HHS Public Access

Author manuscript

*Ann Emerg Med*. Author manuscript; available in PMC 2018 June 01.

Published in final edited form as:

*Ann Emerg Med*. 2017 June ; 69(6): 718–725.e4. doi:10.1016/j.annemergmed.2016.09.034.

# SODIUM NITRITE AND SODIUM THIOSULFATE ARE EFFECTIVE AGAINST ACUTE CYANIDE POISONING WHEN ADMINISTERED BY INTRAMUSCULAR INJECTION

**Vikhyat S. Bebarta**[#1], **Matthew Brittain**[#2], **Adriano Chan**[3], **Norma Garrett**[4], **David Yoon**[5], **Tanya Burney**[5], **David Mukai**[5], **Michael Babin**[2], **Renate B. Pilz**[3], **Sari B. Mahon**[5], **Matthew Brenner**[5], and **Gerry R. Boss**[3]

[1]Department of Emergency Medicine, University of Colorado, School of Medicine, Aurora, CO

[2]Battelle Memorial Institute, Columbus, OH

[3]Department of Medicine, University of California, San Diego, La Jolla, CA

[4]Medical Toxicology and the Department of Emergency Medicine, San Antonio Military Medical Center/59th MDW, San Antonio, TX

[5]Beckman Laser Institute and Medical Clinic, University of California, Irvine, Irvine, CA

[#] These authors contributed equally to this work.

## Abstract

**Study Objective—**The two antidotes for acute cyanide poisoning in the United States must be given by intravenous injection. In the pre-hospital setting, intravenous injection is not practical, particularly for mass casualties, and intramuscular injection would be preferred. The purpose of this study was to determine if sodium nitrite and sodium thiosulfate are effective cyanide antidotes when given by intramuscular injection.

**Methods—**We used a randomized, non-blinded, parallel group study design in three mammalian models: cyanide gas inhalation in mice, with treatment post exposure; intravenous sodium cyanide infusion in rabbits, with severe hypotension as the trigger for treatment; and intravenous potassium cyanide infusion in pigs, with apnea as the trigger for treatment. The drugs were administered by intramuscular injection, and all three models were lethal in the absence of therapy.

**Results—**We found that sodium nitrite and sodium thiosulfate individually rescued 100% of the mice, and that the combination of the two drugs rescued 73% of the rabbits and 80% of the pigs.

Corresponding author: Gerry R. Boss, M.D., gboss@ucsd.edu; Tel: (858) 534-8805; FAX: (858) 534-1421.



Author contributions: GRB conceived the study, and VSB, M Brittain, M Babin, RBP, SBM, and M Brenner each made substantial contributions to study design, and assisted in writing the manuscript. M Brittain, AC, NG, DY, TB, DM, and SBM conducted the experiments. VSB, M Brittain, RBP, M Brenner, and GRB conducted the data analysis. GRB takes responsibility for the work as a whole.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

In all three species, survival in treated animals was significantly better than in control animals (log rank test, $p < 0.05$). In the pigs, the drugs attenuated a rise in the plasma lactate concentration within five minutes post-antidote injection (difference: plasma lactate, saline-versus nitrite-thiosulfate-treated 1.76 [95% confidence interval 1.25-2.27]).

**Conclusion—**We conclude that sodium nitrite and sodium thiosulfate given by intramuscular injection are effective against severe cyanide poisoning in three clinically relevant animal models of prehospital emergency care.

# INTRODUCTION

## Background and Importance

Cyanide is a highly toxic chemical used in a wide variety of industries, and is generated in industrial and residential fires (1-3). It can be made relatively easily, and terrorists could poison food or medicines or detonate a cyanide bomb in an enclosed space (4;5). Thus, cyanide exposure can occur under a variety of situations and mass casualties are possible. Available treatments for cyanide poisoning such as hydroxocobalamin (Cyanokit®) and the combination of sodium nitrite with sodium thiosulfate (Nithiodote®) are administered by intravenous injection, which is not practical for treating a large number of cyanide-poisoned victims in the pre-hospital setting. In a mass casualty scenario, probably the best treatment mode of critically ill patients would be intramuscular injection of antidote by first responders, preferably from a pre-filled autoinjector. This requires that the antidote(s) is/are: (*i*) stable in solution; (*ii*) sufficiently soluble to be administered in 1-3 ml; (*iii*) highly potent such that only a relatively small amount needs to be administered; and (*iv*) rapidly absorbed after intramuscular injection. Sodium nitrite and sodium thiosulfate are very soluble in water and stable under anaerobic conditions. Nithiodote contains 12.5 grams of sodium thiosulfate, an amount far greater than could be given by intramuscular injection, and published data indicate that neither sodium nitrite nor sodium thiosulfate are effective via intramuscular injection (6;7). However, as part of our work of developing the cobalamin analog cobinamide as a cyanide antidote, we found recently that sodium nitrite and sodium thiosulfate showed some anti-cyanide activity when given by intramuscular injection (8-10).

