Sodium Nitrite and Sodium Thiosulfate Are Effective Against Acute Cyanide Poisoning When Administered by Intramuscular Injection

Vikhyat S. Bebarta, MD; Matthew Brittain, PhD; Adriano Chan, BS; Norma Garrett, PhD; David Yoon, MS; Tanya Burney, BS; David Mukai, BS; Michael Babin, DVM; Renate B. Pilz, MD; Sari B. Mahon, PhD; Matthew Brenner, MD; Gerry R. Boss, MD*

*Corresponding Author. E-mail: gboss@ucsd.edu.

Study objective: The 2 antidotes for acute cyanide poisoning in the United States must be administered by intravenous injection. In the out-of-hospital setting, intravenous injection is not practical, particularly for mass casualties, and intramuscular injection would be preferred. The purpose of this study is to determine whether sodium nitrite and sodium thiosulfate are effective cyanide antidotes when administered by intramuscular injection.

Methods: We used a randomized, nonblinded, parallel-group study design in 3 mammalian models: cyanide gas inhalation in mice, with treatment postexposure; 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 3 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 2 drugs rescued 73% of the rabbits and 80% of the pigs. In all 3 species, survival in treated animals was significantly better than in control animals (log rank test, $P<.05$). In the pigs, the drugs attenuated an increase in the plasma lactate concentration within 5 minutes postantidote injection (difference: plasma lactate, saline solution–treated versus nitrite- or thiosulfate-treated 1.76 [95% confidence interval 1.25 to 2.27]).

Conclusion: We conclude that sodium nitrite and sodium thiosulfate administered by intramuscular injection are effective against severe cyanide poisoning in 3 clinically relevant animal models of out-of-hospital emergency care. [Ann Emerg Med. 2017;69:718-725.]

Please see page 719 for the Editor's Capsule Summary of this article.

Readers: click on the link to go directly to a survey in which you can provide **feedback** to *Annals* on this particular article.
A **podcast** for this article is available at www.annemergmed.com.

0196-0644/$-see front matter

http://dx.doi.org/10.1016/j.annemergmed.2016.09.034

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 and sodium thiosulfate (Nithiodote) are administered by intravenous injection, which is not practical for treating a large number of cyanide-poisoned victims in the out-of-hospital setting. In a mass casualty scenario, probably the best treatment mode for critically ill patients would be intramuscular injection of antidote by first responders, preferably from a prefilled autoinjector. This requires that the antidote be stable in solution, sufficiently soluble to be administered in 1- to 3-mL doses, highly potent such that only a relatively small amount needs to be administered, and rapidly absorbed after intramuscular injection. Sodium nitrite and sodium thiosulfate are very soluble in water and stable under anaerobic conditions. Nithiodote contains 12.5 g of sodium thiosulfate, an amount far greater than could be administered by intramuscular injection, and published data indicate that neither sodium nitrite nor sodium thiosulfate is effective by 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 anticyanide activity when administered by intramuscular injection.[8-10]

Editor's Capsule Summary

What is already known on this topic

Cyanide poisoning requires rapid antidote administration, although the current antidotes require intravenous access.

What question this study addressed

Can cyanide antidotes sodium nitrite and sodium thiosulfate be effective if administered intramuscularly?

What this study adds to our knowledge

Using 3 distinct nonblinded animal models with objective endpoints, postexposure treatment with intramuscular cyanide antidote after lethal cyanide poisoning was highly effective at improving surrogate markers and preventing death.

How this is relevant to clinical practice

This animal model does not directly translate into human use, but suggests that additional study is warranted, particularly in the mass casualty setting.

Goals of This Investigation

Our goal was to rigorously assess whether sodium nitrite and sodium thiosulfate are effective when administered by intramuscular injection by testing them in 3 well-established lethal mammalian models of cyanide poisoning: a mouse model of inhalational cyanide exposure that simulates a scenario of gaseous cyanide poisoning,[8,10,11] a rabbit model in which severe hypotension is the trigger for treatment,[9,12,13] and a pig model in which apnea is the trigger for treatment.[14-16]

MATERIALS AND METHODS

Study 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 before or during evacuation from the contaminated area. In consideration of these worst-case scenarios, our 3 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 ill animal (Figure E1, available online at http://www.annemergmed.com). We studied 3 different species because we wanted to ensure that our findings were not limited to 1 or 2 species; because efficacy testing cannot be performed in human beings for a cyanide antidote, greater assurance is important in preclinical studies compared with that for most drug development programs.

The investigation was conducted as a randomized, nonblinded, parallel-group study. Animals were randomized to the control or treated group with a block randomization procedure to ensure equal numbers of animals in each group. Sample size was determined by a χ^2 test, with α=.05 and power=0.9 for the mice and rabbits and 0.8 for the pigs (the pigs were 50 kg, and we wanted to use a minimum 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 3 animal models; full experimental details are in Appendix E1, available online at http://www.annemergmed.com.

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 only visual monitoring of the animals. The mice were exposed to 587 ppm hydrogen cyanide (HCN) gas for 15 minutes, injected with test antidote, and then reexposed to the gas for 25 minutes (Figure E1*A*, available online at http://www.annemergmed.com). This model assumes that approximately 15 minutes are required for emergency medical personnel to arrive at a disaster scene and another 25 minutes are required to treat and evacuate the victims. As required by our Institutional Animal Care and Use Committee (IACUC), the mice were anesthetized by injection of isoflurane into the chamber to a final concentration of 2%; at 30°C (86°F), the isoflurane rapidly vaporizes and anesthetizes the mice. We used 8- to 12-week-old male C57BL/6J mice, purchased from Jackson Laboratories (Bar Harbor, ME), and injected them in the right gastrocnemius muscle with either 50 μL saline solution (control group) or 50 μL of the indicated concentrations of sodium nitrite or sodium thiosulfate.

