Efficacy of Intravenous Hydroxocobalamin for Treatment of Sodium Methanethiolate Exposure in a Swine Model (*Sus scrofa*) of Severe Methanethiol Toxicity

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Abstract

Introduction Methanethiol is a highly toxic chemical present in crude oil and natural gas. At high concentrations, methanethiol causes metabolic acidosis, seizures, myocardial infarction, coma, and death. Occupational Health and Safety Administration lists methanethiol as a potential terrorist weapon. Methanethiol blocks the electron transport chain, resulting in lactic acidosis and acidemia. There is no specific treatment for methanethiol. Our objective was to measure the efficacy of intravenous (IV) hydroxocobalamin (HOC) versus no treatment (control) in methanethiol-induced apnea in a swine model.

Methods Sixteen anesthetized swine received IV sodium methanethiolate to apnea and were randomized to receive either IV HOC or no treatment. Physiologic and laboratory parameters were monitored throughout the study. Power analysis indicated that 8 animals per group would be sufficient to find a moderate effect ($f = 0.24$) with 2 groups, $\alpha = 0.05$, and 80% power.

Results Both groups were similar in baseline characteristics. Following treatment, the HOC group had significantly higher heart rate and blood pressure at 5–10 minutes post-apnea, higher systemic vascular resistance at 5 minutes post-apnea, higher tidal volume, higher end-tidal carbon dioxide, and lower end-tidal oxygen 10–15 minutes post-apnea compared with controls. None of the animals survived to the end of the study (60 minutes). The Kaplan-Meier survival curves were significantly different between cohorts (log-rank $p = 0.0321$), with the HOC group surviving longer than controls (32.4 ± 7.3 vs. 25.8 ± 1.0 minutes).

Conclusions In our model of intravenous methanethiolate poisoning, IV HOC administration resulted in a transient improvement in vital signs and prolonged time to death; however, it did not improve survival.

Keywords Methanethiol · Hydroxocobalamin · Swine · Toxicity · Apnea

Introduction

Methanethiol is a gas which occurs naturally in crude oil, certain foods, various physiologic processes, and flatus

[1]. Methanethiol has an odor threshold of only 0.002 parts per million (ppm) and is added to natural gas as an odorant for the purpose of warning individuals of natural gas leaks [2]. Methanethiol is also used in farming, in mining operations, and in the production of methionine, a dietary component in poultry and animal feed [2]. In 2014, four people were killed when methanethiol was released at a Texas plant [3]. Accidental release during transportation has also been reported [4]. According to the Occupational Health and Safety Administration (OSHA) and the US Environmental Protection Agency (EPA), methanethiol is highly toxic and has been associated with workplace injuries and deaths [4, 5]. Exposure to a low dose of 5 ppm of methanethiol can produce difficulty breathing, agitation, confusion, nausea, vomiting, and heart palpitations. At higher levels, it is lethal and can cause central apnea, hypotension,

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myocardial infarction, unconsciousness, seizures, and acidemia [1]. Moreover, OSHA lists methanethiol as a potential terrorist weapon [6].

Methanethiol has a similar chemical structure to another potentially fatal gas, hydrogen sulfide (H_2S), with both chemicals possessing a thiol group [7, 8]. Additionally, both methanethiol and hydrogen sulfide induce central apnea, metabolic acidosis, seizures, myocardial infarction, coma, and death [1, 9–11]. Currently, there is no antidote specifically for hydrogen sulfide or methanethiol poisoning [1, 10]. However, several studies have demonstrated hydroxocobalamin (HOC) to be an effective therapy for hydrogen sulfide, presumably due to the ability of HOC to bind to the sulfide molecule [10–12]. Cobinamide, which is chemically similar to HOC, has also been effective against H_2S poisoning and binds well to hydrogen sulfide [12]. Given that the structure and mechanism of action of methanethiol is similar to hydrogen sulfide (H_2S), we postulate that the pharmacologic treatment for toxicity may be similar. HOC is available in the USA for treatment of cyanide toxicity and its effectiveness has been demonstrated in cases of cyanide poisoning [13–15]. Moreover, due to its safety and efficacy, it can be administered in pre-hospital and hospital settings and may be available to emergency response personnel or physicians treating a methanethiol-poisoned patient [14, 16, 17].