## Goals

Our goal was to rigorously assess if sodium nitrite and sodium thiosulfate are effective when administered by intramuscular injection by testing them in three well-established lethal mammalian models of cyanide poisoning: (*i*) a mouse model of inhalational cyanide exposure that simulates a scenario of gaseous cyanide poisoning (8;10;11); (*ii*) a rabbit model where severe hypotension is the trigger for treatment (9;12;13), and (*iii*) a pig model where apnea is the trigger for treatment (14-16).

# METHODS

## Study Overview and Design

According to general HAZMAT principles, persons exposed to toxic chemicals should be evacuated immediately from the contaminated area, but it would be difficult to remove a large number of victims quickly from a confined, hard-to-access location such as a subway

station. In these cases, it would be useful to treat the victims as quickly as possible, prior to or simultaneous with evacuation from the contaminated area. In consideration of these worse-case scenarios, our three animal models incorporate continued exposure to cyanide, even after treatment. This makes the models extremely rigorous, because the antidote has to neutralize not just the amount of cyanide that triggered treatment—cardiovascular and respiratory collapse in the rabbits and pigs, respectively—but also cyanide that continued to be administered to a profoundly sick animal (Fig. S1a-c). We studied three different species, because we wanted to ensure our findings were not limited to one or two species: since efficacy testing cannot be performed in humans for a cyanide antidote, greater assurance is important in pre-clinical studies compared to most drug development programs.

The investigation was conducted as a randomized, non-blinded, parallel-group study. Animals were randomized to the control or treated group using a block randomization procedure, to assure equal numbers of animals in each group. Sample size was determined by a Chi-square test, setting alpha at 0.05 and power at 0.9 for the mice and rabbits and 0.8 for the pigs (the pigs are 50 kg in size and we wanted to use the absolute minimal number of animals). From pilot studies and previous work, we expected 100% lethality in untreated mice and pigs, and 80% lethality in untreated rabbits (8-16). Assuming 90% survival in treated animals, sample sizes were calculated for the mice, rabbits, and pigs as 6, 11, and 5, respectively.

The following sections provide a general description of the three animal models; full experimental details are in Supplementary Data.

## Mouse Model

Mice are small enough that they can be exposed to cyanide gas within a sealed chamber; this minimizes the risk of exposing laboratory personnel to cyanide, but it allows for only visual monitoring of the animals. The mice were exposed to 587 ppm HCN gas for 15 min, injected with test antidote, and then re-exposed to the gas for 25 min (Fig. S1a). This model assumes about 15 min are required for emergency medical personnel to arrive at a disaster scene, and another 25 min are required to treat and evacuate the victims. As required by our Institutional Animal Care and Use Committee (IACUC), the mice were anesthetized by injecting isoflurane into the chamber to a final concentration of 2%; at 30ºC, the isoflurane rapidly vaporizes and anesthetizes the mice. We used 8-12 week old male C57BL/6J mice, purchased from Jackson Laboratories, and injected them in the right gastrocnemius muscle with either 50 μl saline (control group), or 50 μl of the indicated concentrations of sodium nitrite or sodium thiosulfate.

## Rabbit and Pig Models

The heart and central respiratory center are major cyanide targets, and we wanted to determine if sodium nitrite and sodium thiosulfate could rescue animals from both cardiovascular and respiratory collapse (1;17). Since it would be technically difficult to have a single model for both endpoints, we developed separate models in rabbits and pigs. Because rabbits have a relatively high metabolic rate and can sustain only very short periods of apnea— providing a very short window for treatment—they are not a good model of

respiratory collapse, but can be used as a model of cardiovascular collapse as the trigger for treatment. Since pigs can sustain longer periods of apnea, we were able to use respiratory collapse as the trigger for treatment in pigs.

Due to their sheer size, it is difficult to expose rabbits and pigs to cyanide gas safely, and we infused a cyanide salt intravenously at a constant rate. The $pK_a$ of HCN is 9.2; hence, at physiological pH, cyanide exists almost exclusively as HCN and infusing a cyanide salt rapidly generates HCN, the same form of cyanide that is inhaled and absorbed from the lungs. Thus, a cyanide infusion model yields the same end product as an inhalation model.

**Rabbit Model—**New Zealand white rabbits about 4 kg in size were anesthetized with ketamine and xylazine, and mechanically ventilated. After a 10 min baseline equilibration period, sodium cyanide was injected intravenously at 0.08 mg/kg/min. When the mean arterial blood pressure decreased to < 70% of baseline, which generally occurred after infusing cyanide for ~ 40 min, the animals were injected intramuscularly either with saline or simultaneously with sodium nitrite and sodium thiosulfate (Fig. S1b). The sodium nitrite and sodium thiosulfate doses were 0.62 and 22.3 mg/kg, respectively (0.12 ml 300 mM $NaNO_2$ and 0.12 ml 3.0 M $Na_2S_2O_3$). The cyanide infusion was continued for an additional 30 min post treatment, and the animals were observed for an additional 60 min, at which time surviving animals were euthanized. To assess the effect of a different pharmacokinetic profile on animal survival, the same sodium nitrite and sodium thiosulfate doses were given by intravenous injection to five animals; other than the different administration route, the protocol was otherwise identical.