The heart and central respiratory center are major cyanide targets, and we wanted to determine whether sodium nitrite and sodium thiosulfate could rescue animals from both cardiovascular and respiratory collapse.[1,17] Because 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. Because pigs can sustain longer periods of apnea, we were able to use respiratory collapse as the trigger for their treatment.

Because of their 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 physiologic 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.

New Zealand White rabbits weighing approximately 4 kg were anesthetized with ketamine and xylazine and mechanically ventilated. After a 10-minute baseline equilibration period, sodium cyanide was injected intravenously at 0.08 mg/kg per minute. When the mean arterial blood pressure decreased to less than 70% of baseline, which generally occurred after infusing cyanide for approximately 40 minutes, the animals were injected intramuscularly with either saline solution or sodium nitrite and sodium thiosulfate (Figure E1*B*, available online at http://www.annemergmed.com). The sodium nitrite and sodium thiosulfate doses were 0.61 and 22.3 mg/kg, respectively (0.12 mL 300-mM sodium nitrite and 0.12 mL 3.0-M sodium thiosulfate). The cyanide infusion was continued for an additional 30 minutes posttreatment, and the animals were observed for an additional 60 minutes, 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 administered by intravenous injection to 5 animals; other than the different administration route, the protocol was otherwise identical.

Yorkshire pigs weighing approximately 50 kg 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 premedication 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, potassium cyanide was injected intravenously at 0.17 mg/kg per minute. The duration of potassium cyanide infusion and the time of antidote administration were based on the physiologic trigger of apnea, defined as no breathing for 20 seconds, as determined by a capnograph. At 1 minute after onset of apnea, the animals were injected intramuscularly with saline solution or simultaneously with sodium nitrite at 0.69 mg/kg and sodium thiosulfate at 20.8 mg/kg (0.5 mL 1-M sodium nitrite and 1.4 mL 3-M sodium thiosulfate for a 50-kg animal). Five minutes posttreatment, the cyanide infusion was stopped, ie, the cyanide infusion was continued for 6 minutes after onset of apnea (Figure E1*C*, available online at http://www.annemergmed.com). Surviving pigs were observed daily for 2 weeks and then were euthanized, after which their brains were examined histopathologically.

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.

Primary Data Analysis

Survival curves were plotted and analyzed by a log rank (Mantel-Cox) test with Prism 5 (version 5; GraphPad, La Jolla, CA); $P<.05$ was considered significant. For plasma lactate concentration, the 95% confidence interval of mean differences between saline solution– and nitrite or thiosulfate-treated animals was calculated with Prism 5.

RESULTS

To determine whether sodium nitrite and sodium thiosulfate were 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 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 (Figure 1; survival at the high dose of each drug was significantly different from that of saline solution–treated animals by a log rank test). All rescued animals appeared normal for 2 weeks, at which time they were euthanized. The high drug doses were less than those used previously in mice—sodium nitrite at 100 mg/kg and sodium thiosulfate at 1,000 mg/kg; we will address this point in the rabbit model and consider it in the "Discussion" section.

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

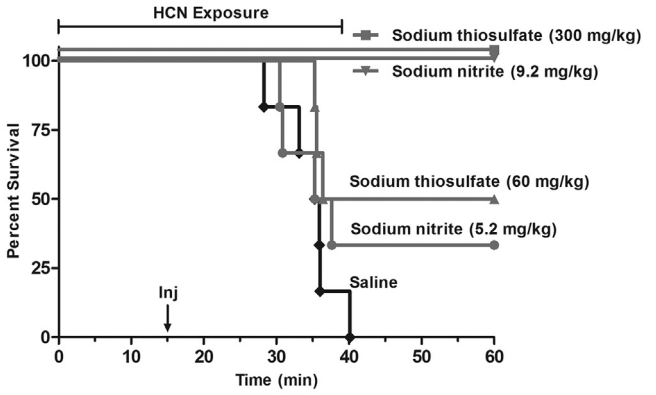

Figure 1. Survival in a mouse model. Mice were exposed to cyanide gas and, after 15 minutes, received an intramuscular injection of saline solution (black diamonds), sodium nitrite at 5.2 or 9.2 mg/kg (gray circles and inverted triangles, respectively), or sodium thiosulfate at 60 or 300 mg/kg (gray triangles or squares, respectively). Six animals were studied per condition. All of the saline solution–treated animals died, and the difference between them and animals treated with the higher sodium nitrite and sodium thiosulfate doses was significant by a log rank test.

