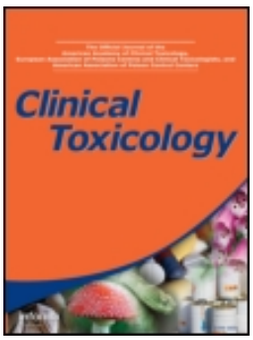

Clinical Toxicology

ISSN: 1556-3650 (Print) 1556-9519 (Online) Journal homepage: https://www.tandfonline.com/loi/ictx20

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To cite this article: Stephen W. Borron, Michael Stonerook & Frances Reid (2006) Efficacy of Hydroxocobalamin for the Treatment of Acute Cyanide Poisoning in Adult Beagle Dogs, Clinical Toxicology, 44:sup1, 5-15, DOI: 10.1080/15563650600811672

To link to this article: https://doi.org/10.1080/15563650600811672

Clinical Toxicology, 44:5–15, 2006

ISSN: 1556-3650 print / 1556-9519 online
DOI: 10.1080/15563650600811672

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ARTICLE

Efficacy of Hydroxocobalamin for the Treatment of Acute Cyanide Poisoning in Adult Beagle Dogs

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Introduction. **The efficacy of hydroxocobalamin for acute cyanide poisoning was compared with that of saline vehicle in dogs.** ***Methods.*** **Anesthetized adult beagle dogs were administered potassium cyanide (0.4 mg/kg/min, IV) until 3 min after the onset of apnea. Hydroxocobalamin (75 mg/kg [n = 19] or 150 mg/kg [n = 18], IV) or saline vehicle [n = 17] was then infused over 7.5 min while animals were ventilated with 100% oxygen, which was stopped after 15 min.** ***Results.*** **In vehicle-treated animals cyanide produced deterioration that culminated in a moribund state requiring euthanasia within 4 h in 10 of 17 animals and in neurological deficits necessitating euthanasia within 2–4 d in an additional 4 animals (mortality rate 82%). Survival through 14 d was observed in 15 of 19 animals administered hydroxocobalamin 75 mg/kg (mortality rate 21%), and 18 of 18 administered hydroxocobalamin 150 mg/kg (mortality rate 0%).** ***Conclusion.*** **Hydroxocobalamin reversed cyanide toxicity and reduced mortality in a canine model.**

Keywords Hydroxocobalamin; Cyanides; Antidotes

INTRODUCTION

The cyanide antidote hydroxocobalamin, the natural form of vitamin B_{12a}, is being investigated for possible introduction in the United States (1–3). Hydroxocobalamin was first licensed for the treatment of cyanide poisoning in 1996 in France, where it is used in both prehospital and hospital settings. Hydroxocobalamin detoxifies cyanide by forming cyanocobalamin, which is excreted in urine. Unlike the currently available Cyanide Antidote Kit (comprising amyl nitrite, sodium nitrite, and sodium thiosulfate), hydroxocobalamin does not compromise the oxygen-carrying capacity of the blood (via the induction of methemoglobinemia) or cause hypotension (due to vasodilatation) (4,5).

Received 4 May 2006; accepted 11 May 2006.
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Successful prehospital and hospital use of hydroxocobalamin for cyanide poisoning from ingestion, smoke inhalation, and occupational exposure has been documented in case reports and series and in a prospective study of fire victims (5–10). However, the antidotal efficacy of hydroxocobalamin for cyanide poisoning cannot be evaluated in a placebo-controlled clinical trial for obvious ethical reasons. In cases in which well-controlled studies in humans cannot be conducted, appropriate studies in animals constitute a vital source of information about effectiveness (11). This article reports the results of a study conducted to compare the efficacy of hydroxocobalamin with that of saline vehicle for the treatment of acute cyanide poisoning in adult beagle dogs.

Cyanide poisoning has been studied in many species including mice, rats, guinea pigs, rabbits, dogs, and primates. The selection of the canine model over other animal models for this study was based on several considerations. The relatively large size of the dog enhances the comparability of modeled cyanide toxicity to cyanide toxicity in humans. Smaller animals metabolize xenobiotics more rapidly than larger animals (allometry) (12), and the window of time between the onset of severe intoxication and death or recovery is narrow (13,14). Therefore, in small species, the ability to administer an antidote after intoxication to model the clinical situation in humans is limited. The large size of the dog also allowed for an indwelling probe for measurement of hemodynamic parameters and sampling over time of an amount of blood sufficient to evaluate pharmacokinetics, which were key secondary study endpoints. Moreover, the pharmacokinetics and pharmacodynamics of hydroxocobalamin in dogs have been thoroughly elucidated, and the canine model is considered predictive of antidotal effects in humans (15,16).

Human exposure to cyanide can occur via various routes including inhalation, ingestion, and dermal exposure. In this study, intravenous infusion was chosen over other means of administering cyanide for several reasons: 1) the ability to titrate the dose to a specific effect; 2) the ability to avoid incomplete absorption of the

toxicant; and 3) the comparability among the various exposure routes of signs, symptoms, and tissue concentrations of cyanide.

MATERIALS AND METHODS

The study was a parallel-group, randomized assessment of the antidotal efficacy of hydroxocobalamin versus saline vehicle administered by intravenous infusion to adult beagle dogs poisoned with an intravenous dose of potassium cyanide. The protocol was approved by the Institutional Animal Care and Use Committee at Battelle Memorial Institute (Columbus, Ohio). The study was conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations for nonclinical studies (21 CFR Part 58), guidelines published in the Guide for the Care and Use of Laboratory Animals (17), and the US Department of Agriculture Animal Welfare Act (Public Law 99–198).

Animals

Healthy, experimentally naïve, male and female beagle dogs from Covance Research Products, Inc, Denver, Pennsylvania, USA, were individually housed before and during the study in stainless-steel cages in a room with a light/dark cycle of 12 h/12 h, temperature between 64° and 84°F, and relative humidity between 30% and 70%. Food (Certified Canine Diet® 2025C, Harlan Teklad global diet) was provided daily and was withheld overnight before the day of antidote or vehicle administration (Day 1). Water was provided *ad libitum* in a stainless steel bowl. Animals were identified with cage cards labeled with animals' tattoo numbers and study-specific animal identification numbers assigned by the Xybion PATH/TOX System (Version 4.2.2). These animal identification numbers, used for recording of raw data, provided no information about antidote dose. Mean (SD) body weights of the animals on the day of experimentation are shown in Table 1. The numbers of animals treated with hydroxocobalamin 75 mg/kg, 150 mg/kg, and saline were 19, 18, and 17, respectively. Randomization to treatment groups was done using a table of random numbers with assignment by the study director.