**Pig Model—**Yorkshire pigs about 50 kg in size were acclimated to being suspended in a sling and breathing through a nose cone; the acclimation allowed the animals to be anesthetized directly with isoflurane, eliminating the need for parenteral pre-medication with ketamine and xylazine. Anesthesia was induced with 5% isoflurane, and then animals were intubated and maintained on 2% isoflurane throughout the experiment. Once animals were hemodynamically stable, KCN was injected intravenously at a rate of 0.17 mg/kg/min. The duration of KCN infusion and the time of antidote administration were based on the physiological trigger of apnea, defined as no breathing for 20 sec, as determined by a capnograph. At 1 min after onset of apnea, the animals were injected intramuscularly with saline or simultaneously with 0.69 mg/kg $NaNO_2$ and 20.8 mg/kg $Na_2S_2O_3$ (0.5 ml 1 M $NaNO_2$ and 1.4 ml 3 M $Na_2S_2O_3$ for a 50 kg animal). Five minutes post-treatment, the cyanide infusion was stopped, .i.e., the cyanide infusion was continued for six minutes after onset of apnea (Fig. S1C). Surviving pigs were observed daily for two weeks, and then were euthanized and their brains examined histopathologically.

## Institutional Animal Care and Use Committee Approval

Mouse studies were approved by the University of California, San Diego IACUC, rabbit studies by the University of California, Irvine IACUC, and pig studies by the Battelle Memorial Institute IACUC. All experiments 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.

### Statistical Analysis

Survival curves were plotted and analyzed by a log-rank (Mantel-Cox) test using Prism 5 software; p values < 0.05 were considered significant. For plasma lactate concentration, the 95% confidence interval of mean differences between saline- and nitrite/thiosulfate-treated animals was calculated by Prism 5 software.

## RESULTS

### Mouse Model

To determine if sodium nitrite and sodium thiosulfate were each effective against cyanide poisoning when administered by intramuscular injection, we tested the drugs separately in a 100% lethal mouse model. Sodium nitrite rescued 33% of animals at a dose of 5.2 mg/kg and 100% of animals at 9.2 m/kg; sodium thiosulfate rescued 50 and 100% of animals at doses of 60 and 300 mg/kg, respectively (Fig. 1; the high dose of each drug was significantly different from saline-treated animals by a log rank test). All rescued animals appeared normal for two weeks, at which time they were euthanized. The high drug doses are less than those used previously in mice—100 mg/kg sodium nitrite and 1000 mg/kg sodium thiosulfate; we will address this point in the rabbit model and consider it in the Discussion.

For sodium nitrite and sodium thiosulfate to be administered by intramuscular injection, they would need to cause minimal muscle damage at the injection site. We found that the two drugs caused mild muscle necrosis when assessed at 24 h; animals injected with saline showed no muscle necrosis.

### Rabbit Model

Sodium nitrite and sodium thiosulfate are known to be synergistic against cyanide poisoning and since the drugs would likely be used together in humans, we tested the drug combination in rabbits and pigs (18-20). We found that two of 11 rabbits injected with saline survived, with the other nine animals dying between 5 and 38 min post-saline injection (Fig. 2). This is to be contrasted with survival in eight of 11 animals that received simultaneous intramuscular injections of 0.61 mg/kg sodium nitrite and 22.3 mg/kg sodium thiosulfate, which was significantly different from controls as determined by a log-rank test (Fig. 2). The sodium nitrite and sodium thiosulfate doses were established in pilot experiments.

The sodium nitrite and sodium thiosulfate doses that rescued rabbits are considerably less than those used previously by intravenous injection in animals (18-20). We hypothesized that intramuscular administration of the drugs might provide more favorable pharmacokinetics for treating cyanide poisoning than intravenous injection; sodium thiosulfate is cleared relatively rapidly from blood and relatively high intravenous doses may be needed to maintain an effective antidotal concentration (21;22). To test this hypothesis, we used the same cyanide poisoning model and the same sodium nitrite and sodium thiosulfate doses as above, but administered the drugs by intravenous injection. We found

that the intravenously-injected drugs still rescued animals: five of five animals treated with 0.61 mg/kg sodium nitrite and 22.3 mg/kg sodium thiosulfate survived.

We found similar methemoglobin concentrations in saline-treated rabbits as in sodium nitrite/sodium thiosulfate-treated rabbits: in the nitrite/thiosulfate-treated rabbits, the methemoglobin concentration was 2.3% (± 0.19%) at baseline, and ranged from 1.7 to 2.0% at 5, 15, 30, 45, 60, and 90 min post sodium nitrite injection; these values are similar to those in saline-treated animals ($p > 0.50$ for comparison between the two groups).

### Pig Model

Five of five pigs that received an intramuscular injection of saline died between 9 and 48 min after the onset of apnea, whereas four of five animals injected with 0.69 mg/kg sodium nitrite and 20.8 mg/kg sodium thiosulfate survived; the difference between the two groups was statistically significant by a log-rank test (Fig. 3a). As in the rabbits, the sodium nitrite and sodium thiosulfate doses were determined in pilot experiments.

