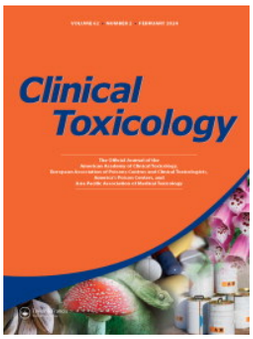

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CLINICAL RESEARCH

Intratracheal cobinamide (vitamin B_{12} analog) administration increases survivability in rabbits exposed to a lethal dose of inhaled hydrogen sulfide

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ABSTRACT

Background: Hydrogen sulfide is a highly toxic, flammable, and colorless gas. Hydrogen sulfide has been identified as a potential terrorist chemical threat agent in mass-casualty events. Our previous studies showed that cobinamide, a vitamin B_{12} analog, effectively reverses the toxicity from hydrogen sulfide poisoning. In this study, we investigate the effectiveness of intratracheally administered cobinamide in treating a lethal dose hydrogen sulfide gas inhalation and compare its performance to saline control administration

Methods: A total of 53 pathogen-free New Zealand White rabbits were used for this study. Four groups were compared: (i) received no saline solution or drug intratracheally ($n = 15$), (ii) slow drip saline intratracheally ($n = 15$), (iii) fast drip saline intratracheally ($n = 15$), and (iv) slow drip cobinamide intratracheally ($n = 8$). Blood pressure was continuously monitored, and deoxy- and oxyhemoglobin concentration changes were monitored in real-time *in vivo* using continuous wave near-infrared spectroscopy.

Results: The mean (± standard deviation) weight for all animals ($n = 53$) was 3.87 ± 0.10 kg. The survival rates of the slow cobinamide and the fast saline groups were 75 percent and 60 percent, respectively, while the survival rates in the slow saline and control groups were 26.7 percent and 20 percent, respectively. A log-rank (Mantel-Cox) test showed that survival in fast saline and slow cobinamide groups were significantly greater than those of no saline control and slow saline groups ($P < 0.05$). The slow and no saline control groups were not significantly different ($P = 0.59$). The slow cobinamide group did significantly better than the slow saline group ($P = 0.021$).

Discussion: The ability to use intratracheal cobinamide as an antidote to hydrogen sulfide poisoning is a novel approach to mass-casualty care. The major limitations of this study are that it was conducted in a single species at a single inhaled hydrogen sulfide concentration. Repeated investigations in other species and at varying levels of hydrogen sulfide exposure will be needed before any definitive recommendations can be made.

Conclusions: We demonstrated that intratracheal cobinamide and fast saline drip improved survival for hydrogen sulfide gas inhalation in rabbit models. Although further study is required, our results suggest that intratracheal administration of cobinamide and fast saline may be useful in hydrogen sulfide mass-casualty events.

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Cobinamide; hydrogen sulfide toxicity; hydrogen sulfide antidote; intratracheal antidote administration; cerebral near infrared spectroscopy

Introduction

Hydrogen sulfide (H_2S) is an easily obtainable gas that is colorless, flammable, highly toxic and has a rotten-egg odor. It is produced naturally by decaying organic materials and is also found in many industrial settings such as oil and gas refining, mining, pulp and paper processing, sulfur hot springs, carbon disulfide production, hot asphalt fumes, and pools of sewage sludge or liquid manure [1]. Inhalation is the major route of exposure to H_2S, and because it is heavier than air, it collects in enclosed, low-lying areas, making it dangerous to work within confined spaces. It is the second most common workplace cause of fatal gas inhalation exposures after carbon monoxide [2]. In addition, it has recently been reported to be used for suicide or terrorism purposes, and it has also been included in terrorist training manuals for chemical weapons use by terrorist groups [3]. The United States government considers hydrogen sulfide a high-priority chemical threat, both industrially and as a potential weapon of mass destruction by terrorists [4].

