Title
The combination of cobinamide and sulfanegen is highly effective in mouse models of cyanide poisoning

Journal
Clinical Toxicology, 49(5)

ISSN
1556-3650

Authors
Chan, Adriano
Crankshaw, Daune L
Monteil, Alexandre
et al.

Publication Date
2011-06-01

DOI
10.3109/15563650.2011.584879

Published in final edited form as:
Clin Toxicol (Phila). 2011 June ; 49(5): . doi:10.3109/15563650.2011.584879.

The Combination of Cobinamide and Sulfanegen Is Highly Effective in Mouse Models of Cyanide Poisoning

Adriano Chan[1,2], **Daune L. Crankshaw**[3], **Alexandre Monteil**[3], **Steven E. Patterson**[3], **Herbert T. Nagasawa**[3], **Jackie E. Briggs**[3], **Joseph A. Kozocas**[4], **Sari B. Mahon**[5], **Matthew Brenner**[5], **Renate B. Pilz**[1], **Timothy D. Bigby**[1,2], and **Gerry R. Boss**[1,6]

[1]Department of Medicine, University of California, San Diego, La Jolla, CA 92093-0652

[2]Medicine Service, VA San Diego Healthcare System, San Diego, CA 92161

[3]Center for Drug Design, University of Minnesota, Minneapolis, MN 55455-0213

[4]SRI International, Menlo Park, CA, 94025-3493

[5]Beckman Laser Institute and Medical Clinic, University of California, Irvine, CA 92612-1475

SUMMARY

Context—Cyanide poisoning is a major contributor to death in smoke inhalation victims and accidental exposure to cyanide occurs in a variety of industries. Moreover, cyanide has the potential to be used by terrorists, particularly in a closed space such as an airport or train station. Current therapies for cyanide poisoning must be given by intravenous administration, limiting their use in treating mass casualties.

Objective—We are developing two new cyanide antidotes—cobinamide, a vitamin B_{12} analog, and sulfanegen, a 3-mercaptopyruvate prodrug. Both drugs can be given by intramuscular administration, and therefore could be used to treat a large number of people quickly. We now asked if the two drugs would have an augmented effect when combined.

Materials and Methods—We used a non-lethal and two different lethal models of cyanide poisoning in mice. The non-lethal model assesses neurologic recovery by quantitatively evaluating the innate righting reflex time of a mouse. The two lethal models are a cyanide injection and a cyanide inhalation model.

Results—We found that the two drugs are at least additive when used together in both the non-lethal and lethal models: at doses where all animals died with either drug alone, the combination yielded 80 and 40% survival in the injection and inhalation models, respectively. Similarly, drug doses that yielded 40% survival with either drug alone yielded 80 and 100% survival in the injection and inhalatiion models, respectively. As part of the inhalation model, we developed a new paradigm in which animals are exposed to cyanide gas, injected intramuscularly with antidote, and then re-exposed to cyanide gas. This simulates cyanide exposure of a large number of people in a closed space, because people would remain exposed to cyanide, even after receiving an antidote.

Conclusion—The combination of cobinamide and sulfanegen shows great promise as a new approach to treating cyanide poisoning.

Keywords

Inhalation exposure; intramuscular injection; lethal model; non-lethal model

[6]Corresponding Author: 9500 Gilman Dr., La Jolla, CA 92093-0652, gboss@ucsd.edu, Phone: (858) 534-8805, FAX: (858) 534-1421.

INTRODUCTION

Over five billion pounds of cyanide are produced annually worldwide for use in a variety of industries, including gold extraction from metal ore, electroplating, and paint production.[1] Other sources of cyanide exposure include residential and industrial fires, improperly prepared cassava root, and the drug sodium nitroprusside, which releases five cyanide ions for every nitric oxide molecule.[2-5] Generally, only a small number of people are exposed to cyanide at a time, but large scale cyanide exposures are possible. For example, some authors have suggested a possible role of cyanide in addition to methyl isocyanate in the Bhopal, India disaster in 1984.[6, 7] Moreover, the Japanese Aum Shinrikyo cult tried to release cyanide gas in a Tokyo subway station in 1995, Joseph Konopka, alias "Dr. Chaos," sequestered cyanide in a Chicago subway in 2002, and an Al-Qaeda plot was uncovered for cyanide release in the New York City subway system.[8-10] Thus, the potential exists for a large number of people to be exposed to cyanide, including cyanide gas release by terrorists in enclosed public spaces.

Current drugs for treating cyanide poisoning are hydroxocobalamin, a direct cyanide scavenger, sodium nitrite, which oxidizes hemoglobin to methemoglobin—the latter being a cyanide scavenger, and sodium thiosulfate, which provides substrate for the cyanide detoxifying enzyme rhodanese.[11-13] All three agents must be given intravenously over 5-15 minutes, and, therefore, do not lend themselves well to treating mass casualties. We are developing two new drugs as cyanide antidotes that can be given by intramuscular injection —cobinamide, a hydroxocobalamin analog, and sulfanegen, a 3-mercaptopyruvate prodrug (structures of the two compounds are shown in Fig. 1). Compared to hydroxocobalamin, cobinamide: (*i*) binds two cyanide ions (hydroxocobalamin binds only one), (*ii*) has a much higher binding affinity for cyanide (K_a overall 10^{22} M^{-2} compared to 10^{12} M^{-1}), and (*iii*) is several-fold more water soluble.[14] These chemical differences translate into important biological differences, because we have shown that cobinamide is a considerably more potent cyanide antidote than hydroxocobalamin in a non-lethal rabbit model and a lethal mouse model.[15, 16] We should note that cobinamide has a higher affinity for nitric oxide than hydroxocobalamin, which could contribute to cobinamide's greater efficacy as a cyanide antidote, because nitric oxide regulates cytochrome c oxidase, the main cyanide target [17-20]. Sulfanegen is also a potent cyanide antidote, because 3-mercaptopyruvate is a substrate for 3-mercaptopyruvate sulfur transferase (3-MPST), a ubiquitous cyanide detoxifying enzyme that converts cyanide to thiocyanate. Although sulfanegen acts similarly to thiosulfate, it is more effective, because rhodanese is limited in tissue distribution compared to 3-MPST and is present only in mitochondria, whereas 3-MPST is present in both cytosol and mitochondria.[21-24] We have shown sulfanegen to be effective in non-lethal mouse and rabbit models.[25, 26] Since cobinamide and sulfanegen have different mechanisms of action, we hypothesized that combining them would yield an augmented effect.

METHODS

Materials

Mice—Swiss-Webster ND-4 male mice weighing 25-34 g were used in the non-lethal studies at the University of Minnesota, and C57/BL6J male mice weighing 20-25 g were used in the lethal studies at the University of California, San Diego. The former were from Harlan Labs (Indianapolis, IN) and were fed *ad libitum* Teklad #8604 rodent chow; the latter were from Jackson Laboratories (Bar Harbor, ME) and were fed *ad libitum* Teklad #7001. All studies were carried out according to NIH Guidelines for the Care and Use of Laboratory Animals; the University of Minnesota studies were approved by the University of Minnesota Institutional Animal Care and Use Committee and the UCSD studies were approved by the

Veterans Administration San Diego Healthcare System's Institutional Animal Care and Use Committee.