For the first five minutes post apnea, the plasma lactate concentration increased similarly in the control and nitrite/thiosulfate-treated groups, but then the rate of increase slowed in the treated group; by 10 min, no further increased occurred in the treated group, while in the control group, the lactate concentration continued to increase (Fig. 3b). The difference between the control and nitrite/thiosulfate-treated animals was already significant within five minutes after antidote injection (difference: plasma lactate, saline-versus nitrite-thiosulfate-treated 1.76 [95% confidence interval 1.25-2.27]). Changes in cardiac and respiratory parameters in control and treated animals are described in the Supplement, and shown in Fig. S2. No significant change occurred in the arterial concentrations of methemoglobin, sodium, potassium, calcium, or chloride in either the saline- or nitrite/thiosulfate-treated animals.

The four treated animals that survived the cyanide exposure were observed for two weeks, appearing completely normal. Necropsies performed on the animals showed no gross pathological abnormalities in the brain or spinal cord. Two of the four animals had minimal cerebral gliosis on microscopic examination, and one animal had a mixed cellular perivascular infiltrate in both the brain and spinal cord. Similar changes were observed in three animals that did not receive cyanide, but were otherwise treated the same, including anesthesia time: one animal exhibited minimal cerebral gliosis and two animals exhibited a mixed cellular perivascular infiltrate in the brain. Since all of the saline-treated animals died acutely, neuropathological examinations were not performed on these animals.

## LIMITATIONS

Because cyanide can cause pain or distress to animals, we were required by each institutional IACUC to anesthetize the animals. Anesthesia could have impacted our studies, but we found similar results in rabbits, which were anesthetized with ketamine and xylazine, as in mice and pigs, which were anesthetized with isoflurane. Both xylazine and isoflurane can lower blood pressure, but we found no significant change in blood pressure in the rabbits and pigs after induction of anesthesia.

Because of safety and technical considerations, we were able to expose only mice to cyanide gas and we administered cyanide salts intravenously to the rabbits and pigs. Although intravenous administration of cyanide is unlikely in humans, >98% of a cyanide salt is converted to HCN at physiological pH. This conversion would be expected to occur as soon as the salt mixes with blood, and, since HCN is highly soluble in aqueous-based solutions (Henry's Law Constant for HCN is high), all of the cyanide in blood would be expected to be in the form of dissolved HCN (23). Thus, whether cyanide is inhaled as a gas or infused as a salt, the end product of dissolved HCN in blood is the same. Additionally, intravenous cyanide infusion was accepted by the FDA as a valid exposure model in approval of hydroxocobalamin as a cyanide antidote (24).

We did not compare intramuscularly-administered sodium nitrite and sodium thiosulfate to another intramuscularly-administered drug, because no such approved drug exists. However, in the mice and rabbits, sodium nitrite and sodium thiosulfate yielded similar results as intramuscular injection of the investigational drug cobinamide (10).

Although we observed the mice and pigs for two weeks post-cyanide exposure and performed necropsies on the pigs, we did not perform formal neurological tests. It is possible, therefore, that we could have missed subtle neurological changes induced by cyanide.

Because of the expense of conducting experiments on large pigs, we set power at 0.8, yielding five animals per group. Although this is a relatively small number of animals, 80% power is generally acceptable; moreover, we found similar results in the mice and rabbits, where power was set at 90%.

## DISCUSSION

We have developed lethal models of cyanide poisoning in three mammalian species. In all three models, animals are exposed to cyanide, injected intramuscularly with antidote, and then re-exposed to cyanide. For the following four reasons, we feel our models more accurately reflect real-life scenarios of cyanide exposure than other models, where antidote was given either before or after cyanide exposure, but never both (6;7;18-20;24-28). First, the two most likely modes of human exposure to cyanide would be inhalation of cyanide gas or oral ingestion of a cyanide salt. Inhalational exposure occurs in residential and industrial fires, and could occur in a terrorist attack from poisoning a building's ventilation system or detonating a cyanide bomb in an enclosed space. Oral ingestion of cyanide could occur from deliberate tampering with food or medicines or poisoning a city's water supply (29;30). In both modes of exposure, victims could be exposed to cyanide even after treatment, since it takes time to evacuate people from a cyanide gas-contaminated area, and cyanide remaining in the gastrointestinal tract will continue to be absorbed. Hence models that incorporate continued cyanide exposure post treatment reflect real-life scenarios. Second, our models expose animals to cyanide for periods ranging from 10 to 75 min, in contrast to most other models where animals receive a single bolus injection of cyanide (6;7;18-20;25-27). Bolus injection of cyanide generally does not occur clinically, other than in the rare case of homicide or suicide, where it is unlikely a person can be rescued; in likely scenarios of

cyanide exposure—gas inhalation or oral ingestion, treatable victims will be exposed to cyanide for many minutes. Third, we observed the mice and pigs for two weeks after cyanide exposure, followed by necropsy of the pig brains. In the vast majority of animal models, the animals were euthanized at the end of the experiment prior to the development of delayed structural changes. And finally, our models are rigorous, because we treat the rabbits and pigs at times when they are profoundly hypotensive or apneic, respectively.