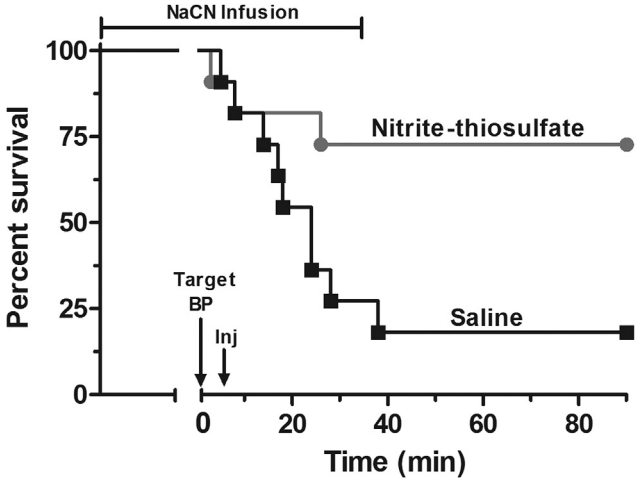

Figure 2. Survival in a rabbit model. Rabbits received an intravenous infusion of sodium cyanide, and 5 minutes after the blood pressure decreased to the target value, they received an intramuscular injection of saline solution (black squares) or sodium nitrite at 0.61 mg/kg and sodium thiosulfate at 22.3 mg/kg (gray circles). Eleven animals were studied per condition; the difference between saline solution–treated animals and nitrite- or thiosulfate-treated animals was significant by a log rank test.

found that the 2 drugs had caused mild muscle necrosis by 24 hours after injection; animals injected with saline solution showed no muscle necrosis.

Sodium nitrite and sodium thiosulfate are known to be synergistic against cyanide poisoning, and because the drugs would likely be used together in human beings, we tested the drug combination in rabbits and pigs.[18-20] We found that 2 of 11 rabbits injected with saline solution survived, with the other 9 animals dying between 5 and 38 minutes post–saline solution injection (Figure 2). This is to be contrasted with survival in 8 of 11 animals that received simultaneous intramuscular injections of sodium nitrite at 0.61 mg/kg and sodium thiosulfate at 22.3 mg/kg, which was significantly different from that of controls, as determined by a log rank test (Figure 2). The sodium nitrite and sodium thiosulfate doses were established in pilot experiments.

The sodium nitrite and sodium thiosulfate doses that rescued rabbits were 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: 5 of 5 animals treated with sodium nitrite at 0.61 mg/kg and sodium thiosulfate at 22.3 mg/kg survived.

We found methemoglobin concentrations in saline solution–treated rabbits similar to those in sodium nitrite– or sodium thiosulfate–treated rabbits: in the nitrite- or thiosulfate-treated rabbits, the methemoglobin concentration was 2.3% (SD 0.19%) at baseline and ranged from 1.7% to 2.0% at 5, 15, 30, 45, 60, and 90 minutes post–sodium nitrite injection; these values are similar to those in saline solution–treated animals ($P>.50$ for comparison between the 2 groups).

Five of 5 pigs that received an intramuscular injection of saline solution died between 9 and 48 minutes after the onset of apnea, whereas 4 of 5 animals injected with sodium nitrite at 0.69 mg/kg and sodium thiosulfate at 20.8 mg/kg survived; the difference between the 2 groups was statistically significant by a log rank test (Figure 3*A*). As with the rabbits, the sodium nitrite and sodium thiosulfate doses were determined in pilot experiments.

For the first 5 minutes postapnea, the plasma lactate concentration increased similarly in the control and nitrite- or thiosulfate-treated groups, but then the rate of increase slowed in the treated group; by 10 minutes, no further increased occurred in the treated group, whereas in the control group, the lactate concentration continued to increase (Figure 3*B*). The difference between the control

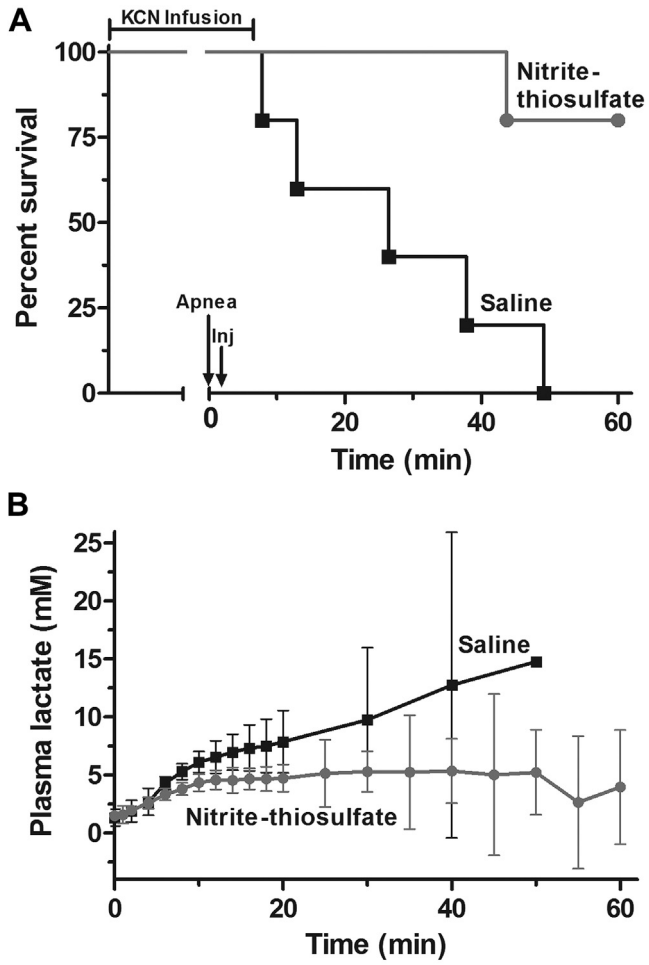

Figure 3. Survival and plasma lactate concentration in a pig model. *A*, Pigs received an intravenous infusion of potassium cyanide and, at 1 minute postapnea, an intramuscular injection of saline solution (black squares) or sodium nitrite at 0.69 mg/kg and sodium thiosulfate at 20.8 mg/kg (gray circles). Five animals were studied in each condition; the difference between saline solution–treated animals and nitrite- or thiosulfate-treated animals was significant by a log rank test. *B*, Mean arterial plasma lactate concentration is shown: saline solution–treated animals are designated by black squares and nitrite- or thiosulfate-treated animals by gray circles. Error bars denote 95% confidence interval.