Hydrogen sulfide is an extremely rapid-acting and poisonous gas that is toxic to many organ systems. It has a role in neuromodulation, smooth muscle relaxation, anti-inflammatory effects, insulin regulation, and the physiological response to oxidative stress via inhibiting the cytochrome oxidase

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enzyme [5,6]. The H_2S exposure–response curve for lethality is extremely steep and gives little margin of safety [7]. In addition, the gas concentration is much more important than the duration of exposure [8]. Taken together, H_2S can produce an extremely rapid depression of the central nervous system and respiratory systems compared with other inhaled toxic substances. Physiological symptoms and signs of H_2S exposure begin immediately, even in small exposures. Hydrogen sulfide toxicity presents in a unique, reliable, and characteristic toxidrome consisting, in ascending order of exposure, of mucosal irritation, especially of the eye (gas eye), olfactory paralysis, and sudden but reversible loss of consciousness (knockdown) [9]. Higher concentration exposures (> 500 parts per million [ppm]) can cause shock, convulsions, apnea, coma, and death [10].

Unfortunately, there is currently no proven antidote treatment for H_2S. Treatment generally consists of support of respiratory and cardiovascular functions. Recently, two cyanide antidotes, hydroxocobalamin and sodium nitrite, have been tested for reversing H_2S toxicity [11–13]. Hydroxocobalamin is approved for cyanide poisoning as an intravenous infusion, serving as a cyanide scavenger, and it also can bind sulfide. It has been shown to decrease plasma sulfide concentrations. Although both antidotes have shown some efficacy in animal models, it is important that they need to be administered immediately after sulfide exposure because of their mechanism of action [14,15], and they are required to be administered intravenously. Moreover, the administration of nitrites carries its own risks because nitrites oxidize the ferrous ion in hemoglobin producing methemoglobin and thereby leading to reduced oxygen-carrying capacity and tissue hypoxia.

Previously, we have completed several investigations of cobinamide treatment for cyanide poisoning [16–18] and provided evidence that cobinamide should be effective in reversing H_2S toxicity *in-vitro* and murine models via intravenous and intramuscular administration [19]. Based on these results, and because the solubility of H_2S is relatively low, we hypothesized that tracheal instillation of cobinamide would be a faster and less-invasive method of delivering drugs.

In this study, we investigate the efficacy of intratracheal cobinamide treatment to reverse hydrogen sulfide exposure effects in a rabbit model as a potential treatment for mass casualty resulting from exposure to hydrogen sulfide since intratracheal treatment can be delivered rapidly in such scenarios.

Materials and methods

Study approval

Pathogen-free New Zealand white rabbits weighing 3.5–4.5 kg (Western Oregon Rabbit Supply) were used for this study. The Institutional Animal Care and Use Committee at the University of California, Irvine, approved the rabbit study (AUP-18-087), and the rabbits were cared for and treated according to the Association for Assessment and Accreditation of Laboratory Animal Care standards.

We chose New Zealand white rabbits as our animal models because of their relatively proper size, which serves to bridge the gap between smaller rodents (mice and rats) and larger animals (dogs and pigs), tame disposition, and low cost. The US Food and Drug Administration Animal Rule (21 CFR Parts 314 and 601, 2002) allows the equivalent of human phase III clinical trials to be performed in animal studies when human studies are not ethical or feasible. More importantly, the fact that rabbit animal models allow us to collect relevant physiological data (e.g., heart rate, blood pressure, and oxygenation) from serial blood samples or using monitoring devices makes rabbit animal models optimal for translational research, such as pre-clinical testing of drugs.

Animal preparation

Animals were initially anesthetized with an intramuscular injection of a solution (2:0.5 ratio) of ketamine (100 mg/mL, Ketaject, Phoenix Pharmaceutical Inc., St. Joseph, Michigan) and xylazine (20 mg/mL, Anased, Lloyd Laboratories, Shenandoah, Iowa). After the initial anesthesia, a 24 gauge by 0.75 inch catheter (Terumo, Laguna, Philippines) was placed in the marginal ear vein for additional intravenous anesthesia (0.2 mL–0.4 mL of mixture of 12.5 mg/kg ketamine and 0.5 mg/kg xylazine). All animals were intubated using a 3.5 mm cuffed endotracheal tube (Sheridan, Patterson Veterinary) and anesthetized with 1.5% isoflurane (2.0 L/min). A pulse oximeter probe was placed across the animals' cheek to measure oxygen saturation and heart rate (Cooper and Brown). The right femoral artery and vein were isolated using blunt dissection, and 4 French by 12 cm femoral arterial catheters (C-PMS-400-FA, Cook Medical, Bloomington, Indiana) were inserted. The arterial catheter was used for blood pressure monitoring (BioPac MP100, Biopac Systems Inc. Goleta, California), and blood sampling throughout the study (Figure 1).