Procedures

The procedures and parameters for this canine model were arrived at through a series of pilot studies, some of

TABLE 1
Animals, cyanide treatment, and outcomes

	Vehicle	Hydroxocobalamin 75 mg/kg	Hydroxocobalamin 150 mg/kg
Animals			
N	17	19	18
Sex, n			
Male	8	10	9
Female	9	9	9
Mean (SD) weight on Day 1, kg			
All animals	8.9 (1.9)	9.2 (1.4)	9.0 (1.4)
Males	10.3 (2.1)	10.0 (1.3)	9.9 (1.5)
Females	7.6 (0.3)	8.2 (1.0)	8.1 (0.6)
Cyanide treatment			
Mean (SD) total dose of potassium cyanide, mg/kg			
All animals	2.3 (0.2)	2.4 (0.2)	2.2 (0.2)
Males	2.3 (0.1)	2.4 (0.2)	2.2 (0.2)
Females	2.3 (0.2)	2.3 (0.2)	2.3 (0.2)
Mean (SD) time to apnea, min			
All animals	2.8 (0.4)	2.9 (0.5)	2.6 (0.5)
Males	2.8 (0.3)	2.9 (0.6)	2.4 (0.4)
Females	2.8 (0.5)	2.9 (0.4)	2.8 (0.5)
Outcomes			
Died, n			
No recovery from poisoning[†]	10	1	0
Euthanized after Day 1	4	3	0
Survived to Day 15	3	15	18

[†]Euthanized because of persistent signs of poisoning at 4 h post-infusion of cyanide.

which are described elsewhere (18). Animals were fasted overnight before Day 1. On Day 1, animals were preanesthetized with ketamine (10 mg/kg, IV) and diazepam (0.5 mg/kg, IV). Animals were endotracheally intubated and maintained under isoflurane (1.3% to 2%, inhalation) on spontaneous ventilation with medical-grade air at a surgical stage III level distinguished by lack of sensory reflexes or gross motor movement and steady cardiac and respiratory rates. Isoflurane delivery was monitored by an anesthetic gas monitor (Puritan Bennet).

After a 15-min baseline period during which respiratory and cardiac data were obtained, dogs were intravenously administered potassium cyanide (Sigma-Aldrich, 1 mg/mL in 0.9% NaCl vehicle) at a rate of 0.4 mg/kg/min until 3 min after the onset of apnea (Fig. 1). Immediately upon the conclusion of the potassium cyanide infusion, hydroxocobalamin (Cyanokit®, Merck Santé, Lyon, France; either 75 mg/kg at an infusion volume of 3 mL/kg or 150 mg/kg at an infusion volume of 6 mL/kg) or saline vehicle (Baxter and Hospira, 0.9% Sodium Chloride for Injection, USP at an infusion volume of 6 mL/kg) was administered by intravenous infusion in a peripheral vein over 7.5 min while applying supplemental 100% oxygen and mechanical ventilation (10–15 breaths/min at a tidal volume of ~15 mL/kg; actual values set to approximate baseline minute-volume values for each dog) (Fig. 1). The target number of animals for each dosing group (hydroxocobalamin 75 mg/kg, hydroxocobalamin 150 mg/kg, vehicle) was 18 (9 males and 9 females). Because 1 animal was inadvertently dosed with 75 mg/kg instead of vehicle, the numbers of animals in the hydroxocobalamin 75 mg/kg group and the vehicle group were 19 and 17, respectively.

Hydroxocobalamin (Batch No. 2081, Merck Santé, France) was supplied as a lyophilized powder, which was reconstituted by adding 100 mL of vehicle to a 2.5-g bottle. Hydroxocobalamin doses were chosen to produce exposure in dogs comparable to that in humans given an antidotal dose. Hydroxocobalamin 75 mg/kg and 150 mg/kg administered to dogs are comparable on a dose-to-body-mass basis (assuming an average 70-kg human) to hydroxocobalamin 5 g and 10 g, respectively, doses typically administered to humans in France. Hydroxocobalamin was administered by intravenous infusion. Mechanical ventilation and supplemental oxygen were administered to mimic the supportive care given to human victims of acute cyanide poisoning.

Mechanical ventilation was stopped after 15 min, and animals were supplied medical-grade air via the endotracheal tube. Repeated brief cycles of assisted ventilation were applied as necessary to support resumption of spontaneous breathing in surviving animals. This method is often required to wean dogs after a period of assisted ventilation even in the absence of poisoning. If apnea persisted for 45 s after mechanical ventilation was stopped, ventilation was reinstated for 15 s and then stopped. Assisted ventilation was repeated up to 6 times. Failure to resume ventilation after 6 cycles of assistance was considered consistent with irreversible apnea. Once animals were weaned from the ventilator, they were supplied medical-grade air by endotracheal tube for 2 h. Animals surviving 2 h after infusion of antidote or vehicle were weaned from anesthesia, had catheters and monitoring equipment removed, and were returned to home cages where they were maintained for an additional 14 d. Animals that did not recover consciousness within 2 h after cessation of anesthesia were euthanized.

Assessments

Efficacy was evaluated on the basis of survival during a 14-day follow-up period as well as cardiovascular, respiratory, neurologic, and hematologic effects. Animals were assessed for changes in clinical observations by observers not informed of treatment assignments nor the purpose of the study at least twice on Day 1 (predose and at least once after the 2-h postdose data collection) and at least once daily on subsequent days unless recovery did not occur.

Cardiovascular and respiratory data were collected on Day 1 with an automated physiologic data-collection system (Notocord, Version 4.1). Cardiovascular parameters (mean arterial, systolic, and diastolic blood pressures and heart rate) were continuously monitored with a Millar catheter placed via the femoral artery into the abdominal aorta and attached to a transducer. Electrocardiogram (ECG) electrodes were attached for collection of a minimum of a Lead II waveform, which was saved digitally throughout the monitoring period. Respiratory parameters (respiratory rate, tidal volume, minute volume)

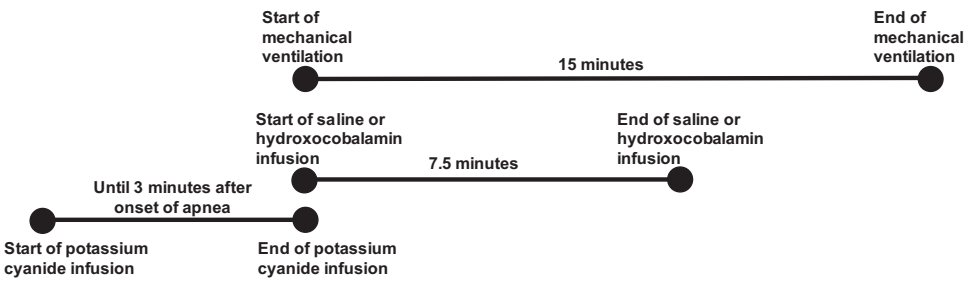

FIG. 1. Study procedures.

were measured with a pneumotach attached to the endotracheal tube. Animals were assessed for neurological injury by a clinical veterinarian not informed of treatment assignments nor the purpose of the study before Day 1 and on Days 2, 8, and 15 in surviving animals. Neurological injury was scored with respect to the level of consciousness (scale: 0–100), the respiratory pattern (scale: 0–100), sensory reflexes (scale: 0–5), motor reflexes (scale: 0–25), and behavior (scale: 0–30) (19).