The purpose of our study was to determine the efficacy of intravenous (IV) HOC versus control (no treatment) for improving survival in a lethal methanethiol animal model. We compared the physiologic parameters, laboratory values, and duration of survival of the two study arms.

Methods

Materials

We used intravenous sodium methanethiolate as an alternative, because methanethiol exists only as a gas and administration to a large animal without exposure to research personnel would not be possible at our facility. Sodium methanethiolate was prepared at a concentration of 60 mg/mL (a salt; CAS Number: 5188-07-8; Sigma Aldrich, 281018-25G, St. Louis, MO). Sodium methanethiolate is water soluble so it can be infused using saline. Sodium methanethiolate is a viable alternative given the compound is a mercaptan, has a similar structure to methanethiol, and possesses the same mechanisms of toxicity (central apnea and cytochrome c inhibition) [18–20].

Study Design and Setting

We conducted a randomized controlled trial in a large animal swine model. The protocol was approved by the 59th Medical

Wing (MDW) Institutional Animal Care and Use Committee (IACUC). All procedures in the study were conducted at the Clinical Investigations and Research Support (CIRS) Division. The study was conducted in accordance 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 the Accreditation of Laboratory Animal Care. The animals were housed at the 59th MDW/CIRS vivarium. The study was funded by the Office of the Surgeon General – Graduate Medical Education Division.

Model Development

Because there is no previously validated methanethiol poisoning swine model, we used 10 experiment development animals to determine the concentration of sodium methanethiolate needed to produce apnea without producing immediate death. There was significant variability between swine in the dose of sodium methanethiolate necessary to produce apnea. Furthermore, decreasing the dose following apnea resulted in a return of spontaneous respirations. We found that a starting dose of 3.5 mg/kg/minute for 5 minutes, titrated up by 10% every minute until apnea (20 s), and then continued at that terminal dose would result in a transient recurrence of spontaneous respiration, but reliably produce death over a range of 20–30 minutes.

Animal Subjects and Preparation

On the day of the experiment, prior to the arrival of the swine to the surgical room, they were randomly assigned to the control or treatment arm using the randomization application <http://www.randomization.com>. Yorkshire swine (*Sus scrofa*) weighing between 65 and 85 kg were fasted overnight except for water ad libitum. Prior to induction of anesthesia, all animals were sedated with ketamine at an intramuscular dose of 10 mg/kg, and endotracheal intubation was performed. Mechanical ventilation was commenced and adjusted to maintain the arterial partial pressure of carbon dioxide (PCO_2) between 38 and 42 mmHg using a volume-limited, time-cycled ventilator (Dräger-Siemens, Fabius GS anesthesia machine, New York City, NY). During the placement of catheters, isoflurane was administered in a range between 1 and 3.5%. Arterial and venous access was obtained by cut-down to isolate one internal and two external jugular veins, a carotid artery, and a femoral artery. A Swan-Ganz CCombo V pulmonary artery catheter (777F8, Edwards Life science, Irvine, CA) was placed in the right external or internal jugular to measure central venous and pulmonary artery pressures. Once all lines were placed, isoflurane was maintained between 1 and 2% to mitigate isoflurane-induced hypotension and apnea. Electrocardiogram (ECG) electrodes

were placed for continuous monitoring of heart rate and rhythm. A Foley catheter was placed in the bladder of females, and a suprapubic catheter for males, for urine collection, preventing exposure of lab personnel.