Materials

Aquohydroxocobinamide, referred to as cobinamide hereafter, was produced by collaborators at the University of California, San Diego, with stock solutions at 200 mM. It was produced by base hydrolysis of hydroxocobalamin using cerium hydroxide as described previously and was >98% pure as assessed by high-performance liquid chromatography. It is water soluble up to concentrations of 300 mM. Sodium hydrogen sulfide was produced from (NaSH × H20, Sodium hydrosulfide hydrate, 161527-100 G, Sigma Aldrich Corp, St Louis Mo).

Hydrogen sulfide gas production and exposure

All H_2S production and exposures were done inside a fume hood. One-hundred fifty mL of 3 N hydrochloric acid was prepared by diluting 36.5–38% hydrochloric acid (36.5–38% hydrochloric acid, VWR Chemicals, Radnor, PA) with distilled water. The sodium hydrosulfide solution (321 mM) was dripped into a flask containing 150 mL of 3.0 N hydrochloric acid (150 mL) at 1 mL/min to produce H_2S gas. The mixture of H_2S (~2,000 ppm) and anesthesia gas was delivered to the spontaneously breathing animal via the endotracheal tube (2.25 L/min airflow) using a T-piece set-up. Figure 1 illustrates an overview of H_2S production and exposure to animals.

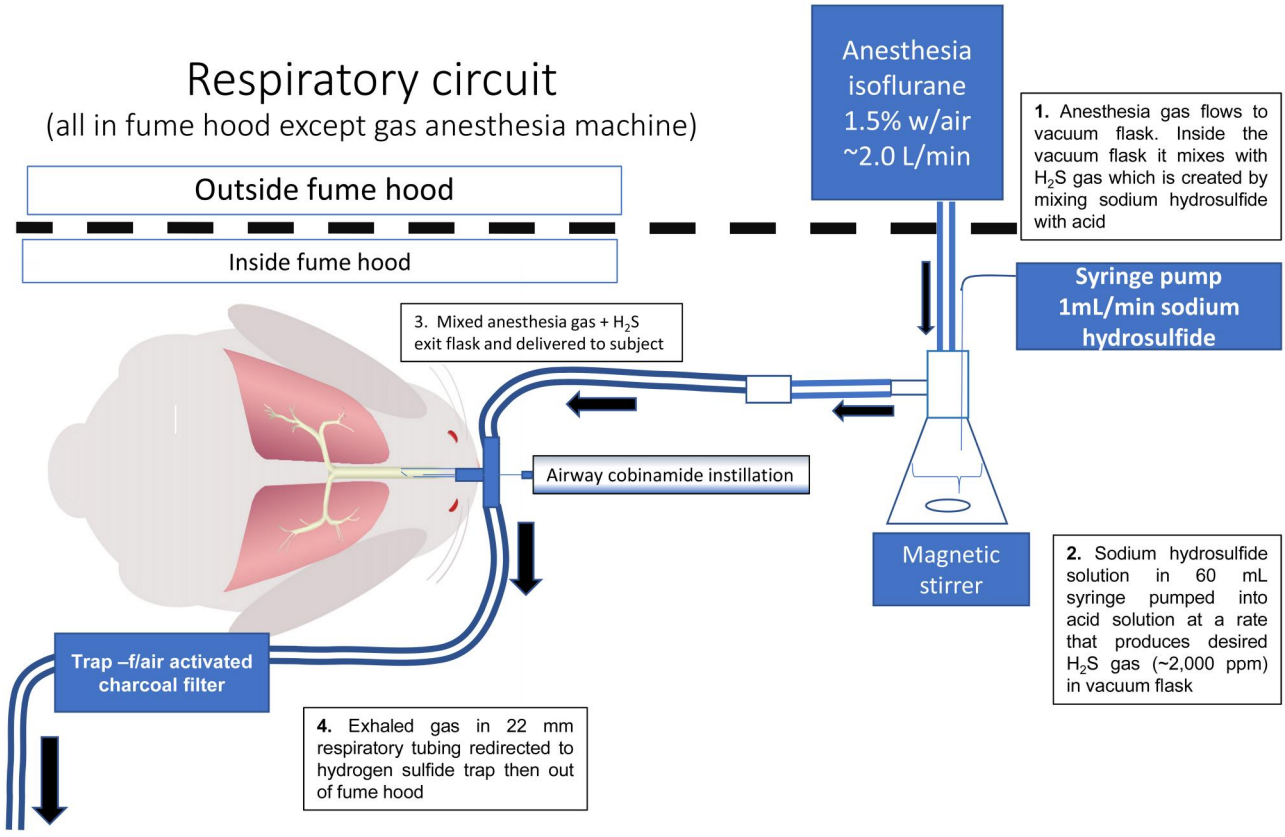

Figure 1. Schematic overview of hydrogen sulfide production and inhalation during experiment.