Chemicals and Cyanide Gas Generation—Aquohydroxocobinamide was produced as described previously and administered with equimolar amounts of sodium sulfite to improve intramuscular absorption;[15] this preparation of sulfitohydroxocobinamide is referred to as cobinamide. The 3-mercaptopyruvate prodrug disodium 3-mercaptopyruvatedithiane was synthesized as described previously and is referred to as sulfanegen.[25] Hydrogen cyanide gas (HCN) was generated in a custom-made, gas-tight, acrylic glass (Plexiglas®) chamber described previously.[15] The chamber has three ports: one for injecting anesthetic (isoflurane), one for injecting potassium cyanide into a stirred beaker containing sulfuric acid, rapidly generating HCN in the chamber, and one for sampling gas from the far side of the chamber from where the HCN is generated. The mice are separated by a plastic screen from the HCN-generating system and from a fan that rapidly equilibrates the anesthetic and HCN content; temperature is maintained at 30°C by a Pelletier heater and feedback loop controller.

Non-Lethal Cyanide Exposure Model

The righting reflex is the innate response of a mouse to move from the underneath side of a wire mesh screen to the top side of the screen.[27, 28] This is tested by placing a mouse on a screen and quickly inverting the screen; a normal mouse will right himself within a few seconds (Fig 2A). However, mice that have been exposed to toxic, but sublethal, doses of cyanide require longer to right themselves. Efficacy of an antidote is measured by the quantitative reduction in the righting reflex time. The advantage of this model is that it tests neurologic function, and neurologic complications are well known to occur in humans exposed to sublethal cyanide concentrations.[12, 29] We found that sodium cyanide injected into the peritoneal cavity at a dose of 0.098 mmol/kg markedly extends the animals' righting reflex time.[25] Cobinamide and sulfanegen were injected into the peritoneal cavity at varying times before the cyanide as indicated in Fig. 2B. At least four animals were studied per condition.

Lethal Cyanide Exposure Models

We used two different lethal models of cyanide poisoning: a parenteral injection model and an inhalation model. The two models complement each other, because the injection model provides an exact measure of cyanide received by the animal, whereas this is not known in the inhalation model; however, the injection model does not simulate most cyanide exposures, whereas the inhalation model does.

General Animal Handling—Mice were anesthetized with isoflurane to minimize pain and distress during cyanide exposure. Conducting this study on awake animals was considered inhumane and was strongly discouraged by the UCSD institutional animal care guidelines. Respiration of anesthetized animals was regular, albeit reduced in rate, and the animals did not require mechanical ventilation. Due to the rapid metabolism and large body surface-to-volume ratios of mice, hypothermia of anesthetized animals was a concern. To address this possibility, animals receiving parenteral cyanide were kept on a water-heated operating platform maintained at 36°C, and, as mentioned above, animals exposed to cyanide gas were in a chamber maintained at 30°C. In all experiments, surviving animals were observed for at least 24 h post experiment to determine if they manifested adverse effects from the cyanide or the drugs. Five or more animals were studied per condition.

Parenteral Injection Models

Intravenous Sulfanegen Pre-Cyanide Exposure: Prophylactic administration of a cyanide antidote could be useful to firefighters, emergency medical personnel, and industrial personnel working with cyanide, and we, therefore, tested sulfanegen in a pre-cyanide exposure model. Mice were anesthetized in an induction chamber with 3% isoflurane and 100% O_2, and then maintained on a nose-cone supply of 2% isoflurane in room air. Sulfanegen dissolved in 0.1 ml sterile saline was injected into the lateral tail vein; control animals received 0.1 ml of intravenous saline. Immediately afterwards, 0.24 mmol/kg of potassium cyanide (KCN) dissolved in 10 mM sodium carbonate, pH 9.5 (final concentration 20 mM) was injected into the peritoneal cavity. This is a fully lethal dose, and, in the absence of antidote, the following sequence of events occurs post cyanide injection: (*i*) animals become apneic within 1 min, i.e., all evidence of breathing ceases; (*ii*) after ~ 1 min of apnea, they develop agonal breathing characterized by diaphragmatic and chest muscle spasm; and (*iii*) after 1-4 min of agonal breathing, all respiratory activity ceases, and the animals are considered dead.

Intramuscular Sulfanegen and Cobinamide Post-Cyanide Exposure: Although prophylactic use of a cyanide antidote is suitable for some conditions, an antidote that can be given both pre- and post-cyanide exposure would be more useful. We, therefore, modified the above protocol and gave sulfangegen after cyanide. We choose an intramuscular route to administer sulfanegen, since that would be the easiest delivery mode in the field, particularly in the case of mass casualties. Because this is a post-cyanide exposure model and because the drugs were given intramuscularly, the KCN dose had to be decreased to 0.16 mmol/kg to provide a feasible time frame when antidotes could be given; this KCN dose is still 100% lethal. Sulfanegen, cobinamide, or both were injected into the gastrocnemius muscle 3 min after the intraperitoneal cyanide injection. The volume injected was 50 μl, and when both drugs were used, they were injected separately into the left and right legs. Control mice received 50 μl of normal saline or sodium sulfite by intramuscular injection.

HCN Gas Exposure Model—Mice were placed in the acrylic glass chamber and anesthetized by injecting sufficient isoflurane into the chamber to achieve a concentration of 2% (v/v) when fully evaporated. The mice become anesthetized within 1-2 min, but 5 min are allowed to be sure they are fully anesthetized and in a homeostatic state before being exposed to cyanide. Hydrogen cyanide is generated by injecting 0.1 M KCN into a beaker in the chamber containing 1 M sulfuric acid. The resulting HCN equilibrates quickly in the chamber, reaching a constant concentration within 10 min that is sustained for at least 30 min. HCN in the chamber was measured as described previously by withdrawing 10 ml of gas from the sampling port.[15] Respiratory status of the mice was monitored constantly throughout the cyanide exposure period.

Intraperitoneal Sulfanegen Pre-Cyanide Exposure: Mice were injected intraperitoneally with 0.1 ml of a sulfanegen solution or normal saline. Fifteen minutes later they were placed in the cyanide exposure chamber, anesthetized, and exposed to 534 ppm HCN; the amount of KCN injected was calculated using the chamber volume and the standard molar volume of an ideal gas, and measured as described above. Surviving animals were allowed to awaken and recover in room air.