and nitrite- or thiosulfate-treated animals was already significant within 5 minutes after antidote injection (difference: plasma lactate, saline solution– versus nitrite- or thiosulfate-treated 1.76 [95% confidence interval 1.25 to 2.27]). Changes in cardiac and respiratory parameters in control and treated animals are described in Appendix E1 and shown in Figure E2 (available online at http://www.annemergmed.com). No significant change occurred in the arterial concentrations of methemoglobin, sodium, potassium, calcium, or chloride in either the saline solution– or nitrite- or thiosulfate-treated animals.

The 4 treated animals that survived the cyanide exposure were observed for 2 weeks, appearing completely normal. Necropsies performed on the animals showed no gross pathologic abnormalities in the brain or spinal cord. Two of the 4 animals had minimal cerebral gliosis on microscopic examination, and 1 animal had a mixed cellular perivascular infiltrate in both the brain and spinal cord. Similar changes were observed in 3 animals that did not receive cyanide but were otherwise treated the same, including anesthesia time: 1 animal exhibited minimal cerebral gliosis and 2 animals exhibited a mixed cellular perivascular infiltrate in the brain. Because all of the saline solution–treated animals died acutely, neuropathologic examinations were not performed.

LIMITATIONS

Because cyanide can cause pain or distress to animals, we were required by each institutional IACUC to anesthetize them. Anesthesia could have affected our studies, but we found that results in rabbits, which were anesthetized with ketamine and xylazine, were similar to those in mice and pigs, which were anesthetized with isoflurane. Both xylazine and isoflurane can decrease 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 human beings, greater than 98% of a cyanide salt is converted to HCN at physiologic pH. This conversion would be expected to occur as soon as the salt mixes with blood, and, because 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 Food and Drug Administration 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 results similar to those of intramuscular injection of the investigational drug cobinamide.[10]

Although we observed the mice and pigs for 2 weeks post–cyanide exposure and performed necropsies on the

pigs, we did not perform formal neurologic tests. It is possible, therefore, that we missed subtle neurologic changes induced by cyanide.

Because of the expense of conducting experiments on large pigs, we set power at 0.8, yielding 5 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 3 mammalian species. In all 3 models, animals are exposed to cyanide, injected intramuscularly with antidote, and then reexposed to cyanide. For the following 4 reasons, we believe our models more accurately reflect actual scenarios of cyanide exposure than other models in which antidote was administered either before or after cyanide exposure, but never both.[6,7,18-20,24-28] First, the 2 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 because it takes time to evacuate persons 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 posttreatment reflect actual scenarios. Second, our models expose animals to cyanide for periods ranging from 10 to 75 minutes, in contrast to most other models, in which 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, in which 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 2 weeks after cyanide exposure, followed by necropsy of the pig brains. In the majority of animal models, the animals were euthanized at the end of the experiment before the development of delayed structural changes. And finally, our models are rigorous because the rabbits and pigs are treated when they are profoundly hypotensive or apneic, respectively.

Nitrite and thiosulfate have been used for more than 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, which competes with cyanide for binding to cytochrome *c* oxidase.[33-35] We found no increase in methemoglobin concentration in the nitrite- or thiosulfate-treated rabbits and pigs, likely because the nitrite dose they received was only 0.61 and 0.69 mg/kg, respectively, compared with sodium nitrite at 4.2 mg/kg in Nithiodote. Thus, our data would be more consistent with nitric oxide 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 2 agents would be working by separate mechanisms. Drug synergism further suggests that nitric oxide generation is the predominant mechanism of nitrite's action because nitric oxide 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 administered by intravenous, intraperitoneal, or subcutaneous injection in animal studies.[18-20,26] A careful review of the literature yielded 2 reports in which sodium nitrite and sodium thiosulfate were administered by intramuscular injection in an animal model of cyanide poisoning; the 2 articles 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- to 12-kg beagle dogs, with sodium nitrite administered 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 administered 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 during 10 to 15 seconds. Human beings are rarely exposed to cyanide by bolus injection, and treating such exposures would require that the antidote be immediately available. Third, the sodium nitrite and sodium thiosulfate were injected 2 to 3 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.[18-20,26] One explanation for the difference may be historical because high doses of the 2 drugs were used in early studies, and subsequent investigators merely used similar doses.[20,32,37] Another explanation is what we discussed previously, ie, that cyanide is generally administered to animals by bolus injection; this will kill animals quickly, necessitating high amounts of antidote. Because our animal models were designed to reflect actual 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, in which 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 nitric oxide and methemoglobin; nitric oxide 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 civilian populations are treated, a wide array of ages and racial and ethnic groups will be represented; some of these persons 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 because of reduced methemoglobin reductase activity, and in persons who are deficient in glucose-6 phosphate dehydrogenase activity.[39,40] Because of the nitric oxide and methemoglobin generation, Nithiodote carries a Food and Drug Administration "black box" warning (http://www.drugs.com/pro/nithiodote.html). Second, sodium nitrite and sodium thiosulfate solutions are unstable when mixed because of oxidation-reduction reactions. Administering the drugs by autoinjector would require either 2 separate prefilled 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 a 1-M solution at 10 μL/kg and sodium thiosulfate as a 3-M solution at 28 μL/kg, the equivalent of 0.7 mL of sodium nitrite and 1.96 mL of sodium thiosulfate in an adult human, for a total volume of approximately 2.7 mL. This volume can be administered into the thigh muscle, but it is relatively large. 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-threatening condition.[10]

In conclusion, sodium nitrite and sodium thiosulfate were effective antidotes when administered by intramuscular injection in 3 clinically relevant, out-of-hospital models of severe cyanide poisoning.