All animals received a bolus of 15 mL/kg of warm normal saline intravenously followed by intravenous heparin (100 units/kg). Arterial pressure was measured continuously through the femoral artery (Dräger Infinity HemoMed Pod). At this point, animals were acclimated and the blood pressure stabilized (at least 10 minutes). Animal temperature was maintained between 37.5 and 39 °C using heating adjuncts as needed (warmed induction and operating room, warm intravenous fluid bolus, bed warmer during the procedure, and warming blankets). The fraction of inspired oxygen (FiO₂) was maintained at 0.40. The Fabius GS's embedded anesthesia data collection software was used for data acquisition at 1-minute intervals. Following animal stabilization, baseline blood samples were collected for serum biochemical analysis. Baseline biochemical measurements included oxygen saturation, PaO₂, PaCO₂, hemoglobin (Hgb), pH, bicarbonate, base excess, and lactate, which were collected in a manner reported in prior publications (ABL 800 Flex blood gas analyzer, Radiometer America, Westlake, OH) [11, 21–24].

Experimental Procedures

Following instrumentation and acclimatization, anesthesia was adjusted until the animals were breathing spontaneously with a FiO₂ of 0.21 (room air) without mechanical ventilation. The animals were observed (with appropriate intervention as necessary) until they could maintain SpO₂ > 90% and respiratory rate between 25 and 35 breaths per minute.

Sodium methanethiolate (Sigma Aldrich, 281018-25G, St. Louis, MO) was prepared immediately preceding each experiment by dissolving it in sterile saline at a concentration of 60 mg/mL. The sodium methanethiolate was administered to each of the 16 protocol animals. Following 20 s of apnea confirmed by capnography, each swine was treated with either intravenous HOC (150 mg/kg infused over 6 minutes) or no treatment (control) (Fig. 1) based upon prior randomization. In

our previous studies using smaller animals (approximately 45–55 kg), we administered 180 mL over 3 minutes [21]. Due to the increase in the weight range for this study, and to keep the solubility at 50 mg/mL, we mixed HOC in 240 mL of normal saline to be administered over 6 minutes. Physiologic parameters were collected every minute including heart rate (HR), mean arterial pressure (MAP), mixed venous saturation (SVO₂), cardiac output (CO), and pulmonary artery pressures. Blood samples for laboratory analysis were collected at baseline, 5 minutes post-infusion of sodium methanethiolate, at apnea + 1 minute (treatment), 2 minutes post-treatment, and every 10 minutes following treatment. The end of study (EOS) time point was anticipated to be 60 minutes following treatment; however, since none of the animals survived to 60 minutes post-treatment, EOS was collected as the last time point prior to euthanasia for each animal.

Once a MAP of < 30 mmHg was reached, a 10-minute period was observed before animals were euthanized. Euthanasia (Sodium Pentobarbital 100 mg/kg, VetOne, MWI Animal Health, Boise, ID) was accomplished by the CIRS veterinarian, veterinary technologists, or trained surgical technicians in accordance with the American Veterinary Medical Association Panel on euthanasia guidelines.

Methods of Measurement and Outcome Measures

Primary Outcome Variable

The primary outcome was survival at 60 minutes after treatment.

Secondary Outcome Variables

Secondary outcome parameters included cardiac output, heart rate, mixed venous oxygenation, pH, bicarbonate, base excess, and lactate. Blood pressure and heart rate were measured every minute from the initiation of the sodium methanethiolate infusion until 60 minutes after treatment. Cardiac output and other invasive parameters were measured

Fig. 1 Experimental design: timeline indicating sequence of events during experimental procedures.

Table 1 Comparison of baseline characteristics, sodium methanethiolate dosage, and time to apnea.

Variable	HOC (<i>n</i> = 8)	Control (<i>n</i> = 8)	Difference (95% CI)	<i>p</i>
Weight, kg	75.50 (7.21)	74.63 (6.37)	0.87 (− 6.42, 8.17)	0.8007
Height, in.	64.25 (2.38)	63.63 (2.77)	0.62 (− 2.14, 3.39)	0.6359
BSA	1.80 (0.08)	1.79 (0.12)	0.01 (− 0.09, 0.12)	0.7302
mL to apnea	49.08 (26.90)	45.76 (24.15)	3.32 (− 24.10, 30.72)	0.7993
mg to apnea	3006.00 (1733.35)	2745.75 (1448.78)	260.25 (− 1513.60, 2034.10)	0.7563
mg/kg to apnea	39.36 (19.09)	36.71 (18.00)	2.65 (− 18.05, 23.35)	0.7866
Time to apnea, s	548.13 (180.95)	537.75 (186.77)	10.38 (− 186.80, 207.60)	0.9118