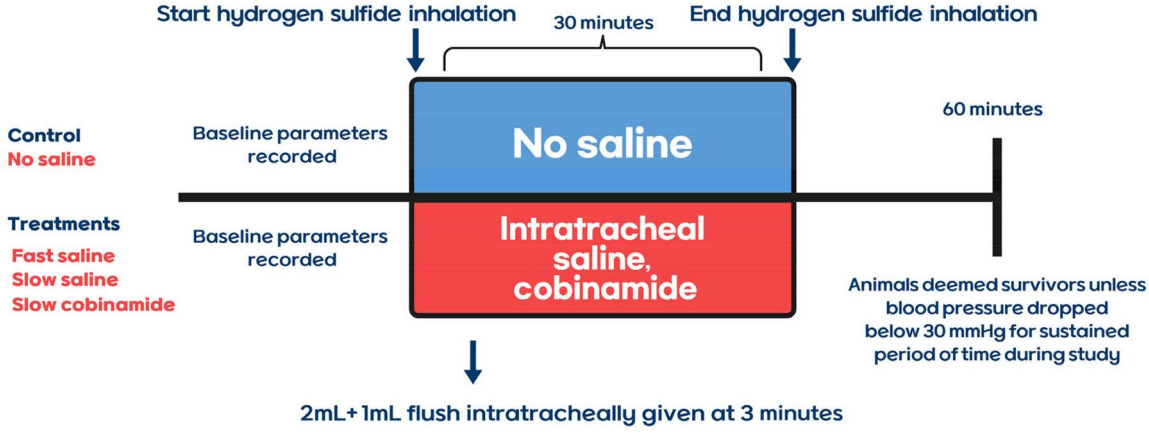

Figure 2. Study design and groups.

Study design

Figure 2 graphically summarizes the study design. After 3 min of H_2S exposure, antidotes (saline or cobinamide) were administered intratracheally, followed by saline flush (1 mL) in the respective animal groups. The administration time was chosen to ensure animals received antidotes before the first apnea. The H_2S gas production rate was increased at 7 min by increasing the sodium hydrosulfide drip rate into the hydrochloric acid reaction vessel to 2 mL/min, and H_2S inhalation continued for 30 min. Animals were sacrificed if systolic blood pressure dropped to less than 30 mmHg over the period of the experiment. Animals were deemed survivors if they did not meet the criteria for sacrifice after 60 min from the start of H_2S exposure.

Animal groups

We studied four animal groups:

- Group 1: No saline control animal group ($n = 15$) received neither fluid treatment (2 mL) nor 1mL saline flush intratracheally.
- Group 2: Slow drip saline animal group ($n = 15$) received a total of 3 mL saline (2 mL as an antidote and 1 mL saline flush). Slow intratracheal administration was completed in 90 seconds or longer.
- Group 3: Fast drip saline animal group ($n = 15$) also received a total of 3 mL saline (2 mL antidote and 1 mL flush). Fast intratracheal administration was completed in less than 15 seconds.

- Group 4: Slow drip cobinamide animal group ($n = 8$) received 2mL cobinamide (200 mM) as an antidote and 1 mL saline flush. Similar to Group 2, slow intratracheal administration was completed in 90 seconds or longer.

The use of an antidote (saline control or cobinamide) was not blinded to the researchers. There were larger numbers of animals in the no saline control group and saline drip (fast and slow) groups because we were surprised by the findings that intratracheal saline appeared more effective than no saline and that the rate at which the saline was instilled in the trachea affected results despite the small volume. So, we studied additional animals in these groups to ensure this was not just a statistical aberration.