Arterial lactate, pH, and blood gases (PO_2, PCO_2) were determined from samples collected before initiation of cyanide infusion; at the end of cyanide infusion; and 4, 7.5, 11, 15, 20, 30, 60, and 120 min after the start of hydroxocobalamin or vehicle infusion. Samples were analyzed immediately or maintained on ice until analysis with a point-of-care blood gas analyzer (I-STAT®, Abbott Point of Care, East Windsor, New Jersey, USA) not more than 20 min after they were obtained. Clinical chemistry and hematology tests were performed on blood samples obtained before Day 1 and on Days 2 and 15 in surviving animals.

Data Analysis

The primary efficacy endpoint was 14-day mortality rate. Mortality rate was compared among the treatment groups with the Fisher exact test 1 h, 4 h, 7 d, and 14 d after the start of hydroxocobalamin or vehicle infusion. A Bonferroni adjustment was made to maintain the overall Type I error rate at ≤ 0.05 for the pairwise comparisons of each active treatment group with the vehicle group. The probability of survival over time in the study was estimated with Kaplan-Meier methodology.

Cardiovascular, respiratory, and blood gas parameters were compared among treatment groups with repeated measures analyses of variance (ANOVA) with treatment group, time point, and their interaction as effects; animal as a random effect; and predose value as a covariate. The Benjamini and Hochberg approach (20) was used to control the false discovery rate to ≤0.05 for all time points simultaneously. For time points with a significant difference among treatment groups, pairwise comparisons between each of the hydroxocobalamin groups and the vehicle group were done with t-tests. A Bonferroni adjustment was made to maintain the overall Type I error rate at ≤ 0.05 for the pairwise comparisons of each active treatment group with the vehicle group. Clinical chemistry and hematology data were compared between each active treatment group and the vehicle group with the Cochran and Cox 2-sample t-test.

RESULTS

Animals

The numbers of animals in the groups receiving vehicle hydroxocobalamin 75 mg/kg, and hydroxocobalamin 150 mg/kg were 17, 19, and 18, respectively. Mean total dose of potassium cyanide ranged from 2.2 mg/kg to 2.4 mg/kg across groups (Table 1). Mean time between start of potassium cyanide infusion and onset of apnea ranged from 2.4 to 2.9 min across groups. All animals were dosed with potassium cyanide until 3 min after the onset of apnea.

Mortality

Mortality rate was significantly lower in both hydroxocobalamin groups compared with the vehicle group 4 h, 7 d, and 14 d after initiation of hydroxocobalamin or vehicle infusion ($P < 0.05$ each hydroxocobalamin group versus vehicle at each time point; Fig. 2). Mortality rate on Day 15 was 82% in the

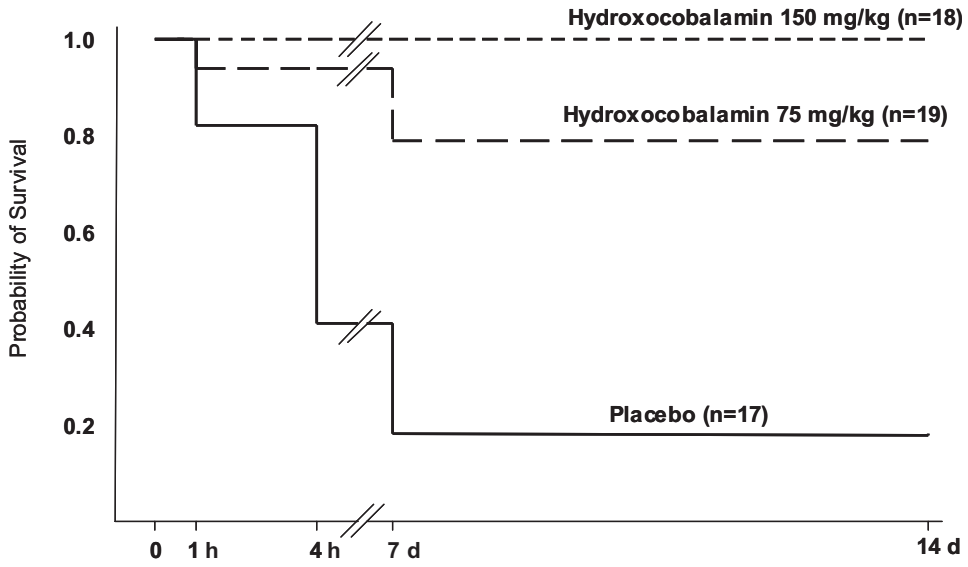

FIG. 2. Probability of survival in beagle dogs treated with hydroxocobalamin 75 mg/kg or 150 mg/kg or saline vehicle for acute cyanide poisoning.

group treated with vehicle compared with 21% in the group treated with hydroxocobalamin 75 mg/kg and 0% in the group treated with hydroxocobalamin 150 mg/kg ($P < 0.05$ each hydroxocobalamin group versus vehicle).

Among the 17 animals in the vehicle-treated group, 10 were euthanized by a veterinarian 4 h after the start of antidotal treatment to avoid suffering with termination of anesthesia because of persistent signs and symptoms of severe poisoning; 4 were euthanized for severe neurological signs after Day 1 (2 on Day 2, 1 on Day 3, and 1 on Day 4), and 3 survived through Day 15 (Table 1). Among the 19 animals in the group treated with hydroxocobalamin 75 mg/kg, 1 was euthanized at 4 h because of signs and symptoms of severe poisoning; 3 were euthanized for severe neurological signs (1 on Day 3 and 2 on Day 4); and 15 survived through Day 15 with no neurological sequelae. All of the 18 animals in the group treated with hydroxocobalamin 150 mg/kg survived through Day 15 with no neurological sequelae.

Neurological Exams

Neurological exam scores before Day 1 were normal for all animals in all groups. Neurological exam scores on Days 2, 8, and 15 in surviving animals were normal in all animals except 3 animals in the vehicle group and 2 animals in the group treated with hydroxocobalamin 75 mg/kg. All 5 of these animals had abnormal neurological exam scores on Day 2 and were subsequently euthanized (as described above) because of the severity of neurological impairment. No neurological exam score beyond the pre-Day 1 score was recorded for the sixth animal (in the hydroxocobalamin 75-mg/kg group) that was euthanized because of neurological impairment.