Intramuscular Sulfanegen and Cobinamide Post-Cyanide Exposure: To simulate a mass exposure to cyanide gas with people remaining in the cyanide-contaminated area even after being treated, we exposed mice to cyanide gas, injected them intramuscularly with sulfanegen and/or cobinamide, and then re-exposed them to the cyanide gas. We chose a 20 min initial exposure period and a 20 min post-treatment exposure period, because we

assume ~ 20 min will be required for emergency medical personnel to arrive at a disaster scene and another 20 will be required to evacuate people. Mice were placed in the exposure chamber, anesthetized, and then exposed to 480 ppm HCN for 20 min. They were removed from the chamber and injected with sulfanegen, cobinamide, or both; control mice received intramuscular saline or sodium sulfite. Immediately after the injections, they were placed back in the chamber, the chamber was recharged with isoflurane and HCN, and they were re-exposed to the HCN for 20 min. Between the first and second HCN exposures, the mice were out of the chamber for < 90 sec, remaining anesthetized during this time.

Data Analysis

The data are presented as Kaplan-Meier Survival Curves generated by Prism 5 Software (GraphPad, Carlsbad, CA). Data were considered statistically significant at a p value of less than 0.05.

RESULTS

Non-Lethal Model

We have shown previously that sulfanegen improves the righting reflex time in mice exposed to non-lethal cyanide doses,[25] and asked whether the combination of sulfanegen and cobinamide yielded better results than either drug alone. In this model, mice that receive no antidote right themselves within 70 min of being exposed to cyanide (Fig. 2B). Mice that received an intraperitoneal injection of either 0.22 mmol/kg of cobinamide or 0.58 mmol/kg of sulfanegen prior to the cyanide injection righted themselves more quickly than animals receiving saline (Fig. 2B). The reduction in righting time correlated with when the antidote was injected: the closer to cyanide exposure the antidotes were injected, the less time required for the mice to right themselves (Fig. 2B). When injected at short times before cyanide exposure, i.e., at 5 and 10 min, the drug combination did not appear any better than either drug alone, which was likely from the single drugs already having a pronounced effect (Fig. 2B). However, combining cobinamide and sulfanegen markedly reduced the righting time when the drugs were given 60 and 30 min before cyanide exposure (Fig. 2B). Thus, the combination of cobinamide and sulfanegen was more potent than either drug alone.

Lethal Models

Having shown a beneficial effect of combining cobinamide and sulfanegen in a non-lethal model of cyanide poisoning, we studied the combination of the two drugs in a lethal injection model and a lethal inhalation model. In both models, we first had to study sulfanegen by itself, because we had not previously studied it in a lethal model of cyanide poisoning.

Injection Model

Intravenous Sulfanegen Pre-Cyanide Exposure: We tested if sulfanegen was effective if given before cyanide, and gave it intravenously in doses ranging from 0.08-0.24 mmol/kg immediately prior to an intraperitoneal injection of 0.24 mmol/kg KCN. All control mice died within 5-7 min of the cyanide injection (Fig. 3A). In contrast, sulfanegen at an equimolar amount as KCN yielded 100% survival, with lower sulfanegen doses yielding lower survival rates (Fig. 3A). By plotting percent survival versus the sulfanegen dose, the effective sulfanegen dose that yeided 50% survival (ED_{50}) was found to be 0.14 mmol/kg (Fig. 3B). In the same model with the same KCN dose, the ED_{50} for cobinamide was 0.054 mmol/kg, indicating cobinamide was 3-4-fold more potent than sulfanegen [15].

Intramuscular Sulfanegen Post-Cyanide Exposure: In these post-cyanide exposure studies, we found that 0.06 mmol/kg of sulfanegen rescued 100% of the mice when given as long as three minutes after the cyanide injection (Fig. 3C). At a dose of 0.04 mmol/kg of sulfanegen, none of the mice survived, pointing to the steep dose response curve of a potent poison like cyanide. Although three minutes is a short window for antidote treatment, it must be remembered that the mice had already been apneic for two minutes at that time. We have shown previously that intramuscular cobinamide also rescues animals in this model.[15]

Intramuscular Cobinamide and Sulfanegen Post-Cyanide Exposure: We studied the combination of cobinamide and sulfanegen given by intramuscular injection post cyanide exposure. At doses of either drug that still resulted in 100% mortality, the combination of the two drugs rescued 80% of the mice, clearly indicating the two drugs had a beneficial effect when added together (Fig. 4A). In a limited dose response experiment, we found that cobinamide and sulfanegen doses that rescued 40% of the mice when used singly rescued 80% of the animals when combined together (Fig. 4B).

Inhalation Model

Sulfanegen Administered Pre-Exposure: Sulfanegen was given by intraperitoneal injection 15 min prior to exposing mice for 30 min to 534 ppm hydrogen cyanide gas. All control mice receiving saline injections died, whereas 0.2 mmol/kg sulfanegen yielded 100% survival; lower doses of sulfanegen between 0.06 and 0.1 mmol/kg had intermediate survival (Fig. 5A). The ED_{50} of sulfanegen was found to be 0.077 mmol/kg (Fig. 5B). In the same model, the ED_{50} for cobinamide was 0.029 mmol/kg, again indicating cobinamide is about three-fold more potent than sulfanegen.[15]

Cobinamide and Sulfanegen Administered Post-Cyanide Exposure with Continued Cyanide Exposure: Control animals injected intramuscularly with saline or sodium sulfite all died within 14 min after the second cyanide exposure (Fig. 6A). Mice receiving 0.03 mmol/kg of cobinamide or 0.04 mmol/kg of sulfanegen also died, but, on average, lived 6-12 min longer than controls. Combining the two drugs together rescued 40% of the animals, once again indicating an augmented effect of the two drugs (Fig. 6A). In a limited dose response experiment, we found that 0.04 mmol/kg of cobinamide and 0.05 mmol/kg of sulfanegen rescued 40% of the mice, and that the combination rescued 100% of the mice (Fig. 6B).

DISCUSSION

Cyanide is a potent and rapidly acting poison. Antidotes must act fast and be capable of being administered quickly in the field. Unfortunately, all of the currently available antidotes must be given intravenously; starting an intravenous line takes time, particularly if the patient is hypotensive as is frequently the case in cyanide poisoning. We are developing two drugs, cobinamide and sulfanegen, to be given by intramuscular injection with an autoinjector. In the present work, we studied the combination of these two agents and found they had at least an additive effect.

Three important aspects of this work need to be emphasized. First, we show that sulfanegen is effective in two different lethal mouse models of cyanide poisoning. In previous work, we showed that sulfanegen was effective in non-lethal mouse and rabbit models of cyanide poisoning [25, 26], but that does not necessarily mean it would be effective in lethal models. As a corollary to this first point, we show that sulfanegen is effective both when given in a prophylactic, pre-cyanide exposure mode, as well as when given post-cyanide exposure. The

latter is particularly important, because a cyanide antidote would be expected to be used predominantly in people already exposed to cyanide.

Second, we present data on a model of cyanide gas exposure in which animals are exposed to cyanide gas, treated with an antidote, and then re-exposed to cyanide gas. The importance of this model is that it closely simulates a real-life scenario of multiple people exposed to cyanide gas in an enclosed space, as could happen in a terrorist attack. Even after emergency medical personnel arrive at a disaster scene and administer an antidote, some time would be required to evacuate people from the cyanide-contaminated area; hence the reason for re-exposing the animals to cyanide gas post antidote treatment. While we chose 20 min of cyanide gas exposure followed by another 20 min of exposure after treatment, the model could be adapted to make the cyanide exposure periods any length of time desired, both before and after antidote administration.