Continuous-wave near-infrared spectroscopy

A custom-designed continuous-wave near-infrared spectroscopy fiber optic probe (16 mm source-detector separation) connected to a light source (HL 2000, Ocean Optics, FL) was placed on a shaved portion of the animals' heads to capture continuous cerebral blood information non-invasively. A charged coupled device spectrometer measured light intensity every second at five specific wavelengths (732, 758, 805, 840, 880 nm) in the near-infrared region. Relative changes of deoxyhemoglobin, oxyhemoglobin, and total hemoglobin in cerebral tissue were calculated using a modified Beer-Lamberts' law. All data were processed with MATLAB (Mathworks, MA, USA) software [20]. A more extensive review of continuous-wave near-infrared spectroscopy technology and instrumentation has been previously reported [21]. Continuous-wave near-infrared spectroscopy enabled us to monitor the effects of H_2S toxicity and assess the efficacy of saline and cobinamide treatment in real-time.

Statistics

Data were reported as mean ± standard deviation, or medians with an interquartile range. Prism Statistics Software (Version 9.0, GraphPad, CA) was used for the analysis. For rabbit survival studies, statistical significance was detected using the Log-Rank (Mantel-Cox) test. We used the unpaired t-test with Welch's correction to test the differences in physiological parameters (mean arterial pressure, heart rate, and oxygen saturation) of slow saline and cobinamide drip groups. A P value <0.05 was deemed statistically significant.

Results

A total of 53 rabbits were studied and divided into four groups; fifteen animals in the control group (no saline), fifteen in both the slow and fast drip saline, and eight in the intratracheal cobinamide group. The mean (±SD) weight for all animals ($n = 53$) was 3.87 ± 0.10 kg (Table 1). The survival times and corresponding total sodium hydrogen sulfide dose received before death or termination of the experiment at 60 min determine the effective dose relative to controls for each antidote.

Table 1 summarizes the weight, survival rate, first apnea, and survival time of all animals in the four study groups. There was no significant difference in weight between the groups. The survival rates of the no saline and slow saline groups were 20% and 26.7%, respectively, while the survival rates in the fast saline drip and slow drip cobinamide groups were 60% and 75%, respectively. The median time of survival was 30.6 min for the no saline control group (95% confidence interval (CI) 21.6–39.6), compared to 54.4 min for the slow cobinamide treatment group (95% CI 45.4–63.6). The mean times of the first apnea were not significantly different for all four groups, ranging from 9.3 to 9.8 min (Table 1).

Figure 3 shows significant differences in survivability among four study groups ($P = 0.0087$) (Figure 3(a)). Slow drip saline and no saline control groups were not significantly different ($P = 0.59$) (Figure 3(b)). In contrast, the survivability of the fast saline group was significantly different from the no saline control and was significantly better than that of the slow saline group ($P = 0.04$). Although the slow cobinamide group tended not-significantly toward longer median survival time, the fast saline and slow cobinamide groups were not significantly different in survivability (Figure 3(c)). The survivability of the slow cobinamide group was significantly better than the slow saline groups ($P = 0.021$) (Figure 3(d)).

Mean arterial pressure was measured at one-minute intervals. Mean arterial pressure decreased ~30% from baseline during the first 15 min of H_2S administration.

Figure 4 shows changes in deoxyhemoglobin, oxyhemoglobin, and total hemoglobin in cerebral tissue measured with continuous-wave near-infrared spectroscopy throughout the experiment. In the no saline control animal (Figure 4(a)) and slow drip saline animal (Figure 4(b)), the cardiovascular collapse was especially noticeable with a precipitous drop in oxyhemoglobin and a rise in deoxyhemoglobin concentration as the animal expired. The drop in oxyhemoglobin was especially pronounced after increased H_2S gas concentration at 7 min.

Table 1. Summary of four treatment groups.

Treatment	Numbers of animals	Weight (kg) (mean ± SD)	Survival rate (%)	Apnea onset (min) (mean ± SD)	Survival time (min) (median) (95% CI)
No saline	15	3.86 ± 0.09	20	9.8 ± 1.4	30.6 (21.6 – 39.6)
Slow saline	15	3.93 ± 0.10	26.7	9.4 ± 0.9	34.8 (24.8 – 44.8)
Fast saline	15	3.74 ± 0.08	60	9.6 ± 1.2	46.1 (36.3 – 55.9)
Slow cobinamide	8	4.02 ± 0.12	75	9.3 ± 0.9	54.4 (45.4 – 63.6)

SD: Standard deviation; CI: Confidence interval.