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

Supervising editor: Lewis S. Nelson, MD

Author affiliations: From the Department of Emergency Medicine, University of Colorado, School of Medicine, Aurora, CO (Bebarta); the Battelle Memorial Institute, Columbus, OH (Brittain, Babin); the Department of Medicine, University of California, San Diego, La Jolla, CA (Chan, Pilz, Boss); Medical Toxicology and the Department of Emergency Medicine, San Antonio Military Medical Center/59th MDW, San Antonio, TX (Garrett); and the Beckman Laser Institute and Medical Clinic, University of California, Irvine, Irvine, CA (Yoon, Burney, Mukai, Mahon, Brenner).

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

Funding and support: By *Annals* policy, all authors are required to disclose any and all commercial, financial, and other relationships in any way related to the subject of this article as per ICMJE conflict of interest guidelines (see www.icmje.org). The authors have stated that no such relationships exist. 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.

Publication dates: Received for publication May 12, 2016. Revisions received July 27, 2016, and September 16, 2016. Accepted for publication September 22, 2016. Available online December 29, 2016.

REFERENCES

1. Way JL. Cyanide intoxication and its mechanism of antagonism. *Annu Rev Pharmacol Toxicol*. 1984;24:451-481.
2. Baud FJ, Barriot P, Toffis V, et al. Elevated blood cyanide concentrations in victims of smoke inhalation. *N Engl J Med*. 1991;325:1761-1766.
3. Alcorta R. Smoke inhalation and acute cyanide poisoning. Hydrogen cyanide poisoning proves increasingly common in smoke-inhalation victims. *JEMS*. 2004;29:suppl 6-15.
4. Eckstein M. Enhancing public health preparedness for a terrorist attack involving cyanide. *J Emerg Med*. 2008;35:59-65.
5. Sauer SW, Keim ME. Hydroxocobalamin: improved public health readiness for cyanide disasters. *Ann Emerg Med*. 2001;37:635-641.

6. Vick JA, Froehlich H. Treatment of cyanide poisoning. *Mil Med*. 1991;156:330-339.
7. Vick JA, Von Bredow JD. Effectiveness of intramuscularly administered cyanide antidotes on methemoglobin formation and survival. *J Appl Toxicol*. 1996;16:509-516.
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.
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.
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;58:1750-1759.
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;49:366-373.
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;15:017001.
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;17:105005.
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;55:345-351.
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;59:532-539.
16. Bebarta V, Tanen D, Boudrea S, et al. Intravenous cobinamide versus hydrocobalamin for acute treatment of severe cyanide poisonong in a swine (*Sus scrofa*) model. *Ann Emerg Med*. 2014;64:612-619.
17. Salkowski AA, Penney DG. Cyanide poisoning in animals and humans: a review. *Vet Hum Toxicol*. 1994;36:455-466.
18. Chen KK, Rose CL. Nitrite and thiosulfate therapy in cyanide poisoning. *J Am Med Assoc*. 1952;149:113-119.
19. Isom G, Way JL. Cyanide intoxication: protection with cobaltous chloride. *Toxicol Appl Pharmacol*. 1973;24:449-456.
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;9:239-251.
22. Braverman B, Ivankovich AD, Shah G. Thiosulfate pharmacokinetics in normal and anuric dogs. *Proc Soc Exp Biol Med*. 1982;170:273-280.
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;44:3028-3034.
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.
25. Evans CL. Cobalt compounds as antidotes for hydrocyanic acid. *Br J Pharmacol Chemother*. 1964;23:455-475.
26. Way JL, Burrows G. Cyanide intoxication: protection with chlorpromazine. *Toxicol Appl Pharmacol*. 1976;36:93-97.
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;32:9-16.
28. Ivankovich AD, Braverman B, Kanuru RP, et al. Cyanide antidotes and methods of their administration in dogs: a comparative study. *Anesthesiology*. 1980;52:210-216.
29. Wolnik KA, Fricke FL, Bonnin E, et al. The Tylenol tampering incident—tracing the source. *Anal Chem*. 1984;56:466A-470A, 474A.
30. Eckstein M. Cyanide as a chemical terrorism weapon. *JEMS*. 2004;29; suppl 22-31.
31. Pedigo LG. Antagonism between amyl nitrite and prussio acid. *Trans Med Soc 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;7:437-440.
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;278:52139-52145.
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;101:101-111.
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; suppl 18-21.
39. Rechetzki KF, Henneberg R, da Silva PH, et al. Reference values for methemoglobin concentrations in children. *Rev Bras Hematol Hemoter*. 2012;34:14-16.
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;25:837-840.