The third important aspect of this work is that we show the combination of two drugs—cobinamide and sulfanegen—is clearly better than either alone in treating cyanide poisoned mice. Since the two drugs work by different mechanisms, *a priori* one would have anticipated an improved effect when combined. However, this is not always true, and some early data suggested that equimolar amounts of hydroxocobalamin and sodium thiosulfate can be antagonistic in cyanide-poisoned mice and rabbits; similarly, cobinamide and sodium thiosulfate were less effective than cobinamide alone. [30] More recent data indicate that hydroxocobalamin and sodium thiosulfate can be given together and be effective.[31, 32] Thus, it was important to determine if the combination of cobinamide and sulfanegen was better than either agent alone in treating cyanide poisoning.

Three potential limitations of this study need to be discussed. First, in the lethal models, we used anesthetized animals. This was done because cyanide treatment of animals is classified as a USDA Pain and Distress Category E condition, and we and our IACUC deemed the studies acceptable only if the animals were anesthetized. While anesthesia could have impacted the outcome of the studies, we think this unlikely, because the results from the non-lethal model--in which the animals were not anesthetized--were similar to the lethal model. Moreover, we and many other investigators have used anesthetized animals when studying cyanide toxicity.[15, 16, 32-35] Second, cobinamide and sulfanegen were injected into different muscles; this was done, because we had found that cobinamide reacts with sulfanegen, reducing the efficacy of both drugs. However, we have since developed a cobinamide formulation that does not react with sulfanegen, allowing co-administration. The third potential limitation is that we did not conduct a full dose response curve of cobinamide and sulfanegen, singly, and in combination, to determine if their combined effect is additive or synergistic. This would have required a very large number of animals, and NIH guidelines require that a minimal number of animals be used. We did not feel the extra animals were justified, because it is not imperative to know if the drug combination is additive or synergistic. The US Food and Drug Administration does not require evidence of synergy in drug combinations, rather that the effect of the two drugs together is better than the effect of either drug alone at the same dosage. Clearly, the combination of cobinamide and sulfanegen meets this requirement.

Using a drug combination to treat cyanide poisoning has several major advantages. First, lower doses of each drug can be used, minimizing potential side effects. Second, as mentioned previously, an intramuscular mode of administration would be preferred in a mass casualty setting, but this limits the total injected volume to 3-5 ml. Two drugs will provide for greater cyanide neutralization capacity in a given volume, because the solubility of each drug is independent of the other. And third, using two drugs with different

mechanisms of action—a direct cyanide scavenger and a 3-MPST substrate--could provide for a more stable effect.

In conclusion, we have found that sulfanegen is an effective cyanide antidote in lethal mouse models, and that the combination of cobinamide and sulfanegen provides at least an additive effect in treating cyanide poisoning.

References

1. Dzomback, DA.; Ghosh, RS.; Wong-Chong, GM. Cyanide in water and soil: chemistry, risk, and management. CRC Press; 2006.
2. Alarie Y. Toxicity of fire smoke. Crit Rev Toxicol. 2002; 4:259–89. [PubMed: 12184505]
3. Baud FJ, Barriot P, Toffis V, Riou B, Vicaut E, Lecarpentier Y, Bourdon R, Astier A, Bismuth C. Elevated blood cyanide concentrations in victims of smoke inhalation. N Engl J Med. 1991; 325:1761–6. [PubMed: 1944484]
4. Ernesto M, Cardoso AP, Nicala D, Mirione E, Massaza F, Cliff J, Haque MR, Bradbury JH. Persistent konzo and cyanogen toxicity from cassava in northern Mozambique. Acta Trop. 2002; 82:357–62. [PubMed: 12039675]
5. Cottrell JE, Casthely P, Brodie JD, Patel K, Klein A, Turndorf H. Prevention of nitroprusside-induced cyanide toxicity with hydroxocobalamin. N Engl J Med. 1978; 298:809–11. [PubMed: 634316]
6. Broughton E. The Bhopal disaster and its aftermath: a review. Environ Health. 2005; 4:6–15. [PubMed: 15882472]
7. Verma DR. Hydrogen Cyanide and Bhopal. Lancet. 1989; 334:567–8.
8. James Martin Center for Nonproliferation Studies. Chronology of Aum Shinrikyo's CBW Activities. http://cns.miis.edu/reports/pdfs/aum_chm.pdf
9. Konopka, Joseph. http://en.widipedia.org/wiki/Joseph_Konopka
10. Suskin, R. The one percent doctrine: deep inside America's pursuit of its enemies since 9/11. Simon & Schuster; New York, NY: 2006.
11. Borron SW, Baud FJ, Barriot P, Imbert M, Bismuth C. Prospective study of hydroxocobalamin for acute cyanide poisoning in smoke inhalation. Ann Emerg Med. 2007; 49:794–801. [PubMed: 17481777]
12. Way JL. Cyanide intoxication and its mechanism of antagonism. Annu Rev Pharmacol Toxicol. 1984; 24:451–81. [PubMed: 6428300]
13. Gracia R, Shepherd G. Cyanide poisoning and its treatment. Pharmacotherapy. 2004; 24:1358–65. [PubMed: 15628833]
14. Hayward GC, Hill HAO, Pratt JM, Vanston NJ, Williams ARW. The chemistry of vitamin B(12). Part IV.1 The thermodynamic trans-effect. J Chem Soc. 1965:6485–93.
15. Chan A, Balasubramanian M, Blackledge W, Mohammad OM, Alvarez L, Boss GR, Bigby TD. Cobinamide is superior to other treatments in a mouse model of cyanide poisoning. Clin Toxicol (Phila). 2010; 48:709–717. [PubMed: 20704457]
16. Brenner M, Kim JG, Mahon SB, Lee J, Kreuter KA, Blackledge W, Mukai D, Patterson S, Mohammad O, Sharma VS, Boss GR. Intramuscular cobinamide sulfite in a rabbit model of sublethal cyanide toxicity. Ann Emerg Med. 2009; 55:352–363. [PubMed: 20045579]
17. Sharma VS, Pilz RB, Boss GB, Magde D. Reactions of nitric oxide with vitamin B12 and its precursor, cobinamide. Biochemistry. 2003; 42:8900–8. [PubMed: 12873151]
18. Broderick KE, Singh V, Zhuang S, Kambo A, Chen JC, Sharma VS, Pilz RB, Boss GR. Nitric oxide scavenging by the cobalamin precursor cobinamide. J Biol Chem. 2005; 280:8678–85. [PubMed: 15632180]
19. Pearce LL, Bominaar EL, Hill BC, Peterson J. Reversal of cyanide inhibition of cytochrome c oxidase by the auxillary substrate nitric oxide. J Biol Chem. 2003; 278:52139–52145. [PubMed: 14534303]