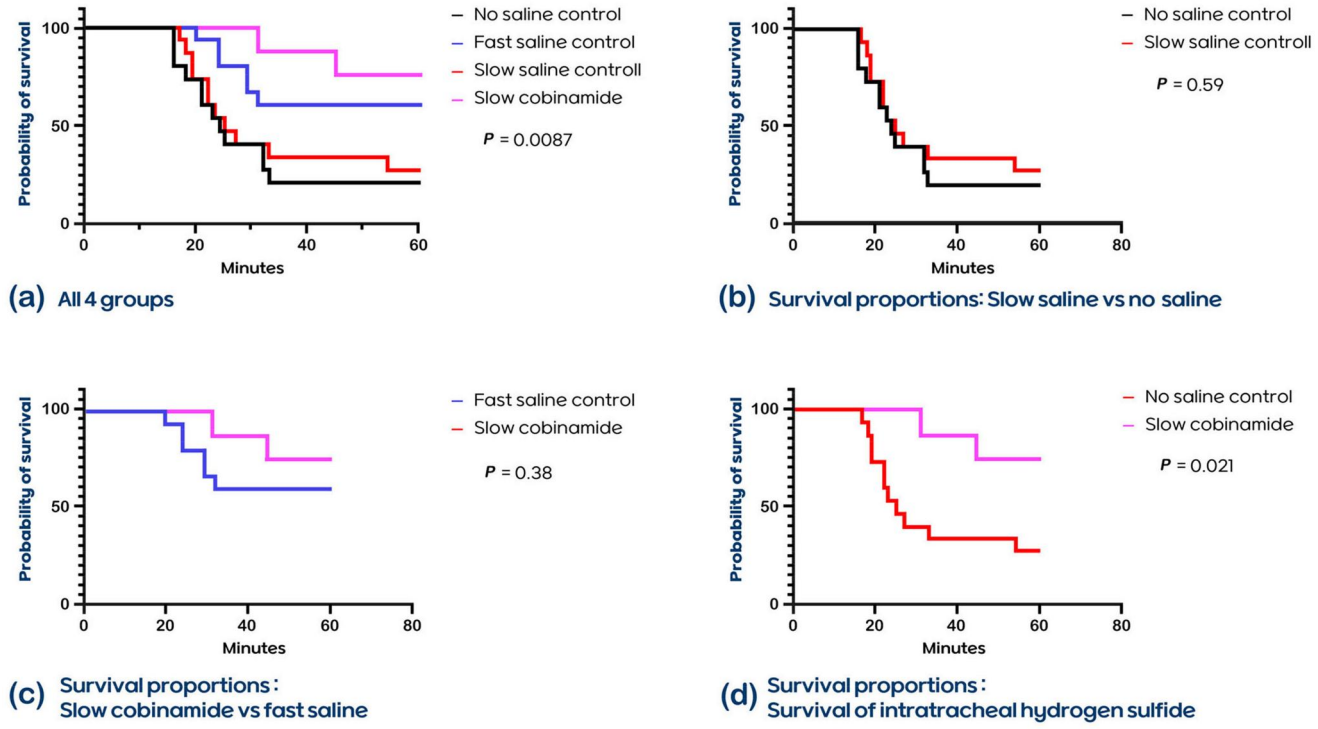

Figure 3. Survival analysis of four study groups.