Values given are mean (standard deviation); *p* values are significant at < 0.05. *CI*s, confidence intervals; *kg*, kilogram; *in.*, inch; *BSA*, body surface area; *mL*, milliliters; *mg*, milligrams; *HOC*, hydroxocobalamin; *s*, seconds

every 5 minutes from the start of the sodium methanethiolate infusion until 60 minutes after treatment.

Data Analysis

We calculated descriptive statistics for animal size and sodium methanethiolate administered using means and standard deviations and compared those variables between groups using independent samples *t* tests (after evaluating variables for normality using quantile plots, skewness, and kurtosis). To compare vital signs and biochemical measurements over time, we used a mixed model repeated measures analysis of variance (RMANOVA) with treatment (HOC vs. control) as a between-subjects factor and 5–9 repeated measurements over time (depending on the variable). Post hoc multiple comparisons included the Tukey-Kramer adjustment to confidence intervals and *p*-values. We compared survival times using Kaplan-Meier survival curves and log-rank *p* value. Results were considered statistically significant at a *p* value of < 0.05. All statistical analyses were conducted in SAS version 9.4 (Cary, NC).

Fig. 2 Survival curve of HOC vs. control animals.

Power Analysis

A power analysis indicated that 8 animals per group (16 total) would be sufficient to find a moderate effect ($f = 0.24$) in a mixed model RMANOVA with 2 groups, 9 time points, an alpha of 0.05, and 80% power. A sample of 8 animals per group was determined to be sufficient to find a hazard ratio of 0.25 in a survival analysis with an alpha of 0.05 and 80% power.

Results

Characteristics of Study Subjects

Baseline characteristics for the two groups were similar, including height, size, body surface area (BSA), amount of sodium methanethiolate received prior to apnea, time to apnea, and duration of breathing post-apnea (Table 1).

Primary Outcome

None of the animals survived to the end of the study period (60 minutes). The Kaplan-Meier survival curves demonstrated a difference in survival time between the HOC and the control group (32.4 ± 7.3 minutes vs. 25.8 ± 1.0 minutes; log-rank $p = 0.0321$; Fig. 2). Given that methanethiol was continuously infused until death, the HOC group received more total sodium methanethiolate prior to death ($11,145 \pm 1943$ mg) compared with the control group (7560 ± 1602.28 mg) (mean difference 3585 mg, 95% CI 1668–5501 mg). The average duration of continued spontaneous respiration following the initial 20 s of apnea was 359.33 ± 185.80 s for the HOC cohort, compared with 288.71 ± 74.12 for controls.

Secondary Outcomes

Compared with the HOC group, the control group had significantly lower heart rate (HR), systolic blood

Table 2 Comparison of hemodynamic and biochemical parameters over time between the HOC vs. control animals.