20. Leavesley HB, Li L, Prabhakaran K, Borowitz JL, Isom GE. Interaction of cyanide and nitric oxide with cytochrome c oxidase: implications for acute cyanide toxicity. Toxicol Sciences. 2008; 101:101–111.
21. Nazifi S, Aminlari M, Alaibakhsh MA. Distribution of rhodanese in tissues of goat (Capra hircus). Comp Biochem Physiol B Biochem Mol Biol. 2003; 134:515–8. [PubMed: 12628382]
22. Sylvester M, Sander C. Immunohistochemical localization of rhodanese. Histochem J. 1990; 22:197–200. [PubMed: 2387754]
23. Nagahara N, Ito T, Minami M. Mercaptopyruvate sulfurtransferase as a defense against cyanide toxication: molecular properties and mode of detoxification. Histol Histopathol. 1999; 14:1277–86. [PubMed: 10506943]
24. Nagahara N, Okazaki T, Nishino T. Cytosolic mercaptopyruvate sulfurtransferase is evolutionarily related to mitochondrial rhodanese. Striking similarity in active site amino acid sequence and the increase in the mercaptopyruvate sulfurtransferase activity of rhodanese by site-directed mutagenesis. J Biol Chem. 1995; 270:16230–5. [PubMed: 7608189]
25. Nagasawa HT, Goon DJ, Crankshaw DL, Vince R, Patterson SE. Novel, orally effective cyanide antidotes. J Med Chem. 2007; 50:6462–4. [PubMed: 18038966]
26. Brenner M, Kim JG, Lee J, Mahon SB, Lemor D, Ahdout R, Boss GR, Blackledge W, Jann L, Nagasawa HT, Patterson SE. Sulfanegen sodium treatment in a rabbit model of sub-lethal cyanide toxicity. Toxicol Appl Pharmacol. 2010; 248:269–276. [PubMed: 20705081]
27. Coughenour LL, Mclean JR, Parker RB. A new device for the rapid measurement of impaired motor function in mice. Pharmacol Biochem Behav. 1977; 6:351–3. [PubMed: 857257]
28. Crankshaw DL, Goon DJW, Briggs JE, DeLong D, Kuskowski M, Patterson SE, Nagaswa HT. A novel paradigm for assessing efficacies of potential antidotes against neurotoxins in mice. Toxicol Lett. 2007; 175:111–117. [PubMed: 18024011]
29. Salkowski AA, Penney DG. Cyanide poisoning in animals and humans: a review. Vet Hum Toxicol. 1994; 36:455–66. [PubMed: 7839575]
30. Evans CL. Cobalt compounds as antidotes for hydrocyanic acid. Br J Pharmacol Chemother. 1964; 23:455–75. [PubMed: 14256807]
31. Hall AH, Rumack BH. Hydroxycobalamin/sodium thiosulfate as a cyanide antidote. J Emerg Med. 1987; 5:115–21. [PubMed: 3295013]
32. Bebarta VS, Tanen DA, Lairet J, Dixon PS, Valtier S, Bush A. 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; 55:345–51. [PubMed: 19944487]
33. 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(Suppl 1):5–15. [PubMed: 16990189]
34. Ivankovich AD, Braverman B, Kanuru RP, Heyman HJ, Paulissian R. Cyanide antidotes and methods of their administration in dogs: a comparative study. Anesthesiology. 1980; 52:210–6. [PubMed: 7369507]
35. Posner MA, Tobey RE, McElroy H. Hydroxocobalamin therapy of cyanide intoxication in guinea pigs. Anesthesiology. 1976; 44:157–60. [PubMed: 1252023]

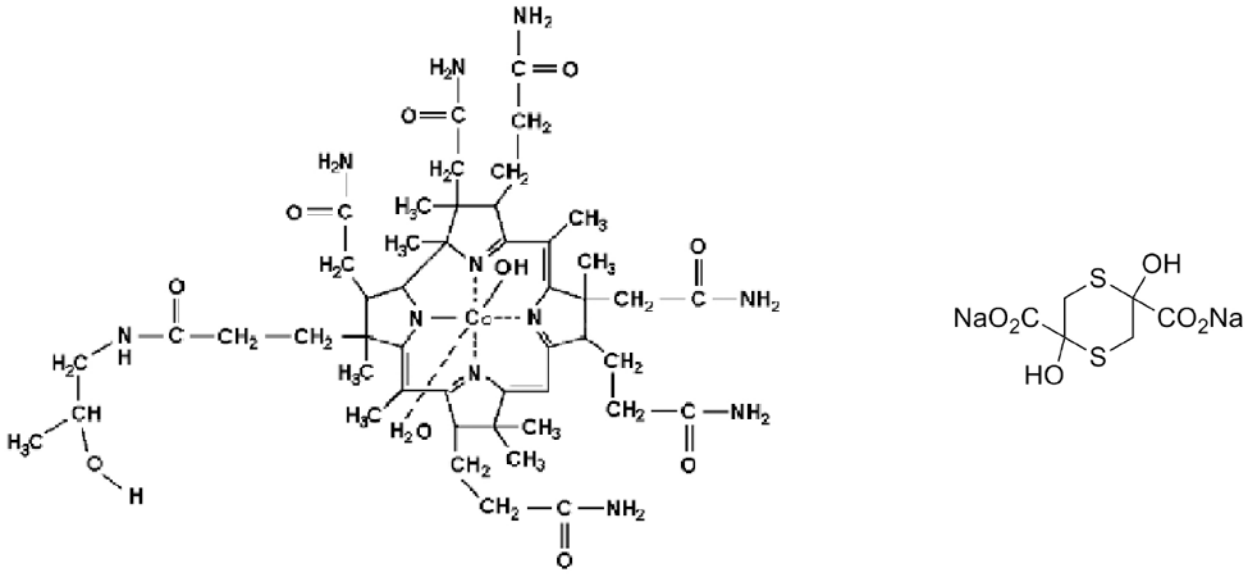

Fig. 1. Structures of Cobinamide and Sulfanegen

The structure of cobinamide is shown on the left and that of sulfanegen on the right. Cobinamide is a vitamin B_{12} (hydroxocobalamin) analog, and sulfanegen is a dimer of 3-mercaptopyruvate.