APPENDIX E1

Sodium nitrite and sodium thiosulfate are effective against acute cyanide poisoning when administered by intramuscular injection

MATERIALS AND METHODS

Sodium nitrite ($NaNO_2$) and sodium thiosulfate ($Na_2S_2O_3$) pentahydrate were obtained from Sigma-Aldrich and were greater than 99% pure. They were dissolved in water to concentrations of 1.0 and 3.0 M, respectively, with the thiosulfate solution close to saturating; the solutions were stable for several months at room temperature. Potassium cyanide (KCN) was used in the mouse and pig studies and was obtained from Fisher Chemical and Sigma-Aldrich, respectively; sodium cyanide was used in the rabbit studies and was obtained from Sigma-Aldrich. The pK_a of cyanide is 9.2, and thus, at physiologic pH, greater than 98% of cyanide is in the form of HCN; the boiling point of HCN is 26.3°C (79.3°F), making HCN partially volatile at room temperature. For the mouse studies, we dissolved the KCN in 0.1 N sodium hydroxide, and for the rabbit and pig studies, in which the cyanide was injected intravenously, we dissolved the cyanide salts in phosphate-buffered saline solution immediately before use in stoppered bottles with minimal headspace; in all cases, we discarded unused material per Environmental Health and Safety–approved protocols. All other chemicals and reagents were of the highest grade available.

Mouse Model of Cyanide Poisoning

C57BL/6J adult male mice were exposed to cyanide gas at 587 ppm in a custom-made sealed gas chamber maintained at 30°C (86°F). Cyanide gas was generated in the chamber by injecting KCN into a beaker of sulfuric acid. We have shown that the HCN concentration reaches equilibrium within 5 minutes of KCN injection and that the HCN concentration remains stable throughout the exposure period.[1]

Muscle Analysis After Intramuscular Injections in Mice

Mice were injected in the gastrocnemius muscle with 50 μL saline solution, 50 μL 200-mM $NaNO_2$, or 50 μL 500-mM $Na_2S_2O_3$. They were euthanized 24 hours later, and the injected muscle was harvested and flash frozen in isopentane precooled in liquid nitrogen. Cryosections (8 μm) were stained with hematoxylin-eosin or for acid phosphatase activity; the latter detects activated macrophages and is an indication of muscle necrosis. Samples were reviewed in a blinded fashion by a veterinary pathologist using a scale of 0 to 4 to evaluate muscle histology: 0, normal muscle; 1, minimally abnormal by evidence of some edema and few polymorphonuclear leukocytes, but no muscle necrosis; 2, mildly abnormal by evidence of many polymorphonuclear leukocytes and mild muscle necrosis; 3, moderately abnormal by evidence of extensive polymorphonuclear leukocyte infiltration and moderate muscle necrosis; and 4, severely abnormal by evidence of extensive muscle necrosis.

Rabbit Model of Cyanide Poisoning

New Zealand White rabbits 6 to 9 months old were purchased from Western Oregon Rabbit Company (Philomath, OR) and weighed 3.5 to 4.5 kg. They were anesthetized with ketamine and xylazine and mechanically ventilated with 100% oxygen at 20 breaths/min and tidal volume of 50 mL. Intra-arterial and intravenous catheters were inserted for measuring blood pressure and injecting sodium cyanide. After 30 minutes of cyanide infusion, the animals were changed to room air at 16 breaths/min. When the mean arterial blood pressure decreased to less than 70% of baseline, the animals were injected in the left pectoral muscle with either 240 μL saline solution or 120 μL 300-mM $NaNO_2$ and 120 μL 3.0-M $Na_2S_2O_3$; the $NaNO_2$ and $Na_2S_2O_3$ injections were spaced 1 cm apart. Cyanide infusion was continued for 30 minutes post–saline solution or antidote injection; animals that survived an additional 60 minutes after the cyanide infusion was stopped were euthanized.

Pig Model of Cyanide Poisoning

Yorkshire swine, *Sus scrofa domesticus,* were purchased from Oak Hill Genetics (Ewing Township, IL). During a 2-week quarantine period, the animals were acclimated to being suspended in a sling and breathing through a nose cone. On the day of study, the animals weighed 50 kg (SD 5 kg). Animals were anesthetized with isoflurane and mechanically ventilated during placement of an arterial Millar catheter in the femoral artery for measuring blood pressure and collecting blood, a central line in the external jugular vein for administering KCN, and an auricular vein catheter for administering 0.9% saline solution at 5 mL/kg body weight while catheters were placed. Breathing rate and tidal volume were monitored with a pneumotachometer, and cardiac rate and rhythm were monitored by ECG.

After catheters were placed, animals were removed from mechanical ventilation and allowed to breathe air spontaneously. Once the animals were stable, respiratory and cardiac data were collected for 2 minutes, and then