Hemodynamic Data

Hemodynamic parameters were comparable among treatment groups from baseline through the end of apnea. Potassium cyanide poisoning was characterized by hypotension with decreases in mean arterial blood pressure to approximately 50% to 80% of baseline at the end of apnea and through the ventilation period in vehicle-treated animals (Fig. 3). Hydroxocobalamin infusion was associated with rapid, dose-related cardiovascular recovery. Mean arterial blood pressure increased beginning within 1 to 3 min of initiation of hydroxocobalamin infusion, peaked at the end of infusion at values 150% to 180% of baseline, was maintained at significantly greater levels than in vehicle-treated animals during the ventilation period, and declined to values comparable to baseline through the post-ventilation period (Fig. 3).

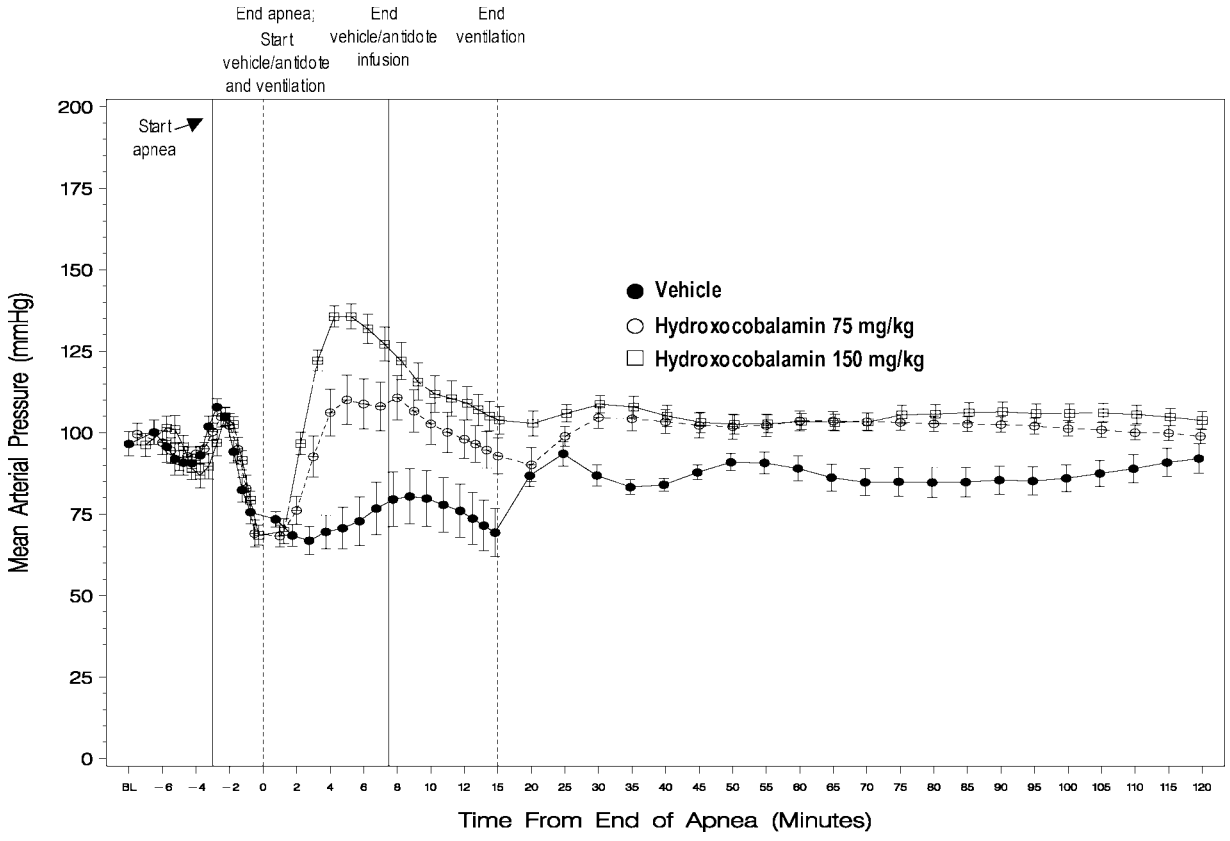

FIG. 3. Mean (SE) arterial pressure as a function of time from the end of apnea in beagle dogs treated with hydroxocobalamin 75 mg/kg, 150 mg/kg, or saline vehicle for acute cyanide poisoning. BL = baseline.

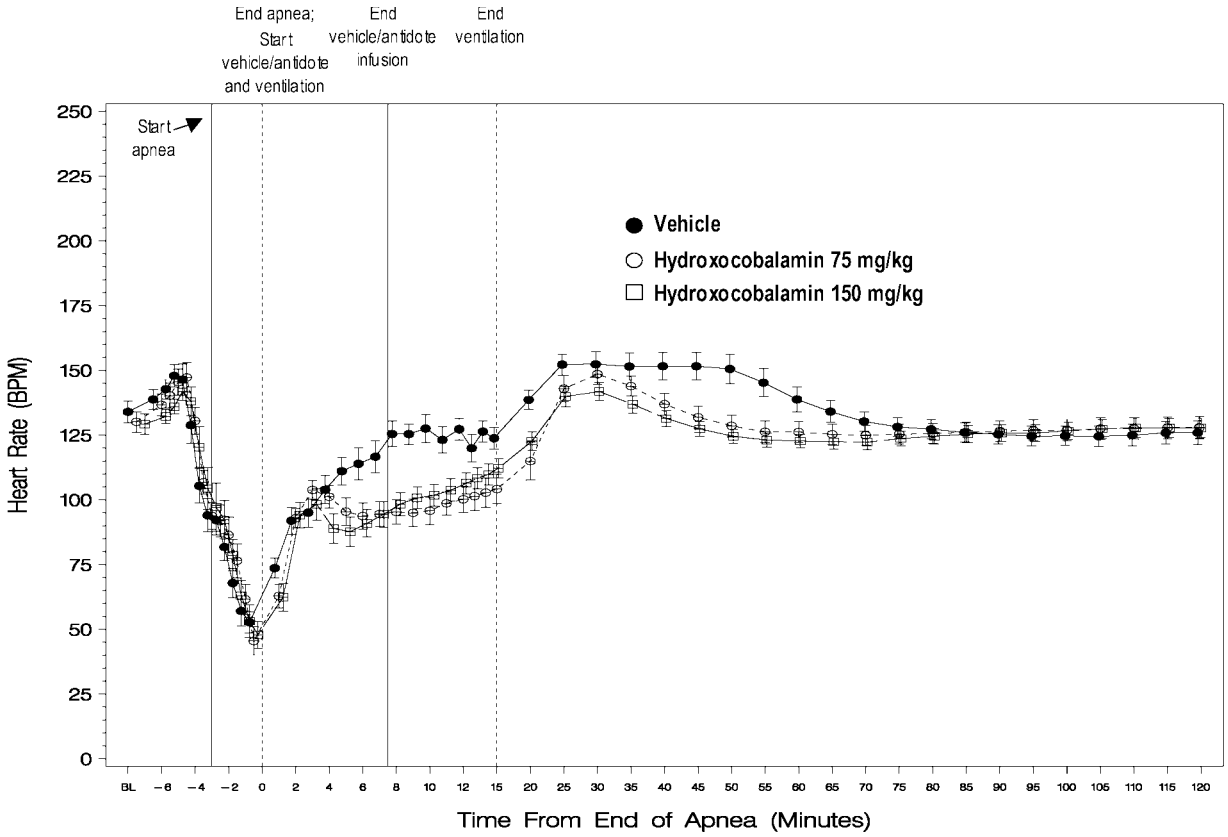

FIG. 4. Mean (SE) heart rate as a function of time from the end of apnea in beagle dogs treated with hydroxocobalamin 75 mg/kg, 150 mg/kg, or saline vehicle for acute cyanide poisoning. BL = baseline.