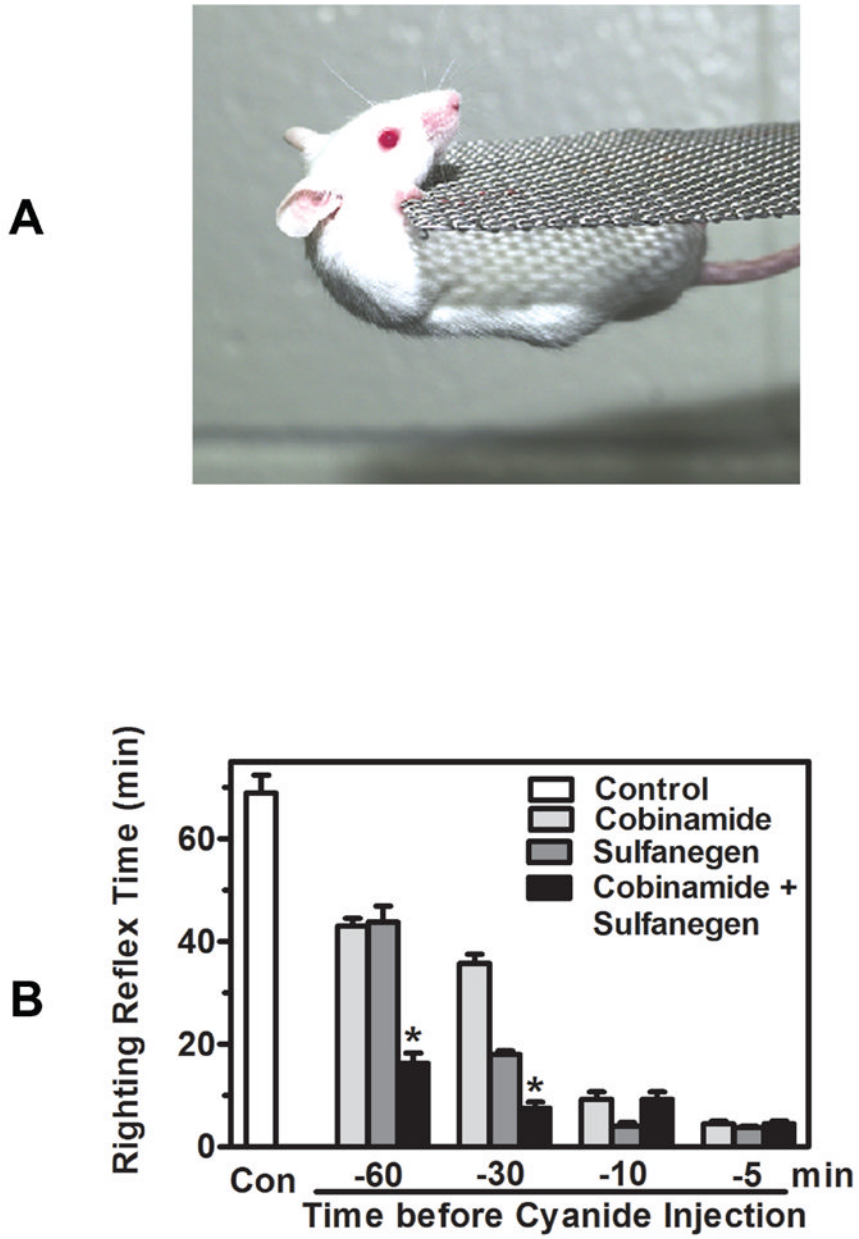

Fig. 2. Non-lethal Cyanide Injection Model

Panel A. A mouse was placed on a horizontal wire mesh screen and the screen was inverted rapidly; the mouse is shown righting itself by crawling from the underside of the screen to the top of the screen. **Panel B.** Mice were injected into the peritoneal cavity with saline (control, Con, open bar), 0.22 mmol/kg cobinamide (light grey bars), 0.58 mmol/kg sulfanegen (dark grey bars), or the combination of the two (black bars) at the indicated times prior to receiving an intraperitoneal injection of 0.098 mmol/kg of sodium cyanide. When the mice had recovered sufficiently to hold onto a mesh screen, the screen was inverted and the total amount of time was recorded until the mice righted themselves. The data for the control mice are for animals injected with saline 60 min prior to the cyanide injection, but similar results were obtained at all the other times. Four mice were studied in each group,

except the control condition where 24 mice were studied. Asterisks indicate a significant difference ($p < 0.05$) between the cobinamide-sulfanegen combination and either drug alone.

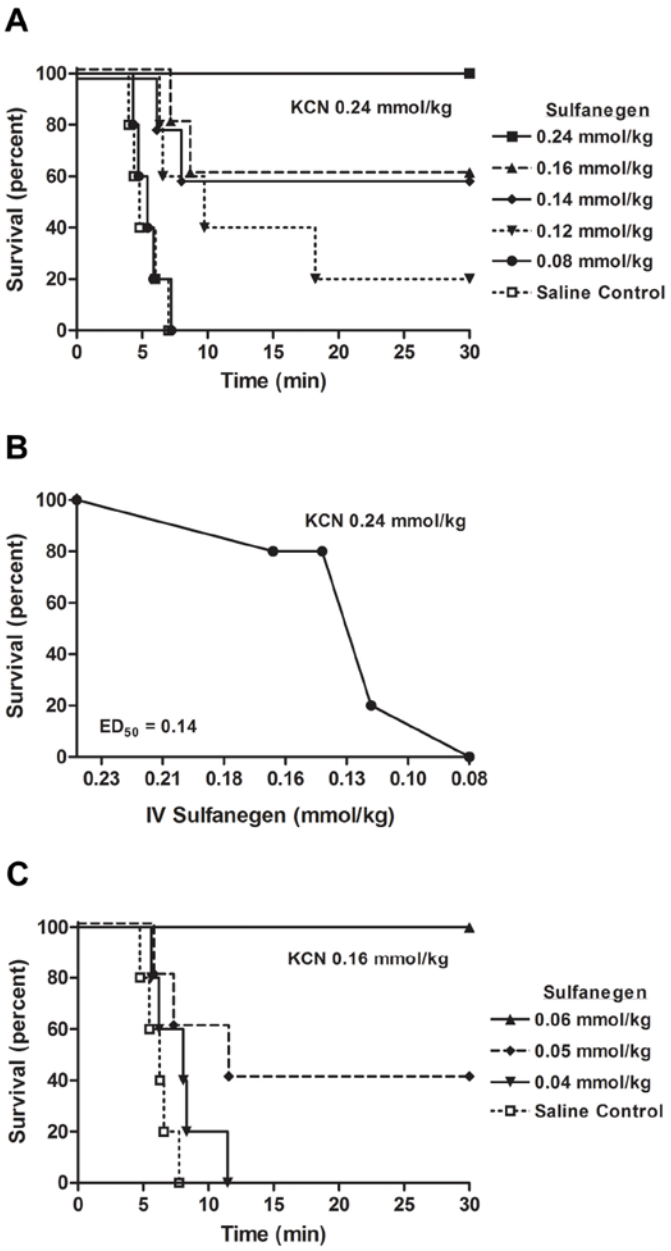

Fig. 3. Effect of Sulfanegen in Lethal Cyanide Injection Model

Panel A. Anesthetized mice were injected intravenously with the indicated amounts of sulfanegen. Immediately thereafter, they were injected in the peritoneal cavity with 0.24 mmol/kg KCN. Mouse survival is plotted on a Kaplan-Meier graph; mice that lived 30 min after cyanide injection remained alive for 24 h. **Panel B.** The data from Panel A were plotted as survival versus sulfanegen dose to determine the ED_{50} of sulfanegen. **Panel C.** Mice were injected in the peritoneal cavity with 0.16 mmol/kg KCN and 3 min later received an intramuscular injection of the indicated doses of sulfanegen. Mouse survival is plotted on a Kaplan-Meier graph. In both Panels A and B, five mice were studied under each condition.