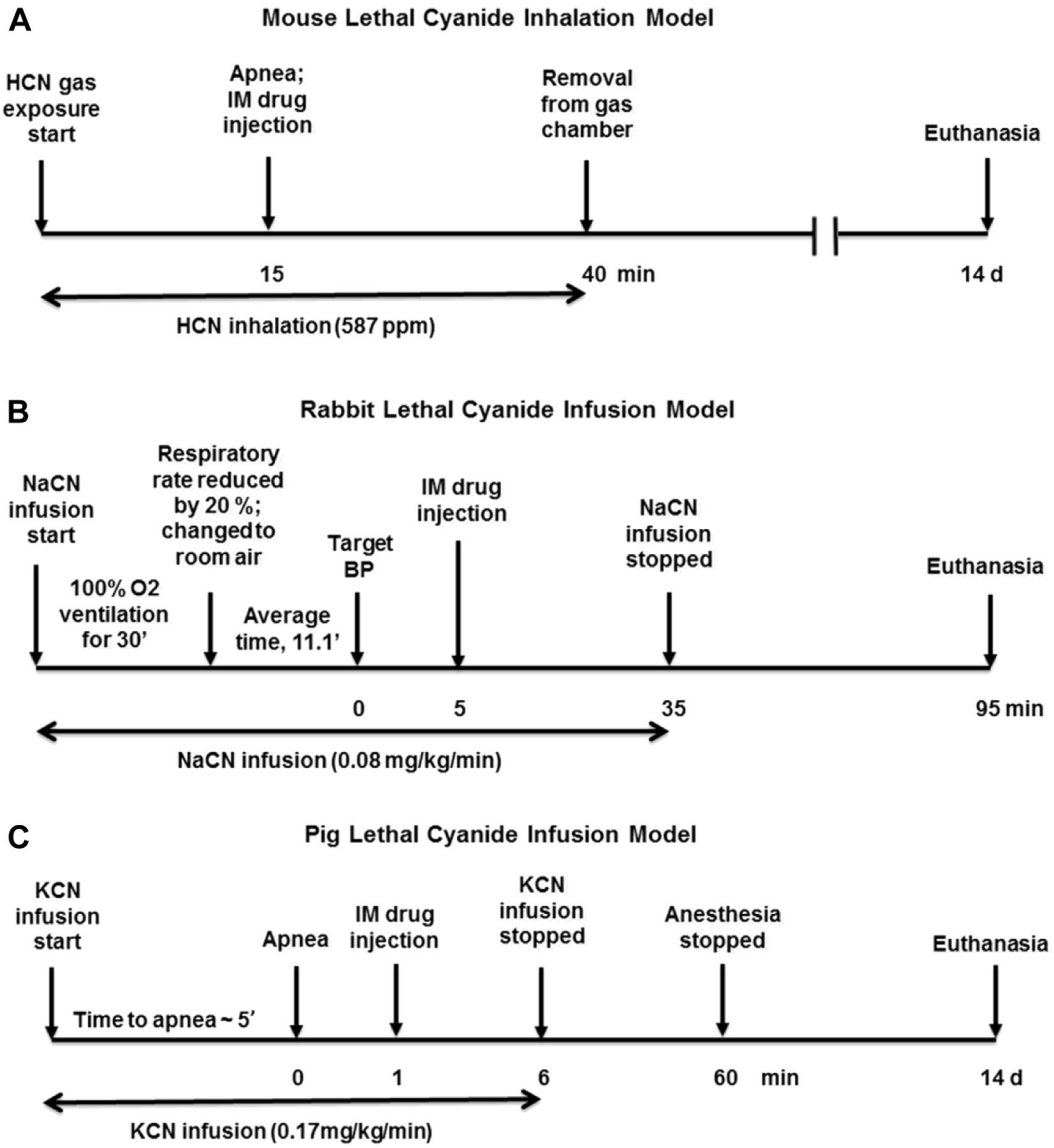

Figure E1. Diagrammatic representation of mouse, rabbit, and pig cyanide exposure models. *A*, A mouse was placed into a sealed plastic chamber and anesthetized with 2% isoflurane. Time zero is the beginning of cyanide exposure, when a solution of sodium cyanide was injected into a beaker of sulfuric acid, generating HCN gas at 587 ppm. After 15 minutes, the mouse was removed, injected intramuscularly with test agent, and placed back in the chamber, which was regenerated with isoflurane and HCN. The mouse was observed continuously, and an animal that showed no evidence of respiration for 3 minutes was declared dead and removed from the chamber. Mice that were still alive at 40 minutes were removed from the chamber, allowed to recover from anesthesia, and observed for 14 days. *B*, Rabbits were anesthetized, intubated, and ventilated with 100% oxygen. Sodium cyanide was then administered intravenously at 0.08 mg/kg per minute. After 30 minutes, the respiratory rate was reduced by 20% and the animals were changed to room air. Time zero was called when the animal's mean arterial pressure decreased to less than 70% of baseline (on average, 11.1 minutes [SD 1.3] after breathing room air). Test drugs were then injected intramuscularly at 5 minutes, and the cyanide was continued for another 30 minutes, ie, 35 minutes after the target blood pressure was reached. Surviving animals were euthanized at 95 minutes. *C*, Pigs were anesthetized and intubated, but not ventilated. KCN was administered at 0.17 mg/kg per minute, and time zero was called when the animals become apneic, on average 5.2 minutes [SD 0.8] after the start of the cyanide infusion. One minute later, the pigs received the test antidote, and 5 minutes later, ie, 6 minutes after the call of apnea, the KCN infusion was stopped. Animals that survived for 60 minutes were removed from anesthesia and observed daily for 14 days, at which time they were euthanized and a necropsy was performed.