Nitrite and thiosulfate have been used for over 100 years to treat cyanide poisoning; amyl nitrite was first used in 1888, and sodium thiosulfate was first used in 1895 (31;32). Nitrite oxidizes hemoglobin to methemoglobin, but its major mechanism of action as a cyanide antidote may be generation of nitric oxide (NO), which competes with cyanide for binding to cytochrome C oxidase (33-35). We found no increase in methemoglobin concentration in the nitrite/thiosulfate-treated rabbits and pigs, likely because the nitrite dose they received was only 1.61 and 0.69 mg/kg, respectively, compared to 4.2 mg/kg sodium nitrite in Nithiodote. Thus, our data would be more consistent with NO generation as nitrite's mechanism of action. Sodium thiosulfate works by serving as a substrate for the endogenous enzyme rhodanese, which transfers the sulfane sulfur of thiosulfate to cyanide, thereby generating thiocyanate. The combination of sodium nitrite and sodium thiosulfate has been shown repeatedly to be synergistic against cyanide poisoning (18-20). If nitrite acted by generating methemoglobin, one would expect only an additive effect with thiosulfate, because the two agents would be working by separate mechanisms. Drug synergism further suggests NO generation is the predominant mechanism of nitrite's action, because NO displacement of cyanide from cytochrome C oxidase could make the cyanide available for thiosulfate neutralization.

Other than amyl nitrite administered by inhalation, sodium nitrite and sodium thiosulfate have been given by intravenous, intraperitoneal, or subcutaneous injection in animal studies (18;18-20;20;26). A careful review of the literature yielded two reports where sodium nitrite and sodium thiosulfate were given by intramuscular injection in an animal model of cyanide poisoning; the two papers were by the same group, and the authors concluded the drugs were not effective by intramuscular injection (6;7). Several possibilities may explain the lack of observed efficacy. First, the studies were conducted in 8-12 kg beagle dogs, with sodium nitrite given as a 2 ml injection and the combination of sodium nitrite and sodium thiosulfate as a 4 ml injection. Scaling these volumes to a 70 kg person would yield 14 and 28 ml, respectively, a volume far too large to be given by intramuscular injection' large injection volumes are associated with relatively slow absorption rates from muscle (36). Second the authors injected the cyanide intravenously, apparently as a bolus over 10-15 sec. Humans are rarely exposed to cyanide by bolus injection, and treating such exposures would require the antidote to be immediately available. And third, the sodium nitrite and sodium thiosulfate were injected two to three minutes after the intravenous cyanide injection, possibly too late to be effective.

The sodium nitrite and sodium thiosulfate doses that rescued animals in our studies were less than those used previously (18;18-20;20;26). One explanation for the difference may be historical, because high doses of the two drugs were used in the early studies, and subsequent investigators merely used similar doses (20;32;37). Another explanation is what

we have discussed previously, i.e., that cyanide is generally given to animals by bolus injection; this will kill animals quickly, necessitating high amounts of antidote. Since our animal models were designed to reflect real-life scenarios of cyanide poisoning, the sodium nitrite and sodium thiosulfate doses we found effective may more accurately represent required doses; this may be particularly true in victims exposed to cyanide gas where cessation of spontaneous respiration will likely limit total cyanide load.

Although sodium nitrite and sodium thiosulfate are effective cyanide antidotes, they do have several drawbacks. First, as already discussed, sodium nitrite generates NO and methemoglobin; NO can induce hypotension, and methemoglobin reduces oxygen carrying capacity of blood—the latter can be of particular concern in smoke inhalation victims who may have high carboxyhemoglobin concentrations, which also reduces blood oxygen carrying capacity (38). Although we did not observe hypotension in our animal models, we used young healthy animals. When treating civilian populations, a wide array of age, and racial and ethnic groups will be represented, some of whom could be sensitive to even low sodium nitrite doses. Thus, hypotension could be a problem in the elderly, or in subjects with autonomic insufficiency, such as diabetics. Methemoglobin production is a concern in infants and young children due to reduced methemoglobin reductase activity, and in people who are deficient in glucose-6 phosphate dehydrogenase activity (39;40). Because of the NO and methemoglobin generation, Nithiodote carries an FDA “Black Box” warning (http://www.drugs.com/pro/nithiodote.html). Second, sodium nitrite and sodium thiosulfate solutions are unstable when mixed together due to oxidation-reduction reactions. Administering the drugs via an autoinjector would require either two separate pre-filled syringes, as in the Mark I®, or a dual-chambered syringe, as in DuoDote®. Both systems add cost and complexity. Third, in the pigs—which come close to approximating the size of a human—we administered sodium nitrite as 10 μl/kg of a 1 M solution and sodium thiosulfate as 28 μl/kg of a 3 M solution. This would translate to 0.7 ml of sodium nitrite and 1.96 ml of sodium thiosulfate in an adult human, for a total volume of about 2.7 ml. This volume can be administered into the thigh muscle, but it is relatively large. And fourth, we found some muscle damage at the injection site in the mouse studies, which may have been due to the hypertonicity of the solutions. From our previous work, the degree of injury was likely fully reversible, and thus acceptable for treating a life-threating condition (10).