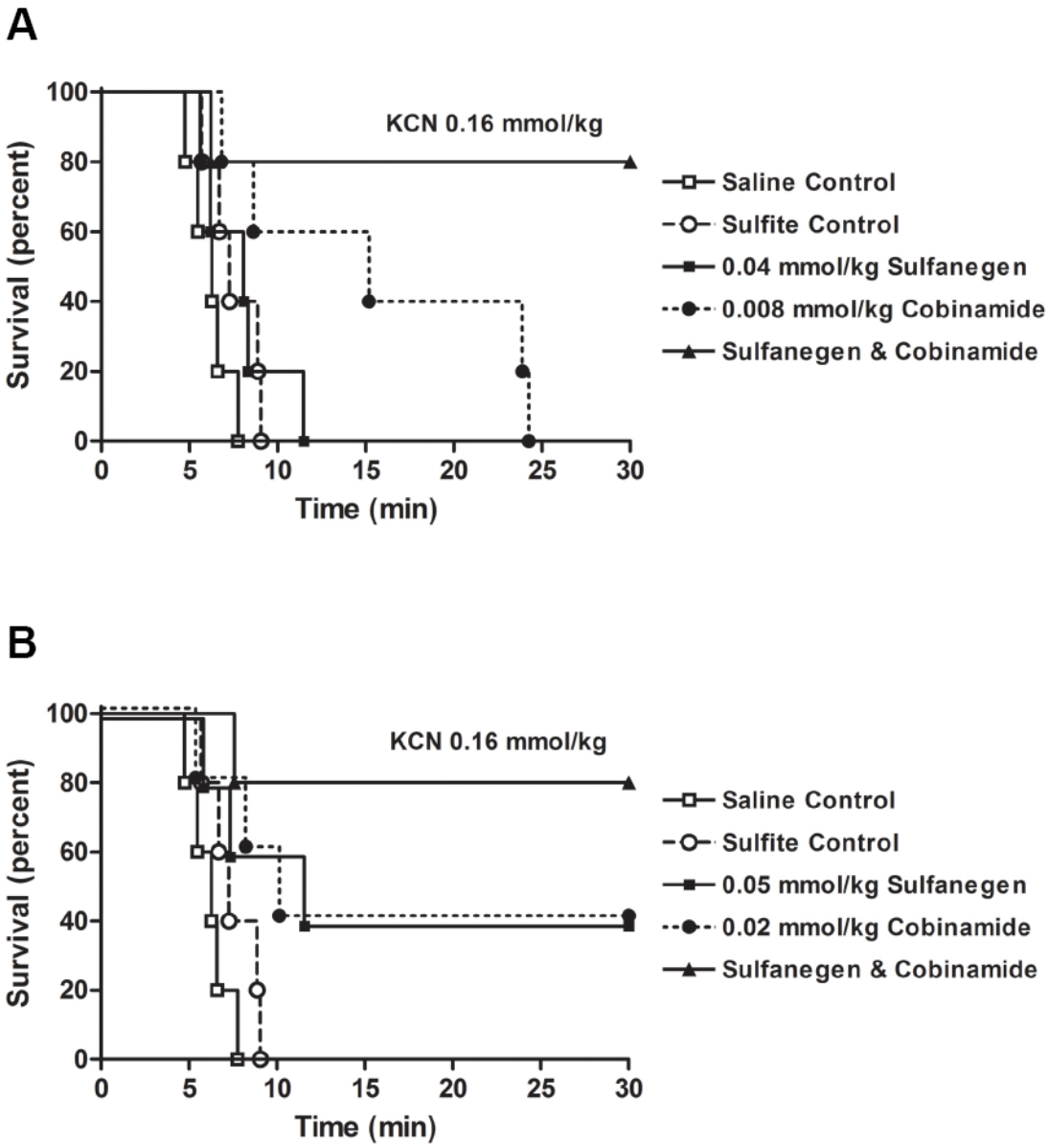

Fig. 4. Effect of the Combination of Cobinamide and Sulfanegen in Lethal Cyanide Injection Model

Anesthetized mice were injected in the peritoneal cavity with KCN as described in Fig. 3, Panel C. Three minutes later they were injected with the indicated amounts of cobinamide and sulfanegen, either alone or in combination (doses in the combination were the same as for the individual drugs). The data are plotted as a Kaplan-Meier survival graph. Mice that survived for 30 min remained alive at 24 h. Higher drug doses were used in Panel B than Panel A to achieve some degree of survival when each drug was used alone. Five mice were used in each condition in both panels.