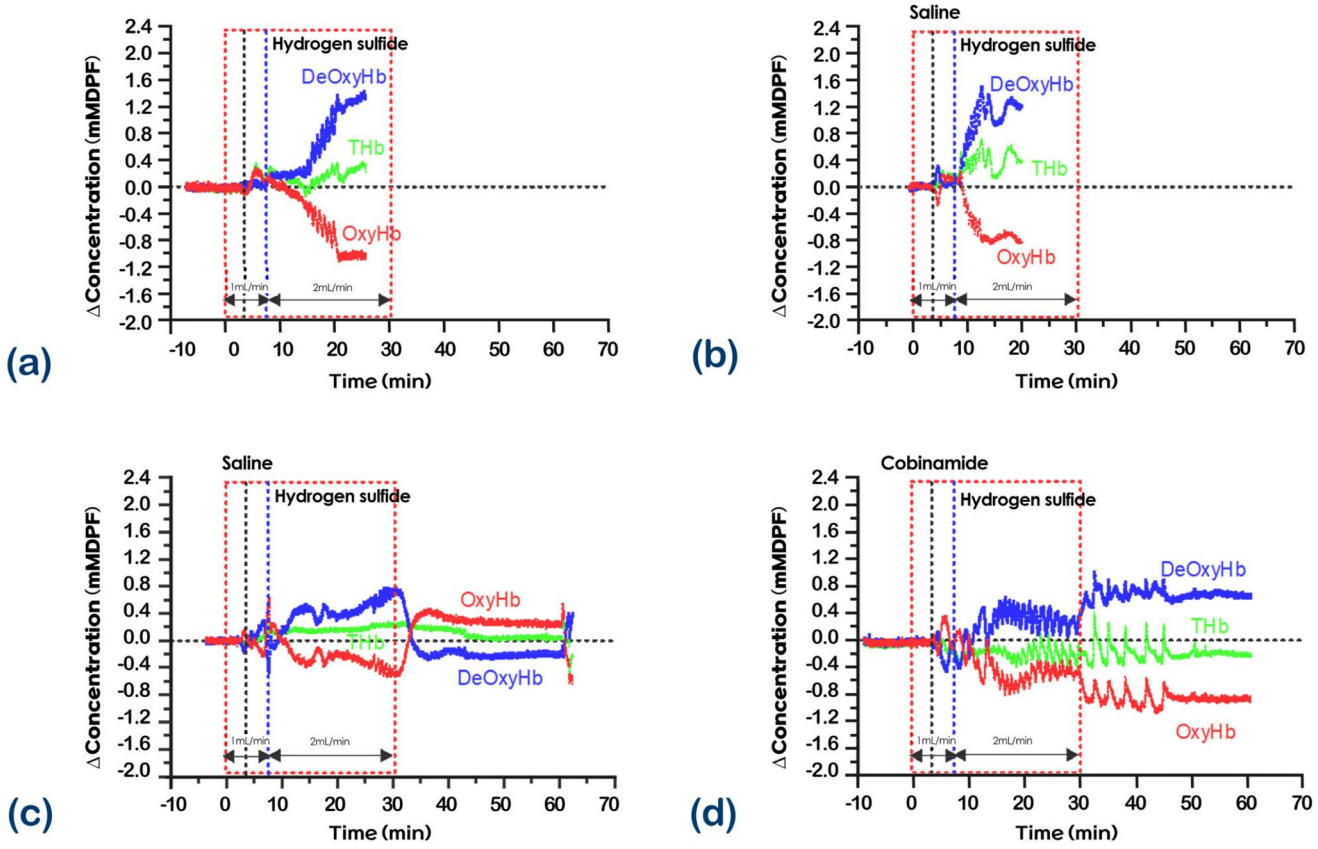

Figure 4. Continuous wave near-infrared spectroscopy measured oxyhemoglobin (OxyHb), deoxyhemoglobin (DeOxyHb), and total hemoglobin (THb) changes during H_2S gas inhalation. (a) no saline control, (b) slow drip saline, (c) fast drip saline, and (d) slow drip cobinamide. Each plot is from a single animal. mM/DPF is the chromophore (hemoglobin) concentration unit in the tissue measured by the continuous-wave near-Infrared spectroscopy (CWNIRS). The differential path length factor (DPF) is the ratio of the mean optical path length the light travels within the tissue to the light source-detector separation distance, which is usually treated as a constant known a priori.

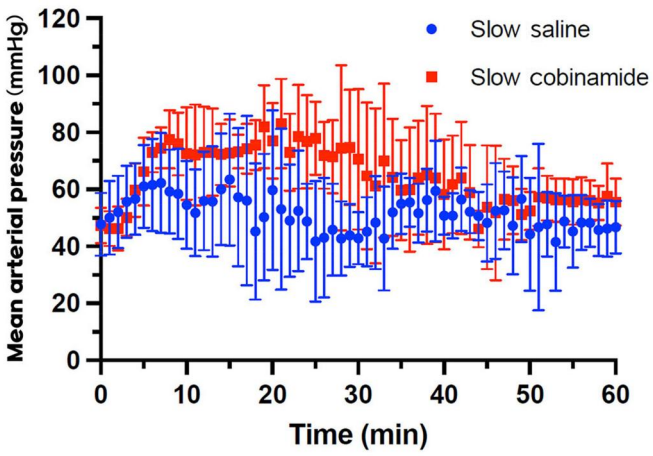

Figure 5. Mean arterial pressure of slow drip cobinamide and slow drip saline groups. Error bars represent lower and upper 95% confidence intervals of mean, respectively.