Heart rate changes were comparable among hydroxocobalamin-treated groups throughout the monitoring period (Fig. 4). Heart rate was slightly lower in the hydroxocobalamin-treated groups than the vehicle group, a finding likely attributable to the higher arterial pressures in the hydroxocobalamin-treated groups.

Respiratory Data

Respiratory parameters were comparable among treatment groups from baseline through the end of apnea. Potassium cyanide poisoning was characterized by initial increases in respiratory rate, tidal volume, and minute volume followed by declines to zero through the apnea period (Fig. 5 for minute volume). Respiratory rate, tidal volume, and minute volume were comparable among treatment groups from the start of potassium cyanide infusion to the end of ventilation (Fig. 5 for minute volume). Cessation of potassium cyanide infusion and initiation of mechanical ventilation were associated with partial recovery of respiratory parameters in all treatment groups. During the post-ventilation period, vehicle-treated dogs had increased tidal volume for up to 60 min and slower compensatory recovery of respiratory rate and minute volume compared with hydroxocobalamin-treated dogs.

Lactate, pH, and Arterial Blood Gases

In hydroxocobalamin-treated animals compared with vehicle-treated animals, lactate concentrations in arterial blood were statistically significantly lower beginning approximately 7.5 min through 60 min from the end of apnea, and arterial pH was statistically significantly higher, beginning approximately 7.5 min through 30 min from the end of apnea (Fig. 6 and 7). Arterial PO_2 and PCO_2 were comparable among treatment groups at all time points with the exception of isolated differences thought to be attributed to random variation (Fig. 8 and 9).

Hematology and Clinical Chemistry

No statistically significant differences among treatment groups were observed for hematology or clinical chemistry values obtained on Days 2 and 15 except potassium on Day 15 (Table 2). The between-group differences in potassium were not deemed medically relevant. No statistically significant differences among treatment groups in hematocrit were observed.

DISCUSSION

The cyanide antidote hydroxocobalamin was efficacious compared with saline vehicle in reducing mortality from potassium

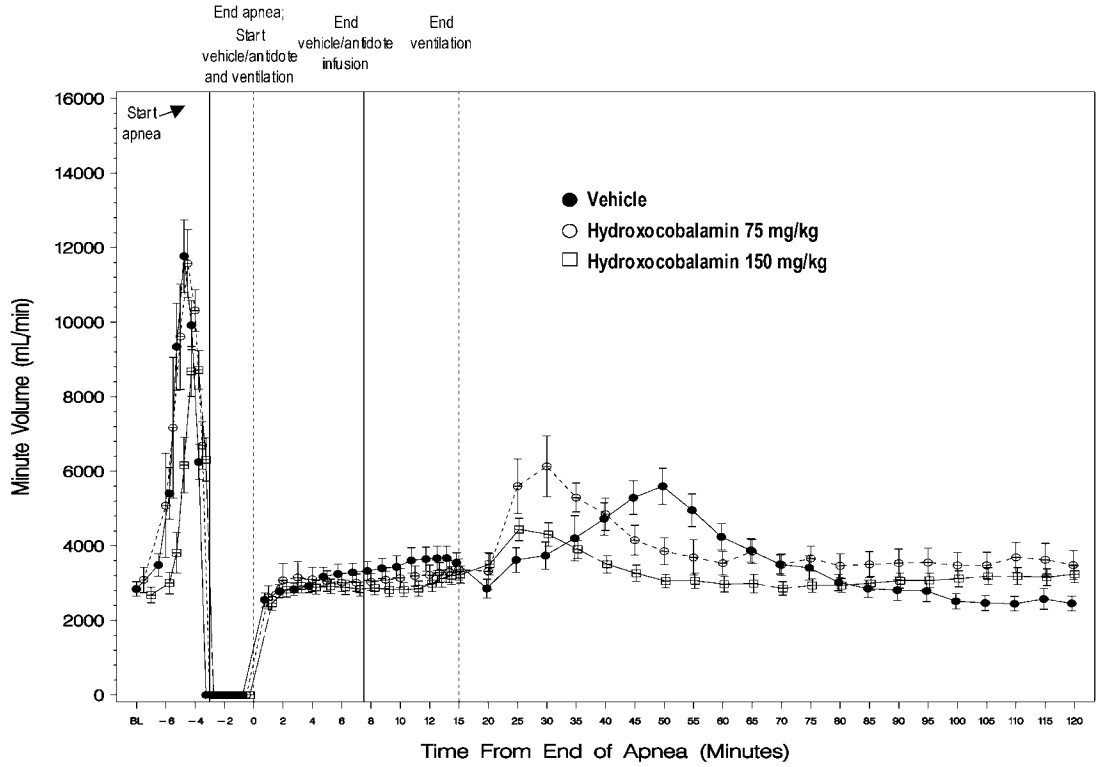

FIG. 5. Mean (SE) minute volume as a function of time from the end of apnea in beagle dogs treated with hydroxocobalamin 75 mg/kg, 150 mg/kg, or saline vehicle for acute cyanide poisoning. BL = baseline.