Variable	Time point	HOC (<i>n</i> = 8)	Control (<i>n</i> = 8)	Difference (95% CI)
HR	Baseline	97.00 (22.72)	100.88 (12.81)	− 3.88 (− 67.12, 59.37)
	Sodium methanethiolate infusion	98.13 (22.70)	102.13 (12.61)	− 4.00 (− 67.24, 59.24)
	5 minutes post-infusion	107.00 (26.02)	105.86 (11.52)	1.14 (− 66.47, 68.75)
	Apnea	102.25 (26.61)	98.38 (12.81)	3.88 (− 59.37, 67.12)
	1 minute post-apnea	96.63 (30.04)	97.38 (13.08)	− 0.75 (− 63.99, 62.49)
	5 minutes	147.71 (34.86)	81.63 (36.18)	66.09 (0.63, 131.55)
	10 minutes	137.43 (59.89)	44.63 (57.32)	92.80 (27.34, 158.27)
	15 minutes	118.86 (57.27)	40.50 (53.97)	78.36 (− 0.92, 157.64)
	20 minutes	72.40 (64.29)	13.00 (0)	59.40 (− 79.16, 197.96)
SBP	Baseline	121.63 (9.07)	117.50 (13.51)	4.13 (− 44.36, 52.61)
	Sodium methanethiolate infusion	123.38 (7.23)	118.25 (13.53)	5.13 (− 43.36, 53.61)
	5 minutes post-infusion	122.29 (11.61)	116.00 (13.83)	6.29 (− 45.55, 58.12)
	Apnea	79.25 (34.47)	65.75 (27.25)	13.50 (− 34.99, 61.99)
	1 minute post-apnea	71.38 (32.73)	57.25 (23.46)	14.13 (− 34.36, 62.61)
	5 minutes	113.50 (54.33)	57.13 (24.67)	56.38 (7.89, 104.86)
	10 minutes	95.57 (44.17)	24.88 (26.33)	70.70 (20.51, 120.89)
	15 minutes	63.57 (33.17)	11.00 (3.32)	52.57 (− 4.21, 109.36)
	20 minutes	31.33 (22.62)	14.00 (0)	17.33 (− 87.41, 122.08)
DBP	Baseline	75.63 (8.93)	72.25 (13.49)	3.38 (− 22.80, 29.55)
	Sodium methanethiolate infusion	77.00 (7.46)	72.75 (13.45)	4.25 (− 21.92, 30.42)
	5 minutes post-infusion	69.71 (9.66)	65.43 (13.55)	4.29 (− 23.69, 32.26)
	Apnea	40.38 (16.41)	34.50 (12.78)	5.88 (− 20.30, 32.05)
	1 minute post-apnea	35.00 (11.96)	29.50 (8.93)	5.50 (− 20.67, 31.67)
	5 minutes	65.13 (34.34)	24.75 (7.01)	40.38 (14.20, 66.55)
	10 minutes	50.00 (22.71)	12.38 (6.28)	37.63 (10.54, 64.71)
	15 minutes	32.71 (14.60)	9.80 (2.59)	22.91 (− 7.73, 53.56)
	20 minutes	19.50 (10.88)	12.00 (0)	7.50 (− 49.04, 64.04)
MAP	Baseline	93.13 (8.85)	89.63 (12.75)	3.50 (− 29.06, 36.06)
	Sodium methanethiolate infusion	94.50 (6.76)	90.75 (12.62)	3.75 (− 28.81, 36.31)
	5 minutes post-infusion	89.71 (9.34)	85.43 (12.16)	4.29 (− 30.52, 39.10)
	Apnea	55.25 (21.45)	47.25 (18.24)	8.00 (− 24.56, 40.56)
	1 minute post-apnea	48.50 (18.34)	40.63 (14.83)	7.88 (− 24.69, 40.44)
	5 minutes	82.75 (40.66)	35.50 (12.20)	47.25 (14.69, 79.81)
	10 minutes	67.86 (29.35)	15.38 (10.00)	52.48 (18.78, 86.19)
	15 minutes	44.57 (21.76)	10.20 (2.59)	34.37 (− 3.76, 72.50)
	20 minutes	24.00 (15.57)	13.00 (0)	11.00 (− 59.34, 81.34)
etCO ₂	Baseline	52.38 (7.09)	58.13 (5.94)	− 5.75 (− 31.88, 20.38)
	Sodium methanethiolate infusion	52.13 (7.55)	57.75 (5.85)	− 5.63 (− 31.76, 20.51)
	5 minutes post-infusion	49.43 (7.02)	54.29 (6.37)	− 4.86 (− 32.79, 23.08)
	Apnea	41.38 (13.08)	41.38 (14.75)	0.00 (− 26.13, 26.13)
	1 minute post-apnea	26.38 (13.74)	32.25 (16.34)	− 5.88 (− 32.01, 20.26)
	5 minutes	42.63 (22.30)	39.88 (18.25)	2.75 (− 23.38, 28.88)
	10 minutes	45.14 (18.04)	11.38 (19.36)	33.77 (6.72, 60.82)
	15 minutes	47.57 (20.63)	3.00 (2.35)	44.57 (13.97, 75.18)
	20 minutes	37.40 (25.76)	2.00 (0)	35.40 (− 21.86, 92.66)
etO ₂	Baseline	16.38 (1.60)	14.13 (1.73)	2.25 (− 4.93, 9.43)
	Sodium methanethiolate infusion	16.75 (2.12)	14.63 (1.69)	2.13 (− 5.06, 9.31)
	5 minutes post-infusion	16.86 (2.34)	15.86 (1.21)	1.00 (− 6.68, 8.68)
	Apnea	19.25 (3.96)	19.50 (5.15)	− 0.25 (− 7.43, 6.93)