Unlike control and slow drip saline animals, animals that received fast drip saline (Figure 4(c)) and slow drip cobinamide (Figure 4(d)) did not suffer cardiovascular collapse. The animal with the slow drip cobinamide administration shows multiple episodes of apnea and rebreathing and oxyhemoglobin concentration is trending upward after ~15 min.

Figure 5 compares the mean arterial pressure of the slow-drip saline and cobinamide groups. The slow drip cobinamide group had significantly greater mean (±SD) arterial pressure ($P < 0.0001$), with blood pressure rising to 64.3 ± 9.79 mmHg, while the mean (±SD) arterial pressure of the slow drip saline group decreased to 49.8 ± 4.72 mmHg.

Discussion

In this study, we demonstrated that intratracheally administered cobinamide and fast saline would effectively increase the survivability of animal models exposed to a lethal dose of inhaled H_2S gas. Our results indicate that intra-tracheal cobinamide may provide protection and potential rescue from H_2S toxicity. The significantly improved survivability of the slow cobinamide group over the slow saline group indicates that cobinamide reverses the metabolic poisoning associated with inhibiting mitochondrial electron transport even without any respiratory stimulation [22]. We also showed simple, rapid administration of a small volume of saline was effective in prolonging survival in lethal H_2S gas exposure. Saline is more easily obtainable than cobinamide and can be administered quickly in emergency situations. Saline also has the advantage of having an extremely low possibility of side effects during administration.

In this report, the rapid administration of saline bolus via intratracheal route appears to stimulate the respiratory response in apneic animals and may improve hypoxemia in these animals despite the increased risk of additional H_2S exposure. These findings appear likely due to the rapid bolus stimulating cough and breathing responses in apneic animals, in contrast to the slow dripping of a similar amount of saline. While the stimulation of breathing leads to inhalation of more H_2S in this model, improvement in hypoxemia in these animals appears to be of greater importance than the risk of additional H_2S exposure. Thus, we speculate that the concurrent hypoxemia from H_2S-induced respiratory depression may be a major contributor to mortality in comparison to the metabolic inhibition of cytochromes from H_2S in this scenario. Further studies will be required to determine if this speculation is valid. If confirmed, the implications for therapeutic approaches to victims of H_2S exposure (even those who cannot be evacuated from the exposure site) may need to prioritize methods for stimulating and supporting breathing. Such information may be most useful for treatment at the pre-hospital sites, such as the location of a terror attack or industrial incident site, and during transport to the hospital.

There are a number of limitations to the study. First, this study was conducted on animals, not humans. Second, the sample size in each group was too small to demonstrate a definite survival benefit with cobinamide treatment for H_2S exposure. Third, animals were euthanized after 60 min of long-term survival, and follow-ups were not accounted for. Fourth, because the intervention must be administered before breathing stop, there may be limitations in its application in large-scale terror situations. However, we believe our data provides support to justify the need to explore longer survival times and investigate the possibility of a complete recovery, careful determination of respiratory response to various stimuli, as well as larger studies to determine whether intratracheally administered cobinamide is superior to equivalent volumes of saline, or other methods to stimulate breathing.

Conclusions

We demonstrated that intratracheally administered fast saline and slow cobinamide drips could potentially increase the survivability of rabbits exposed to a lethal dose of inhaled H_2S gas. The rapid administration of saline bolus via intratracheal route appears to stimulate the respiratory response in apneic animals and lead to improvement in hypoxemia in these animals despite the increased risk of additional H_2S exposure. The results and observations from this study should stimulate research into (1) whether it is primarily respiratory stimulation that provides benefit, (2) whether cobinamide alone or in combination with respiratory stimulation is superior, or (3) whether other forms of respiratory stimulation might be superior, or more easily administered.

Disclosure statement

No potential conflict of interest was reported by the authors.

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