In conclusion, sodium nitrite and sodium thiosulfate were effective antidotes when administered by intramuscular injection in three clinically relevant, prehospital models of severe cyanide poisoning.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGMENTS

The authors thank Drs. David Jett and David Yeung of the National Institutes of Health, CounterACT Program for their unfailing support.

FUNDING

The work was supported by the CounterACT Program, Office of the Director, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Grant # U01 NS058030.

## REFERENCES

(1). Way JL. Cyanide intoxication and its mechanism of antagonism. Annu Rev Pharmacol Toxicol. 1984; 24:451–481. [PubMed: 6428300]

(2). Baud FJ, Barriot P, Toffis V, et al. Elevated blood cyanide concentrations in victims of smoke inhalation. N Engl J Med. 1991; 325(25):1761–1766. [PubMed: 1944484]

(3). Alcorta R. Smoke inhalation & acute cyanide poisoning. Hydrogen cyanide poisoning proves increasingly common in smoke-inhalation victims. JEMS. 2004; 29(8) suppl-15.

(4). Eckstein M. Enhancing public health preparedness for a terrorist attack involving cyanide. J Emerg Med. 2008; 35(1):59–65. [PubMed: 17976798]

(5). Sauer SW, Keim ME. Hydroxocobalamin: improved public health readiness for cyanide disasters. Ann Emerg Med. 2001; 37(6):635–641. [PubMed: 11385334]

(6). Vick JA, Froehlich H. Treatment of cyanide poisoning. Mil Med. 1991; 156(7):330–339. [PubMed: 1922842]

(7). Vick JA, Von Bredow JD. Effectiveness of intramuscularly administered cyanide antidotes on methemoglobin formation and survival. J Appl Toxicol. 1996; 16(6):509–516. [PubMed: 8956097]

(8). Chan A, Balasubramanian M, Blackledge W, et al. Cobinamide is superior to other treatments in a mouse model of cyanide poisoning. Clin Toxicol (Phila). 2010; 48:709–717. [PubMed: 20704457]

(9). Brenner M, Kim JG, Mahon SB, et al. Intramuscular cobinamide sulfite in a rabbit model of sublethal cyanide toxicity. Ann Emerg Med. 2010; 55:352–362. [PubMed: 20045579]

(10). Chan A, Jiang J, Fridman A, et al. Nitrocobinamide, a new cyanide antidote that can be administered by intramuscular injection. J Med Chem. 2015; 58(4):1750–1759. [PubMed: 25650735]

(11). Chan A, Crankshaw DL, Monteil A, et al. The combination of cobinamide and sulfanegen is highly effective in mouse models of cyanide poisoning. Clin Toxicol (Phila). 2011; 49(5):366–373. [PubMed: 21740135]

(12). Brenner M, Mahon SB, Lee J, et al. Comparison of cobinamide to hydroxocobalamin in reversing cyanide physiologic effects in rabbits using diffuse optical spectroscopy monitoring. J Biomed Opt. 2010; 15(1):017001. [PubMed: 20210475]

(13). Kim JC, Lee J, Mahon SB, et al. Noninvasive monitoring of treatment response in a rabbit cyanide toxicity model reveals differences in brain and muscle metabolism. J Biomed Optics. 2012; 17

(14). Bebarta VS, Tanen DA, Lairet J, et al. Hydroxocobalamin and sodium thiosulfate versus sodium nitrite and sodium thiosulfate in the treatment of acute cyanide toxicity in a swine (Sus scrofa) model. Ann Emerg Med. 2010; 55(4):345–351. [PubMed: 19944487]

(15). Bebarta VS, Pitotti RL, Dixon P, et al. Hydroxocobalamin versus sodium thiosulfate for the treatment of acute cyanide toxicity in a swine (*Sus scrofa*) model. Ann Emerg Med. 2012; 59(6): 532–539. [PubMed: 22387086]

(16). Bebarta V, Tanen D, Boudrea S, et al. Intravenous cobinamide versus hydrocobalamin for acute treatment of severe cyanide poisonong in a swine (Suc Scroba) model. Ann Emerg Med. 2014; 64:612–619. [PubMed: 24746273]

(17). Salkowski AA, Penney DG. Cyanide poisoning in animals and humans: a review. Vet Hum Toxicol. 1994; 36(5):455–466. [PubMed: 7839575]

(18). Chen KK, Rose CL. Nitrite and thiosulfate therapy in cyanide poisoning. J Am Med Assoc. 1952; 149(2):113–119. [PubMed: 14917568]

(19). Isom G, Way JL. Cyanide intoxication: protection with cobaltous chloride. Toxicol Appl Pharmacol. 1973; 24(3):449–456. [PubMed: 4704816]

(20). Chen KK, Rose CL, Clowes GHA. Methylene blue, nitrites, and sodium thiosulphate against cyanide poisoning. Proc Soc Exp Biol. 1933; 31:250–251.