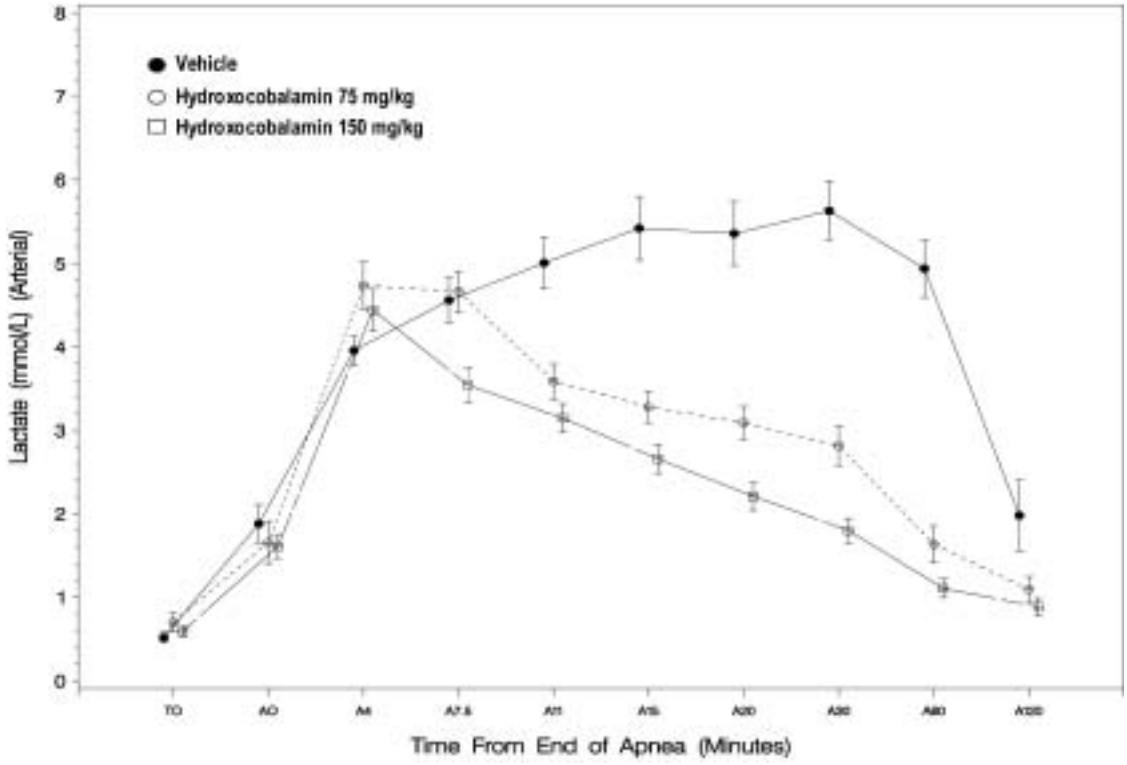

FIG. 6. Mean (SE) arterial lactate as a function of time from the end of apnea in beagle dogs treated with hydroxocobalamin 75 mg/kg, 150 mg/kg, or saline vehicle for acute cyanide poisoning. AO = onset of apnea.

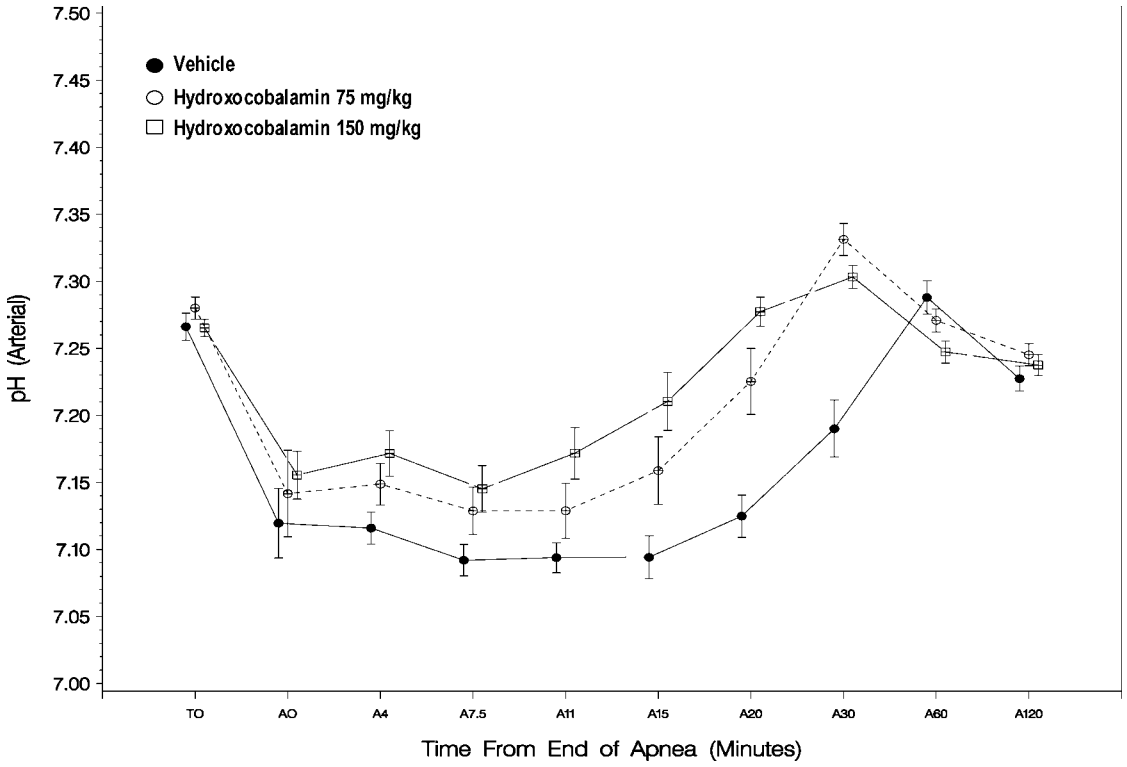

FIG. 7. Mean (SE) arterial pH as a function of time from the end of apnea in beagle dogs treated with hydroxocobalamin 75 mg/kg, 150 mg/kg, or saline vehicle for acute cyanide poisoning. AO = onset of apnea.