Table 2 (continued)

Variable	Time point	HOC (<i>n</i> = 8)	Control (<i>n</i> = 8)	Difference (95% CI)
SVR	1 minute post-apnea	23.13 (4.29)	22.13 (5.62)	1.00 (− 6.18, 8.18)
	5 minutes	18.38 (6.16)	22.38 (4.44)	− 4.00 (− 11.18, 3.18)
	10 minutes	17.57 (6.08)	28.00 (3.12)	− 10.43 (− 17.86, − 2.99)
	15 minutes	19.71 (5.50)	30.00 (2.55)	− 10.29 (− 18.70, − 1.88)
	20 minutes	21.33 (4.46)	29.00 (0)	− 7.67 (− 23.18, 7.85)
	Baseline	920.38 (274.52)	749.57 (175.33)	170.80 (− 202.89, 544.50)
	Sodium methanethiolate infusion	926.25 (251.92)	753.43 (181.19)	172.82 (− 200.88, 546.52)
	5 minutes post-infusion	784.71 (147.25)	658.50 (150.87)	126.21 (− 275.50, 527.93)
	Apnea	446.50 (202.12)	296.00 (151.17)	150.50 (− 223.20, 524.20)
	1 minute post-apnea	414.43 (143.27)	247.00 (153.85)	167.43 (− 218.52, 553.38)
Tve	5 minutes	753.14 (313.68)	236.83 (59.22)	516.31 (114.60, 918.02)
	10 minutes	513.57 (311.83)	162.00 (96.17)	351.57 (− 227.36, 930.50)
	Baseline	407.50 (92.65)	352.88 (52.53)	54.63 (− 382.74, 491.99)
	Sodium methanethiolate infusion	413.25 (74.98)	340.50 (48.90)	72.75 (− 364.62, 510.12)
	5 minutes post-infusion	507.29 (110.84)	458.86 (91.06)	48.43 (− 419.14, 516.00)
	Apnea	245.50 (189.56)	333.13 (270.19)	− 87.63 (− 524.99, 349.74)
	1 minute post-apnea	239.86 (307.51)	230.57 (279.72)	9.29 (− 458.28, 476.85)
	5 minutes	715.43 (372.98)	935.43 (367.25)	− 220.00 (− 687.57, 247.57)
	10 minutes	1025.50 (328.08)	234.00 (459.37)	791.50 (226.86, 1356.14)
	15 minutes	791.33 (399.76)	0.00 (0.00)	791.33 (77.11, 1505.55)
pH	Baseline	7.42 (0.03)	7.46 (0.02)	− 0.03 (− 0.14, 0.07)
	5 minutes post-infusion	7.40 (0.07)	7.42 (0.03)	− 0.01 (− 0.13, 0.10)
	1 minute post-apnea	7.37 (0.09)	7.37 (0.05)	0.00 (− 0.10, 0.10)
	10 minutes	7.30 (0.08)	7.32 (0.08)	− 0.02 (− 0.13, 0.10)
	20 minutes	7.24 (0.08)	7.25 (0.01)	− 0.01 (− 0.16, 0.14)
K	Baseline	3.99 (0.20)	4.13 (0.32)	− 0.14 (− 0.93, 0.66)
	5 minutes post-infusion	4.23 (0.18)	4.05 (0.62)	0.18 (− 0.71, 1.06)
	1 minute post-apnea	5.23 (0.69)	5.34 (0.45)	− 0.11 (− 0.91, 0.68)
	10 minutes	5.10 (0.37)	6.10 (0.56)	− 1.00 (− 1.88, − 0.12)
	20 minutes	4.53 (0.27)	5.53 (1.07)	− 1.00 (− 2.12, 0.12)
Lactate	Baseline	1.24 (0.23)	1.22 (0.35)	0.02 (− 3.36, 3.39)
	5 minutes post-infusion	1.46 (0.22)	1.05 (0.21)	0.41 (− 3.34, 4.17)
	1 minute post-apnea	4.88 (2.72)	6.29 (4.84)	− 1.41 (− 4.79, 1.96)
	10 minutes	6.59 (0.94)	9.90 (0.97)	− 3.31 (− 7.07, 0.44)
	20 minutes	7.97 (0.93)	8.77 (0.92)	− 0.80 (− 5.57, 3.97)
Methgb	Baseline	1.01 (0.87)	1.01 (0.64)	0.00 (− 3.62, 3.62)
	5 minutes post-infusion	0.97 (0.46)	0.97 (0.49)	0.00 (− 4.02, 4.03)
	1 minute post-apnea	2.61 (2.87)	2.34 (2.10)	0.28 (− 3.35, 3.90)
	10 minutes	6.79 (2.78)	4.30 (2.60)	2.49 (− 1.54, 6.51)
	20 minutes	12.97 (4.21)	4.10 (0.53)	8.87 (3.75, 13.99)