(21). Schulz V. Clinical pharmacokinetics of nitroprusside, cyanide, thiosulphate and thiocyanate. Clin Pharmacokinet. 1984; 9(3):239–251. [PubMed: 6375932]

(22). Braverman B, Ivankovich AD, Shah G. Thiosulfate pharmacokinetics in normal and anuric dogs. Proc Soc Exp Biol Med. 1982; 170(3):273–280. [PubMed: 7088953]

(23). Ma J, Dasgupta PK, Blackledge W, et al. Temperature dependence of Henry's law constant for hydrogen cyanide. Generation of trace standard gaseous hydrogen cyanide. Environ Sci Technol. 2010; 44(8):3028–3034. [PubMed: 20302333]

(24). Borron SW, Stonerook M, Reid F. Efficacy of hydroxocobalamin for the treatment of acute cyanide poisoning in adult beagle dogs. Clin Toxicol (Phila). 2006; 44:5–15. [PubMed: 16990189]

(25). Evans CL. Cobalt compounds as antidotes for hydrocyanic acid. Br J Pharmacol Chemother. 1964; 23:455–475. [PubMed: 14256807]

(26). Way JL, Burrows G. Cyanide intoxication: protection with chlorpromazine. Toxicol Appl Pharmacol. 1976; 36(1):93–97. [PubMed: 1273841]

(27). Hatch RC, Laflamme DP, Jain AV. Effects of various known and potential cyanide antagonists and a glutathione depletor on acute toxicity of cyanide in mice. Vet Hum Toxicol. 1990; 32(1):9–16. [PubMed: 2301155]

(28). Ivankovich AD, Braverman B, Kanuru RP, et al. Cyanide antidotes and methods of their administration in dogs: a comparative study. Anesthesiology. 1980; 52(3):210–216. [PubMed: 7369507]

(29). Wolnik KA, Fricke FL, Bonnin E, et al. The Tylenol tampering incident - tracing the source. Anal Chem. 1984; 56(3):466A–470A. 474A.

(30). Eckstein M. Cyanide as a chemical terrorism weapon. JEMS. 2004; 29(8) suppl-31.

(31). Pedigo LG. Antagonism between amyl nitrite and prussio acid. Transactions of Medical Society of Virginia. 1888; 19:124–131.

(32). Lang S. Studien uber Entgiftungstherapie. I. Ueber Entgiftung der Blausaure. Arch Exp Path Pharm. 1895; 36:75–79.

(33). Johnson WS, Hall AH, Rumack BH. Cyanide poisoning successfully treated without 'therapeutic methemoglobin levels'. Am J Emerg Med. 1989; 7(4):437–440. [PubMed: 2567600]

(34). Pearce LL, Bominaar EL, Hill BC, et al. Reversal of cyanide inhibition of cytochrome c oxidase by the auxiliary substrate nitric oxide: an endogenous antidote to cyanide poisoning? J Biol Chem. 2003; 278(52):52139–52145. [PubMed: 14534303]

(35). Leavesley HB, Li L, Prabhakaran K, et al. Interaction of cyanide and nitric oxide with cytochrome c oxidase: implications for acute cyanide toxicity. Toxicol Sci. 2008; 101(1):101–111. [PubMed: 17906319]

(36). Zuidema J, Kadir F, Titulaer HAC, et al. Release and absorption rates of intramuscularly and subcutaneously injected pharmaceuticals (II). Intl J Pharmaceutics. 1994; 105:189–207.

(37). Chen KK, Rose CL, Clowes GHA. Comparative values of several antidotes in cyanide poisoning. Am J Med Sci. 1937; 188:767–781.

(38). Fortin JL, Ruttiman M, Domanski L, et al. Hydroxocobalamin: treatment for smoke inhalation-associated cyanide poisoning. Meeting the needs of fire victims. JEMS. 2004; 29(8) suppl-21.

(39). Rechetzki KF, Henneberg R, da Silva PH, et al. Reference values for methemoglobin concentrations in children. Rev Bras Hematol Hemoter. 2012; 34(1):14–16. [PubMed: 23049377]

(40). Calabrese EJ, Moore GS, Ho SC. Low erythrocyte glucose-6-phosphate dehydrogenase (g-6-PD) activity and susceptibility to nitrite-induced methemoglobin formation. Bull Environ Contam Toxicol. 1980; 25(6):837–840. [PubMed: 7470659]