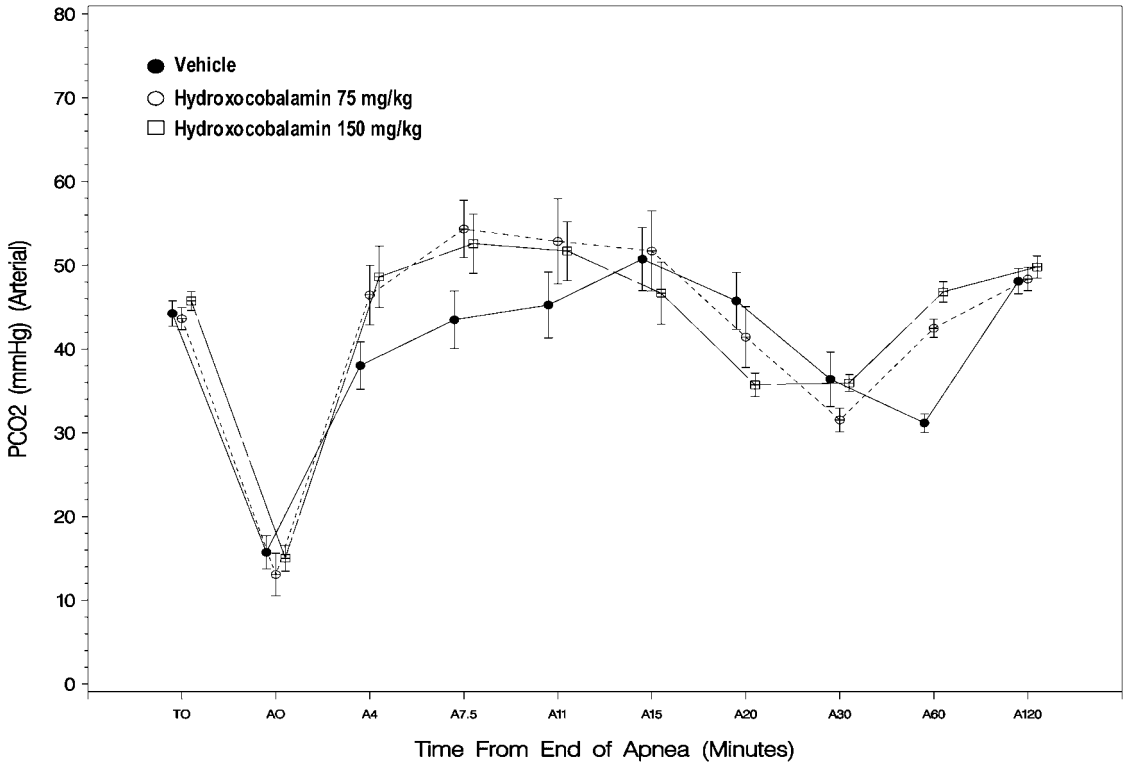

FIG. 8. Mean (SE) arterial PCO_2 as a function of time from the end of apnea in beagle dogs treated with hydroxocobalamin 75 mg/kg, 150 mg/kg, or saline vehicle for acute cyanide poisoning. AO = onset of apnea.

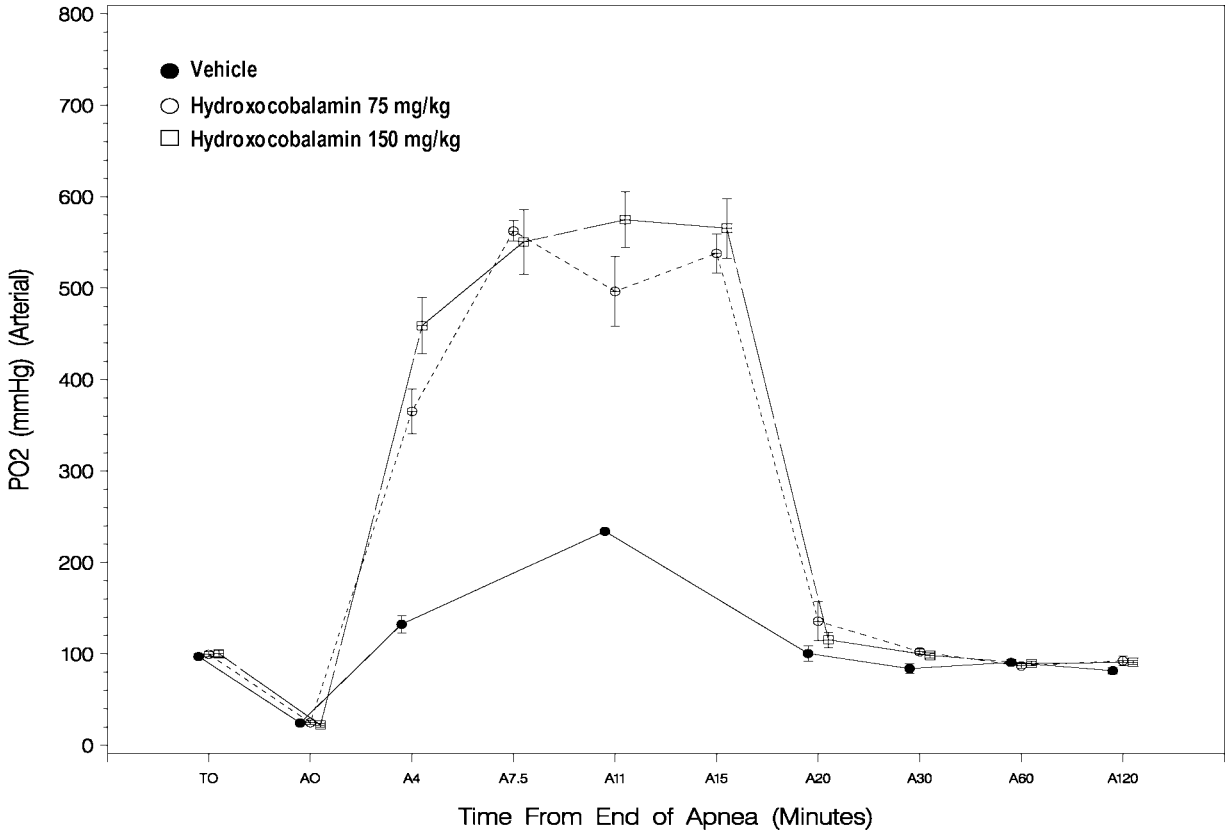

FIG. 9. Mean (SE) arterial PO_2 as a function of time from the end of apnea in beagle dogs treated with hydroxocobalamin 75 mg/kg, 150 mg/kg, or saline vehicle for acute cyanide poisoning. AO = onset of apnea.

cyanide poisoning in this animal model. In vehicle-treated animals, potassium cyanide infusion was associated with severe cardiovascular and respiratory deterioration that culminated in requirement for euthanasia within 4 h of poisoning in 10 of 17 animals and in severe neurological deficits necessitating euthanasia within 2 to 4 d in an additional 4 animals. In contrast, 15 of 19 animals treated with hydroxocobalamin 75 mg/kg and 18 of 18 animals treated with hydroxocobalamin 150 mg/kg survived through 14 d after the poisoning with no clinically observed neurologic or other sequelae and no significant effects on clinical chemistry or hematology tests or untoward events attributed to antidotal administration. Furthermore, hydroxocobalamin treatment resulted in rapid and complete resolution of lactic acidemia induced by cyanide and more rapid improvement in acid-base status. These results were obtained under conditions designed to model treatment of human cyanide poisoning in that animals were 1) given mechanical ventilation with 100% oxygen consistent with that given in human cyanide poisoning; 2) treated with the form of hydroxocobalamin that is commercially available in France and that is being investigated in the United States for antidotal use in humans; 3) given hydroxocobalamin by the intravenous route to mimic the route of administration in humans; and 4) administered hydroxocobalamin doses that produce exposure comparable to that in humans given antidotal doses of hydroxocobalamin. These experimental conditions and the choice of animal species for this study were guided by the goal of maximizing the ability to extrapolate the results to humans. The canine model is considered predictive of antidotal effects of hydroxocobalamin in humans based on well-characterized pharmacokinetics and pharmacodynamics of hydroxocobalamin in dogs (15,16).