Values shown correspond to the mean $\pm$ standard deviation for heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), end-tidal CO₂ (etCO₂), end-tidal O₂ (etO₂), systemic vascular resistance (SVR), tidal volume (T_{VE}), K (potassium), and Methgb (methemoglobin)

pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) at 5–10 minutes post-apnea, lower systemic vascular resistance (SVR) at 5 minutes post-apnea, and lower tidal volume (T_{VE}) 10–15 minutes post-

Fig. 3 Hemodynamic variables obtained throughout the experiment for the HOC (solid line) and control (dotted line) animals. Values shown correspond to the mean $\pm$ standard deviation for **a**) heart rate (HR), **b**) systolic blood pressure (SBP), **c**) diastolic blood pressure (DBP), **d**) mean arterial pressure (MAP), **e**) tidal volume (TVE), **f**) systemic vascular resistance (SVR), **g**) end tidal CO₂ (etCO₂) and **h**) end tidal O₂ (etO₂); statistical significance shown as * corresponding to a $p < 0.05$

apnea (Table 2; Fig. 3a–f). The control group also had lower end-tidal CO_2 (etCO_2) and higher end-tidal O_2 (etO_2) at 10–15 minutes post-apnea (Table 2; Fig. 3g, h). The control group had higher potassium at the end of the study, though the two groups did not differ in pH or lactate (Table 2; Fig. 4a–c). The levels of methemoglobin were different between both cohorts at 20 minutes post-apnea, with a continuous increase from baseline for each cohort (Table 2; Fig. 4d). No differences in complete blood count (CBC) parameters were found between cohorts (data not shown).

Discussion

In our study of HOC for the treatment of acute methanethiol toxicity, we found that administration of HOC during continuous infusion of intravenous sodium methanethiolate prolonged survival but did not prevent death. The treated animals' vital signs improved during the infusion of HOC to the extent of prolonging time to death by approximately 6 minutes compared with the control cohort. It may be that a continuous infusion and/or a

larger dose of HOC may further prevent apnea and prolong survival during methanethiol poisoning.

Given the similarities in the structure and mechanism of poisoning between hydrogen sulfide and methanethiol, we elected to determine the efficacy of HOC in the treatment of sodium methanethiolate toxicity. Intravenous HOC prolonged survival of sodium methanethiolate poisoned animals; however, it did not prevent death during the continued infusion. The use of HOC in an apnea model is likely similar to when the therapy could be conceivably used in a clinical scenario, such as an industrial accident or a terrorist attack.

