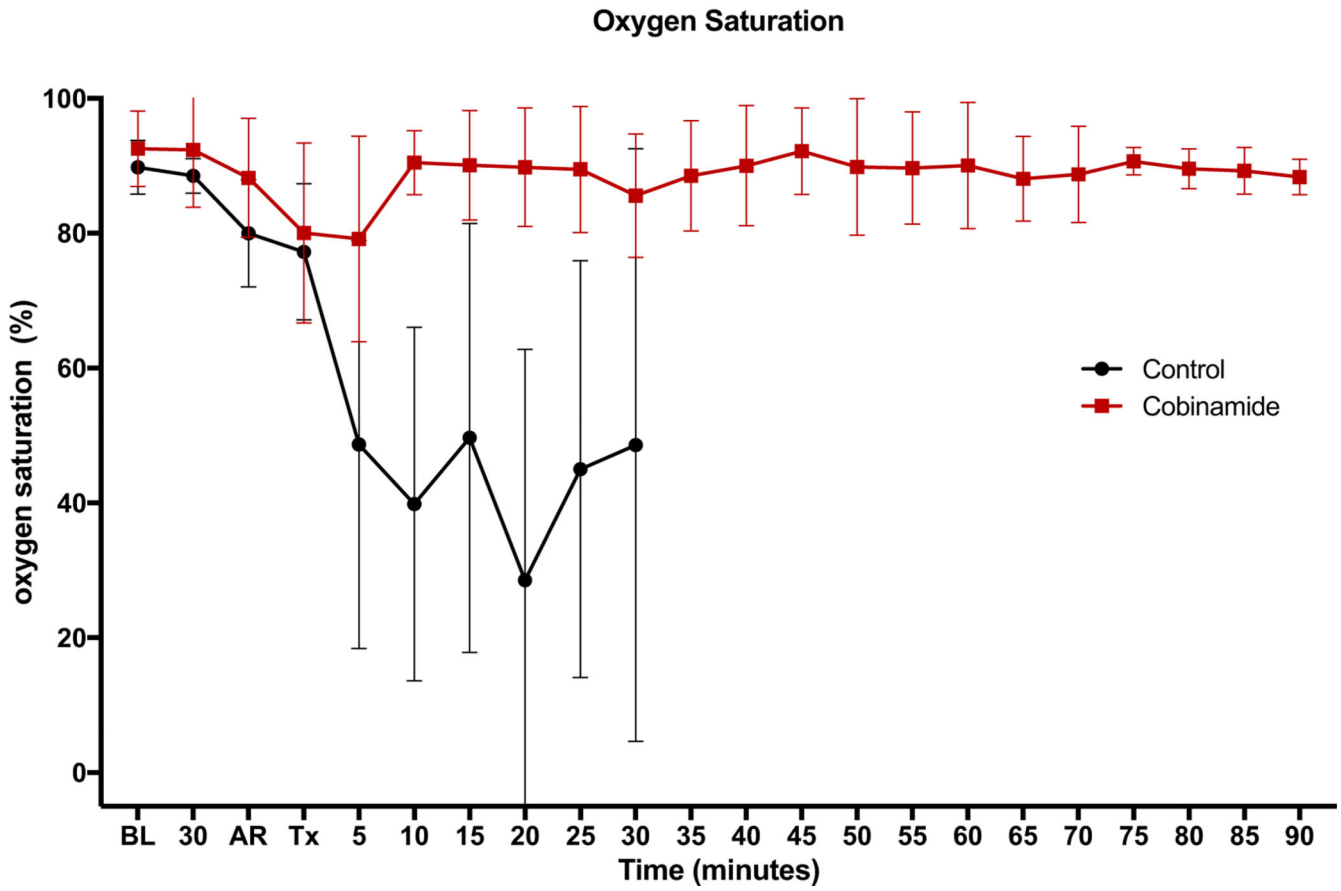

Figure 3.