The results of this study, which included a vehicle control group, supplement data from human studies. In the only prospective study to date of antidotal use of hydroxocobalamin in humans, hydroxocobalamin administered in the prehospital setting in France was associated with survival among 67% (28 of 42) of patients who were confirmed *a posteriori* on the basis of blood cyanide concentrations to have had cyanide poisoning (7). The inclusion of a contemporaneous vehicle control group in the current study allows attribution of the improved survival rate to hydroxocobalamin in this sample of animals.

In the dogs in this study, as in humans, acute cyanide poisoning was associated with severe cardiovascular deterioration. Administration of hydroxocobalamin in this animal model was associated with cardiovascular recovery characterized by increases in blood pressure that began 1 to 3 min after initiation of infusion. This result is consistent with previous observations

TABLE 2
Hematology and clinical chemistry data, mean (SE)

	Vehicle			Hydroxocobalamin 75 mg/kg			Hydroxocobalamin 150 mg/kg		
	Prestudy	Day 2	Day 15	Prestudy	Day 2	Day 15	Prestudy	Day 2	Day 15
				Hematology					
Platelets, $10^3/\mu L$	354.1 (11.6)	262.1 (17.6)	394.3 (33.0)	340.5 (12.4)	277.9 (12.8)	384.2 (21.7)	326.8 (18.6)	263.4 (16.2)	384.3 (21.4)
Red blood cells, $10^6/\mu L$	6.9 (0.1)	6.1 (0.1)	6.9 (0.1)	6.8 (0.1)	6.3 (0.1)	6.8 (0.1)	6.8 (0.1)	6.2 (0.1)	6.5 (0.2)
Total white blood cells, $10^3/\mu L$	12.6 (0.6)	16.3 (1.6)	9.9 (0.7)	12.1 (0.5)	16.1 (0.7)	10.7 (0.5)	12.3 (0.8)	15.6 (0.8)	10.5 (0.5)
				Clinical chemistry					
Alanine aminotransferase, U/L	35.8 (4.2)	54.6 (13.2)	37.0 (9.6)	30.8 (1.9)	49.1 (7.0)	26.5 (2.1)	30.9 (1.7)	37.1 (2.9)	26.7 (1.3)
Aspartate aminotransferase, U/L	35.4 (2.3)	51.1 (6.6)	34.3 (3.0)	34.7 (1.7)	154.8 (59.5)	34.4 (1.6)	33.1 (1.5)	68.1 (5.7)	34.0 (2.1)
Blood urea nitrogen, mg/dL	15.9 (1.3)	8.6 (0.6)	12.0 (2.3)	14.8 (1.2)	9.7 (0.9)	11.9 (0.7)	15.1 (1.3)	9.2 (0.4)	12.2 (0.5)
Chloride mEq/L	111.6 (0.4)	111.7 (1.0)	109.7 (0.9)	111.0 (0.4)	111.5 (0.6)	110.6 (0.4)	110.8 (0.7)	111.2 (0.3)	110.7 (0.4)
Creatine, mg/dL	0.878 (0.017)	0.686 (0.026)	0.833 (0.033)	0.868 (0.022)	0.750 (0.020)	0.847 (0.026)	0.838 (0.018)	0.722 (0.017)	0.839 (0.027)
Glucose, mg/dL	96.7 (1.7)	106.4 (3.7)	89.7 (3.9)	97.2 (1.3)	104.0 (2.2)	97.9 (2.4)	100.1 (1.6)	102.9 (2.6)	98.7 (2.8)
Potassium, mEq/L	4.96 (0.07)	4.16 (0.13)	5.37 (0.03)	4.90 (0.07)	4.38 (0.07)	4.93* (0.06)	5.07 (0.10)	4.49 (0.07)	4.98* (0.10)
Lactate dehydrogenase, U/L	76.1 (7.4)	90.1 (23.2)	184.7 (89.2)	104.5 (18.4)	96.7 (16.6)	140.7 (29.6)	75.5 (9.4)	82.0 (6.7)	131.9 (25.7)
Sodium, mEq/L	149.8 (0.5)	149.1 (0.8)	148.3 (0.3)	149.4 (0.4)	148.0 (0.7)	148.3 (0.6)	149.3 (0.5)	148.3 (0.3)	148.3 (0.4)
Total bilirubin, mg/dL	0.085 (0.006)	0.094 (0.012)	0.103 (0.043)	0.090 (0.008)	0.086 (0.010)	0.078 (0.009)	0.084 (0.010)	0.092 (0.005)	0.086 (0.007)
Total protein, g/dL	5.64 (0.07)	5.46 (0.11)	5.60 (0.17)	5.79 (0.07)	5.58 (0.07)	5.91 (0.10)	5.71 (0.07)	5.57 (0.09)	5.74 (0.09)

*$P < 0.05$ versus vehicle.

in humans (7,8). In 2 retrospective studies in smoke-inhalation victims treated with hydroxocobalamin for suspected cyanide poisoning, infusion of hydroxocobalamin was associated with an increase in blood pressure and improvement of cardiovascular status—effects often observed even among patients who were found in cardiorespiratory arrest or who were otherwise hemodynamically unstable (7,8). The ability of hydroxocobalamin to scavenge nitric oxide might contribute to its transient effect on blood pressure (21,22). The latter mechanism is consistent with the previous finding of transient, self-limiting increases in systolic and diastolic blood pressure after administration of hydroxocobalamin 5 g to healthy volunteers who were heavy smokers (23).

It was determined *a priori* that animals with severe neurological signs would not be permitted to survive, thus requiring euthanasia of animals that appeared to be persistently poisoned at the end of anesthesia. The decision to euthanize animals after the day of dosing was made by a veterinarian who was not informed of treatment assignments nor the intent of the study. Because of the red color of hydroxocobalamin and its alteration of skin color, complete blinding was impossible. However, the decision to euthanize was based solely on the neurological status of the animal and the inability of the affected animal to eat and drink on its own.

In summary, in this animal model, hydroxocobalamin 75 mg/kg and 150 mg/kg reduced mortality from potassium cyanide poisoning that was lethal in the majority of animals treated with saline vehicle. Hydroxocobalamin compared with saline vehicle was associated with improvement in hemodynamic stability beginning 1 to 3 min after the onset of infusion. Hydroxocobalamin-treated animals that survived cyanide poisoning exhibited no neurologic or other sequelae, and no untoward effects of hydroxocobalamin were observed. These data are supportive of the reported findings in humans (5–10).

ACKNOWLEDGMENTS

The authors thank Jane Saiers, Ph.D., for her assistance with the writing of this article. Drs. Borron and Saiers' work on this article was supported by EMD Pharmaceuticals, an affiliate of Merck KGaA.

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