In our model, we provided a continuous infusion of methanethiolate following the administration of the HOC. Given that inhalation exposure is the cause of methanethiol toxicity, treatment of methanethiol-poisoned patients should focus on immediate removal from the exposure location, respiratory support, and current Advanced Cardiac Life Support protocols. While rapid removal from the poisoned environment is a viable therapy, this may not be feasible in all circumstances or sufficient in cases of severe poisoning. Therefore, the administration of intravenous HOC in conjunction with removal and supportive care may improve survival.

Fig. 4 ABG values obtained from blood samples collected at different time points for the HOC (solid line) and control (dotted line) animals. Values shown correspond to the mean $\pm$ standard deviation for **a)**

potassium (K), **b)** pH, **c)** lactate and **d)** methemoglobin (Methgb); statistical significance shown as * corresponding to a $p < 0.05$

The specific mechanism of action of HOC in this model of methanethiol poisoning is unclear. HOC may bind methanethiol directly to detoxify it. Conversely, HOC's regulation of nitric oxide synthase may provide a vasopressor effect, transiently improving blood pressure (BP) and HR and prolonging survival [23]. Our future study efforts will include attempts to measure the binding of HOC and methanethiol. While HOC did transiently improve vital signs (HR, BP, and SVR) and other physiologic parameters, we found no difference in mortality. Even though animals in the HOC group received additional saline as the HOC vehicle, we do not consider additional volume of saline (240 mL) to be a sufficient driver of the slight improvement in vital signs that cohort exhibited compared with control. The increase in methemoglobin observed for both the control and the HOC groups could be partially explained by the insult caused by sodium methanethiolate [25]. However, the higher concentrations of methemoglobin observed in the HOC cohort may be due to the administration of HOC which has been previously reported in a human case report [26].

Compared with our previous studies evaluating cyanide and hydrogen sulfide poisoning in a similar swine model, animals in this study did not develop high blood lactate concentrations prior to apnea. This may suggest a direct effect on the respiratory center of the brain as the primary cause of apnea and the ultimate cause of death in methanethiol poisoning.

Limitations

Our study had several limitations. The first was the need to use intravenous sodium methanethiolate as an alternative to inhaled methanethiol due to laboratory capabilities. However, the clinical course of the poisoning is consistent with methanethiol poisoning. The second limitation was the need to use an animal model instead of using human subjects, as humans could react differently than swine to specific toxins. For ethical reasons, this is permitted by the Food and Drug Administration Animal Efficacy Rule, which allows for the development and testing of xenobiotics to reduce or prevent serious and life-threatening conditions caused by exposure to toxic agents. Third, while HOC is known to bind cyanide, knowledge regarding its ability to bind to thiols is still limited [10, 11]. Fourth, during treatment, animals in the HOC group received a volume of 240 mL of HOC dissolved in saline; this volume of saline was not administered to the control group. At the time of the experiment, we did not consider volume to have a significant impact on the outcome of the experiment; however, future studies will include treating control animals with corresponding volumes of the vehicle. Finally,

the study was not blinded; however, all data collected were objective and recorded via automated computer data algorithms or laboratory analysis, limiting subjective interpretations.

Conclusions

In our large swine model of continuous intravenous methanethiol poisoning, intravenous HOC administration resulted in a transient improvement in vital signs and prolonged time to death; however, it did not improve survival. Further investigation should determine if intravenous HOC administration in combination with termination of or reduction in methanethiol exposure improves survival.

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Compliance with Ethical Standards

The protocol was approved by the 59th Medical Wing (MDW) Institutional Animal Care and Use Committee (IACUC). The study was conducted in accordance 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 the Accreditation of Laboratory Animal Care.

Disclaimer The views expressed are those of the authors and do not reflect the official views or policy of the Department of Defense or its Components.

Conflicts of Interest None.

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