Laboratory values and physiological parameters over time in animals treated with intramuscular cobinamide compared to controls.

(a-e) Laboratory and physiological parameters over time between cobinamide-treated animals and controls. (a) Treated animals returned to normal breathing, while control animals did not and subsequently died. (b) Blood pH decreased over time in control animals until death compared to cobinamide treated animals. (d) Blood lactate increased over time in control animals until death compared cobinamide treated animals. (d) The cobinamide treatment group's mean arterial blood normalized post treatment compared to controls. (e) Oxygen saturation returned to baseline in the cobinamide-treated animals, but fell progressively in control animals.

Data shown exclude control animals after 50% of them had died.

Data are presented as means ± standard deviation. BL: baseline; AR: onset of apnea/agonal respirations; Tx, treatment; mm Hg: millimeters mercury; mmol/L: millimoles/liter.

Table 1.

Physiological parameters at baseline of swine receiving no treatment vs swine treated with cobinamide.

	Control n=6	Cobinamide n=6	Difference between means	95% CI difference
pH	7.43 ± 0.06	7.42 ± 0.02	−0.01 ± 0.02	−0.07, 0.05
Lactate (mmol/L)	0.76±0.24	0.74±0.30	−0.02±0.16	−0.37, 0.33
MAP (mm Hg)	88±6.2	84±10.6	−4.2±5.0	−15.7, 7.4
Oxygen saturation (%)	91±4.0	90±2.4	−0.7±1.9	−5.1, 3.6
Respiratory rate (breaths per minute)	28±8.0	22±3.8	−5.5±3.6	−14.0, 3.0

There is no significant difference in blood pH, lactate, hemodynamics, pulse oximetry or respiratory rate between control and cobinamide-treated animals at baseline.

Data are presented as mean ± standard deviation.

mmol/L: millimole/liter; SBP, systolic blood pressure; MAP, mean arterial blood pressure; mm Hg: millimeters of mercury; CI: confidence interval

Table 2.

Physiological parameters at time of apnea/agonal respirations of swine receiving no treatment vs swine treated with cobinamide.

	Control n=6	Cobinamide n=6	Difference between means	95% CI difference
Time to apnea/agonal respirations (min)	46.0±18.6	45.9±23.0	0.1±12.1	−27.1, 27.0
MAP (mm Hg)	97±15.4	90±6.0	−7.2±6.8	−23.4, 9.1
Oxygen saturation (%)	81±7.6	85±9.6	3.4±5.0	−7.8, 14.6
Respiratory rate (breaths per minute)	5.83 ± 4.58	7.67 ± 3.88	1.83 ± 2.45	−3.67, 7.31

There is no significant difference in time of onset of apnea/agonal respirations between control and cobinamide-treated animals.. Hemodynamic parameters, pulse oximetry, and respiratory rate were not significantly different between control and treated animals.

Data are presented as mean ± standard deviation.

MAP, mean arterial blood pressure; mm Hg: millimeters of mercury; CI: confidence interval

Table 3.

Physiological parameters at death or end of study of swine receiving no treatment vs swine treated with cobinamide.

	Control n=6	Cobinamide n=6	Difference between means	95% CI difference
pH	7.02 ± 0.34	7.45 ± 0.05	0.44 ± 0.17	−0.09, 0.96
Lactate (mmol/L)	10.93±6.02	3.79±2.93	−7.14±2.73	−13.56, −0.72
MAP (mm Hg)	36±33.3	76±11.9	23.0±14.4	−12.0, 58.0
Oxygen saturation (%)	23.1±36.4	90.3±2.2	67.3±14.9	29.1, 105.5

Treated animals survived until the end of the study and showed significant improvement in blood pH, lactate, hemodynamics, and pulse oximetry compared to animals receiving no treatment.

Data are presented as mean ± standard deviation.

mmol/L: millimole/liter; MAP, mean arterial blood pressure; mm Hg: millimeters of mercury; CI: confidence interval