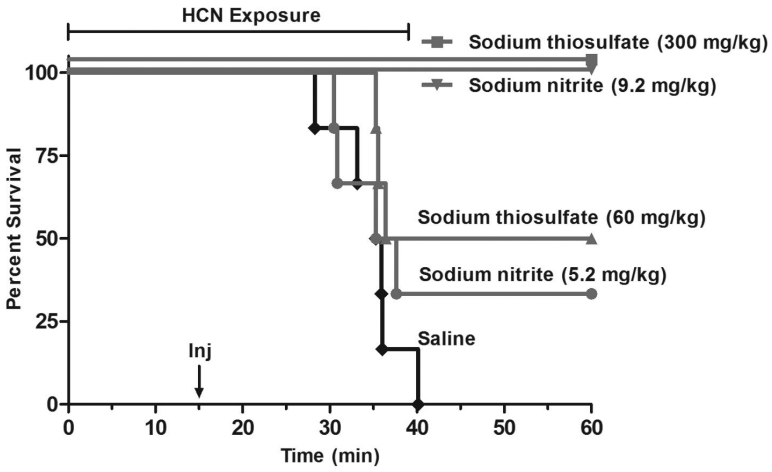


**Fig. 1. Survival in Mouse Model**

Mice were exposed to cyanide gas, and, after 15 min, received an intramuscular injection of saline (black diamonds), 5.2 or 9.2 mg/kg sodium nitrite (grey circles and inverted triangles, respectively), or 60 or 300 mg/kg sodium thiosulfate (grey triangles or squares, respectively). Six animals were studied per condition. All of the saline-treated animals died, and the difference between saline-treated animals and animals treated with the higher sodium nitrite and sodium thiosulfate doses was significant by a log rank test.

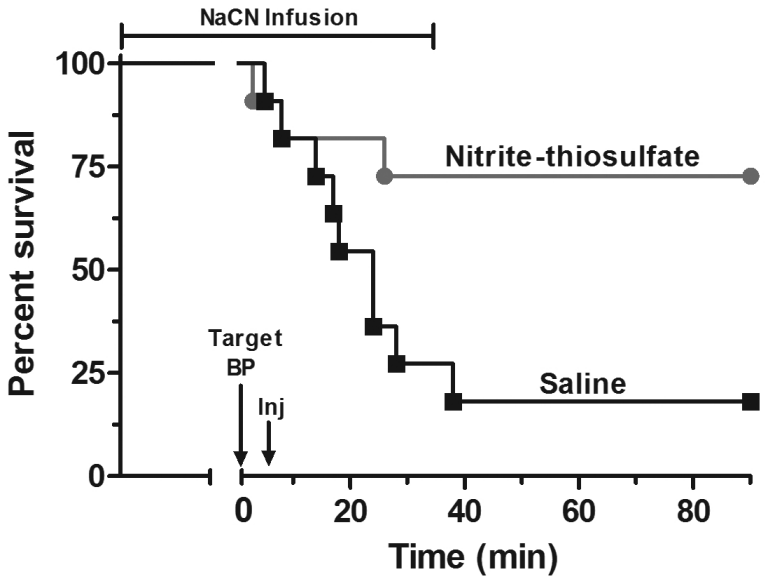


**Fig. 2. Survival in Rabbit Model**

Rabbits received an intravenous infusion of sodium cyanide, and five minutes after the blood pressure decreased to the target value, they received an intramuscular injection of saline (black squares), or 0.61 mg/kg sodium nitrite and 22.3 mg/kg sodium thiosulfate (grey circles). Eleven animals were studied per condition; the difference between saline-treated animals and nitrite/thiosulfate-treated animals was significant by a log-rank test.

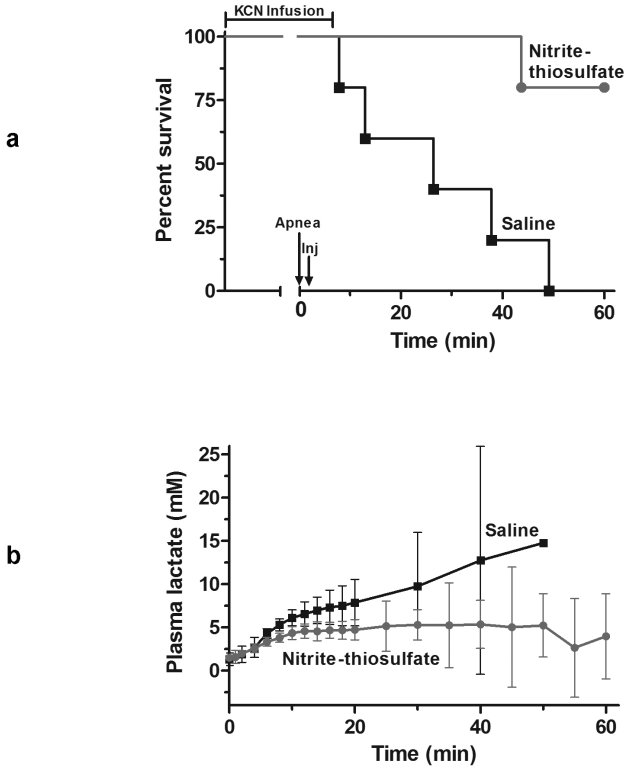


**Fig. 3. Survival and Plasma Lactate Concentration in Pig Model**

**Panel a.** Pigs received an intravenous infusion of potassium cyanide, and, at one minute post apnea, an intramuscular injection of saline (black squares) or 0.69 mg/kg sodium nitrite and 20.8 mg/kg sodium thiosulfate (grey circles). Five animals were studied in each condition; the difference between saline-treated animals and nitrite/thiosulfate-treated animals was significant by a log-rank test. **Panel b.** Mean arterial plasma lactate concentration is shown: saline-treated animals are designated by black squares and nitrite/thiosulfate-treated animals by grey circles. Error bars denote 95% confidence interval.