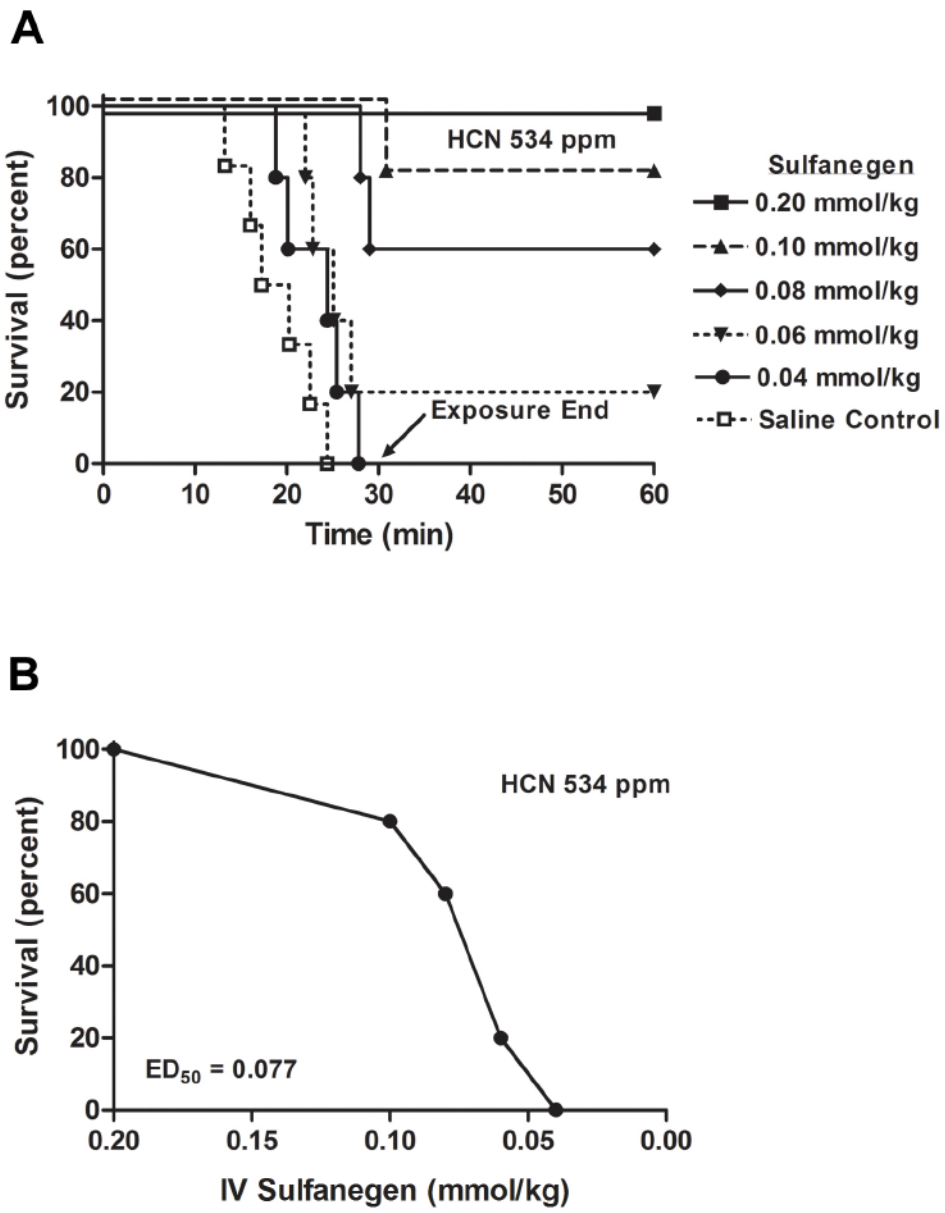

Fig. 5. Effect of Sulfanegen in Lethal Cyanide Inhalation Model

Panel A. Mice were injected in the peritoneal cavity with the indicated amounts of sulfanegen. Fifteen minutes later they were placed in the gas exposure chamber described in Methods and anesthetized with 2% isoflurane. HCN gas (534 ppm) was then generated in the chamber by injecting KCN into a stirred beaker containing sulfuric acid. After 30 min, the mice were removed from the chamber. Survival is plotted as a Kaplan-Meier graph. Five mice were studied under each condition. **Panel B.** The data in Panel A are plotted as survival versus sulfanegen dose to obtain the ED_{50} of the drug.

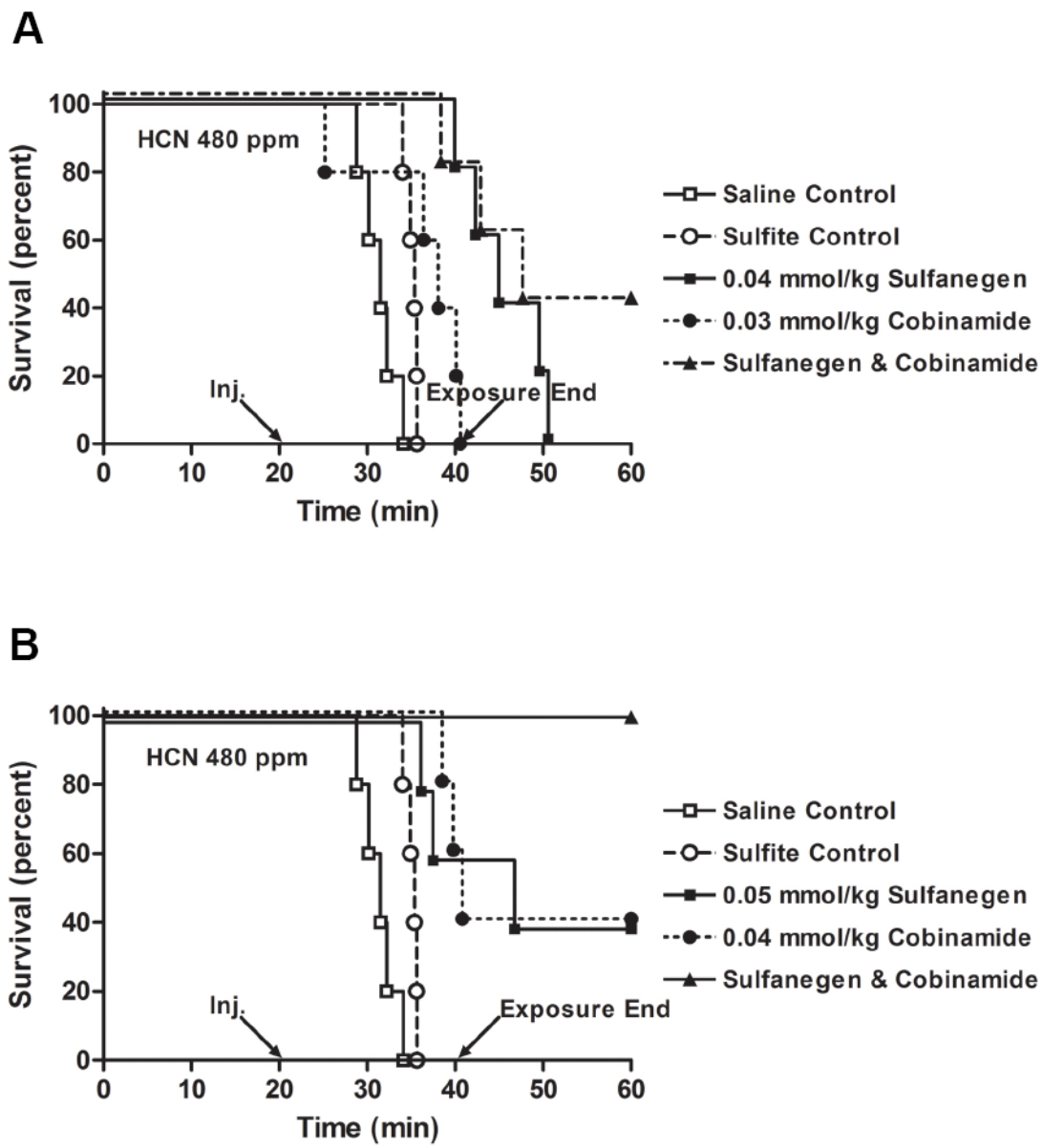

Fig. 6. Effect of the Combination of Cobinamide and Sulfanegen in Lethal Cyanide Inhalation Model: Drugs Given Post-Cyanide Exposure with Continued Cyanide Exposure After Drug Administration

Mice were placed in a gas exposure chamber, anesthetized with isoflurane, and then HCN gas was generated at a concentration of 480 ppm. Twenty minutes later, the mice were removed from the chamber and given an intramuscular injection of cobinamide, sulfanegen, or the combination of the two at the indicated amounts (denoted by “Inj;” doses in the combination were the same as for the individual drugs). They were placed back in the chamber, and the chamber was recharged with isoflurane and HCN. They were exposed to the HCN for an additional 20 min, at which time they were removed from the chamber (denoted by “Exposure End”). Survival is plotted on a Kaplan-Meier graph. As in Fig. 4, higher doses of cobinamide and sulfanegen were used in Panel B to achieve some degree of survival when the drugs were used singly. In both panels, five mice were studied under each condition.