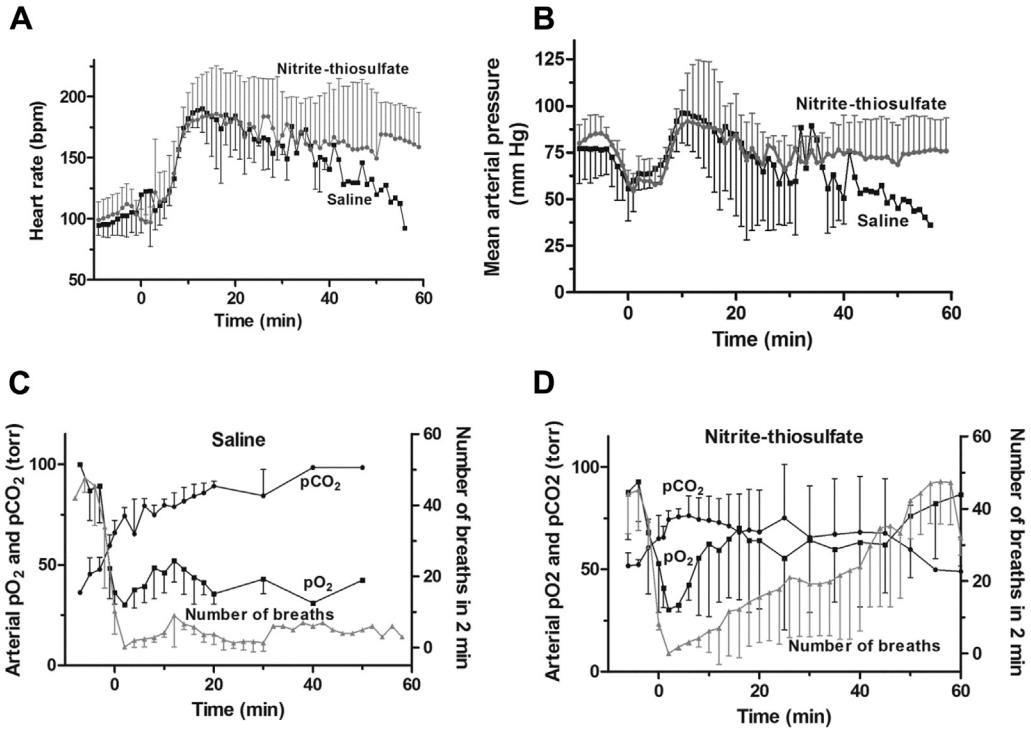

Figure E2. Cardiac and respiratory parameters in a lethal pig model. Pigs were administered an intravenous infusion of KCN and, at 1 minute postapnea, received an intramuscular injection of saline solution or $NaNO_2$ at 0.69 mg/kg and $Na_2S_2O_3$ at 20.8 mg/kg. The pulse rate (*A*), blood pressure (*B*), and respiratory rate and arterial PO_2 and PCO_2 (*C* and *D*) were monitored continuously during the experiment. In *A* and *B*, the saline solution–treated group is designated by black squares and the nitrite- or thiosulfate-treated group by gray circles. *C* and *D*, The saline solution– and nitrite- or thiosulfate-treated groups, respectively. Error bars denote the SD, and only unidirectional bars are shown for the sake of clarity.

KCN was started at 0.17 mg/kg per minute through the external jugular catheter with a syringe pump. Animals became apneic generally after approximately 5 minutes, and then 1 minute after onset of apnea, they were injected in the right vastus medialis muscle with either 1.5 mL saline solution or $NaNO_2$ and $Na_2S_2O_3$; the $NaNO_2$ and $Na_2S_2O_3$ injections were spaced 1 cm apart. The cyanide infusion was continued for an additional 5 minutes and then stopped. This model was shown in pilot experiments to yield 100% lethality. At 1 hour after apnea, catheters were removed and surviving animals were allowed to recover from anesthesia. The animals were transferred back to their kennels, where they were monitored for 14 days.

Measurement of Arterial Oxygenation, Lactate, and Electrolytes

Arterial blood was obtained at the indicated times and analyzed with a commercial blood analyzer/co-oximeter. Parameters measured were pH; PO_2; PCO_2; methemoglobin concentration; and sodium, potassium, calcium, chloride, and lactate concentrations.

Necropsies were performed on animals that survived 2 weeks, concentrating on the brain because it is a major cyanide target. Examinations were performed in a blinded fashion by 2 board-certified veterinary pathologists.

RESULTS

Cardiac and Respiratory Function in the Pigs

In all animals, the pulse rate increased after the cyanide infusion began but decreased at apnea (Figure E2*A*). Within 8 to 12 minutes postapnea, the pulse rate increased to values even higher than preapnea rates in both groups and then decreased slowly during the next 40 minutes in the nitrite- or thiosulfate-treated group. In the control group, the pulse rate decreased more rapidly, but because the values are averages of fewer and fewer animals, the results are skewed toward the longer-surviving ones. The mean arterial pressure followed a pattern similar to that of the pulse rate, but the initial apnea-associated decrease was more pronounced (Figure E2*B*).

After the initial decrease in respiratory rate to zero, some animals in the control group took intermittent breaths, but

none showed sustained breathing (Figure E2*C*, right *y* axis). This is to be contrasted with the nitrite- or thiosulfate-treated group, which showed a progressive increase in respiratory rate to baseline values by approximately 55 minutes postapnea (Figure E2*D*). The PO_2 and PCO_2 values tracked with the respiratory rate, with the PO_2 decreasing and the PCO_2 increasing in the control group but returning to baseline values in the nitrite- or thiosulfate-treated animals (Figure E2*C* and *D*). As would be expected, the arterial pH also tracked with the respiratory rate, as well as with the arterial PCO_2 and lactate concentration, decreasing to approximately 7.3 at apnea in all animals; it continued to decrease in the saline solution–treated animals to approximately 7.0 at death, whereas in the nitrite- or thiosulfate-treated animals, it reached a nadir of approximately 7.2 at 8 to 10 minutes postapnea and then returned to normal by 60 minutes postapnea.

REFERENCE

1. 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.