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Proof of concept efficacy study of intranasal stabilized isoamyl nitrite (SIAN) in rhesus monkeys against acute cyanide poisoning

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ABSTRACT

Cyanide is a fast-acting toxicant that inhibits aerobic cellular respiration in mitochondria. Historically, amyl nitrite has been used to treat cyanide poisoning. The objective of this study was to demonstrate the effectiveness of intranasal (IN) administration of stabilized isoamyl nitrite (SIAN) against lethal potassium cyanide (KCN) intravenous (IV) challenge in a conscious nonhuman primate (NHP) model. Animals received an IV infusion of a 1 mg/mL KCN solution at a dose of 3.25 mg/kg for 15 min. The IN SIAN treatment was administered at 10 or 12 min post-start of KCN challenge and monkeys were monitored for survival, clinical signs, respiratory and cardiovascular changes via continuous telemetry recording. In addition, blood methemoglobin (metHb) levels and blood gases were closely monitored after KCN administration. Mid to mid-high doses (13.5 or 27.0 μL/kg) of SIAN treatments at 10 min post KCN challenge significantly increased animal survival. Improvements were also observed in respiratory and cardiovascular functions. The benefits of SIAN treatment decreased when treatment was delayed to 12 min post cyanide exposure. IN administration of SIAN 10 min following initiation of a lethal dose KCN infusion produced 100% survival (at doses $\geq$ 13.5 μL/kg) in NHP model of lethal cyanide intoxication.

1. Introduction

Cyanide is one of the most rapidly acting lethal poisons currently known and can lead to death within a few minutes to a few hours post exposure. The most common source of cyanide exposure and toxicity is the inhalation of cyanide-containing gases (such as hydrogen cyanide or cyanogen chloride) and dust containing solid or liquid cyanide, most likely from industrial exposures or house fires. These contaminants are typically generated by industrial processes or the combustion of synthetic surfaces in enclosed spaces (Barillo, 2009; Reade et al., 2012). Additionally, cyanide has also been used as a potent poison in both homicides and suicides (Musshoff et al., 2002), as well as a weapon in terrorism or warfare (Eckstein, 2004; Keim, 2006).

Cyanide toxicity can largely be attributed to its ability to stop aerobic cell metabolism. Cyanide reversibly binds to the ferric ion in cytochrome oxidase a_3 in the mitochondria, a crucial component in the reduction of oxygen to water in complex IV of the electron transport chain, and its inhibition effectively disrupts oxidative phosphorylation in the cell (Hall et al., 2007; Hamel, 2011). As a result, the synthesis of adenosine triphosphate (ATP) and the continuation of cellular respiration is halted. Subsequently, the depletion of ATP shifts cellular metabolism to glycolysis through anaerobic metabolism. Glycolysis, however, is inefficient for energy demands of the cell and results in the accumulation of lactic acid, which can rapidly and severely affect the vulnerable tissues of the brain and heart (Shepherd and Velez, 2008; Way et al., 1984). The onset and severity of cyanide toxicity is dependent on multiple factors, such as route of exposure, concentration, and duration of the exposure. Early signs and symptoms of acute cyanide poisoning include hypoxia-associated responses in the nervous, respiratory, and cardiovascular systems (e.g. headaches, hyperventilation, increases in blood pressure and heart rate, etc.), whereas severe poisoning or late symptoms can result in stupor, coma, seizures, hemodynamic shock and

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cardiorespiratory arrest (Kerns and Kirk, 2006; Kirk and Stenhouse, 1953).

Various antidotes have been developed for acute cyanide poisoning, acting to directly or indirectly sequester cyanide to prevent its binding with cytochrome oxidase a_3, and/or neutralize the compound so it can be eliminated safely. For decades cyanide antidote kits have been utilized in the United States (Chen and Rose, 1952; Shepherd and Velez, 2008), and their use has been associated with a decrease in death rates from acute cyanide poisoning (Barillo, 2009). Amyl nitrite has been historically used off-label via inhalation to treat cyanide poisoning but is currently not approved by the FDA for this indication (Petrikovics et al., 2015). Amyl nitrite works as an antidote for cyanide by oxidising the central iron atom of hemoglobin from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) state, producing methemoglobin (metHb) (Butler and Feelisch, 2008; Lavon and Bentur, 2010). Cyanide has a high binding affinity to metHb, and results in the formation of the stable and non-toxic cyanmethemoglobin complex (Chen and Rose, 1952; Chen et al., 1933). This limits cyanide bonding to cytochrome oxidase a_3 and allows aerobic cellular respiration to resume. Extracellular cyanide can be eliminated via metabolism in the liver as thiocyanate, a nontoxic compound that is excreted in the urine (Kirk and Stenhouse, 1953; Megarbane et al., 2003). Another antidotal mechanism of nitrites is attributed to vasodilatory activity of nitric oxide (NO) and their ability to improve peripheral circulation and oxygen supply to vital organs (2011). Organic nitrate, such as isosorbide dinitrate (ISDN), has also shown a beneficial effect in the treatment of cyanide poisoning in rabbit and swine models (Lavon et al., 2017, 2020).

There are some associated risks with nitrite administration in the context of cyanide exposure. Nitrite-induced methemoglobinemia is a side effect and can reduce oxygen carrying capacity of hemoglobin (Cooling, 2014). Additionally, efficacy studies of amyl nitrite in animal models of cyanide toxicity provide limited information regarding the antidotal effects of amyl nitrite on respiratory and hemodynamic parameters, as well as dose- or concentration-response correlations after cyanide exposure. These studies were mostly performed on dogs and mice (Chen and Rose, 1952; Jandorf and Bodansky, 1946; Klimmek and Krettek, 1988; Vick and Froehlich, 1985, 1991). Nonhuman primates (NHPs) are one of the most commonly used preclinical species due to their genetic, cardiovascular and metabolic similarities to humans; however, only a few studies on cyanide antidote testing have used NHPs as a model. The NHP model allows for telemetered respiratory and cardiovascular monitoring, and has been successfully employed and validated in a number of studies (Authier et al., 2007; Benardeau et al., 2000; Gauvin et al., 2006; Hassimoto and Harada, 2003).

The current study aimed to assess the pharmacodynamic response of telemeterized NHPs (*Macaca mulatta*) to cyanide exposure and efficacy of the administration of subsequent antidotal stabilized isoamyl nitrite (SIAN) therapy. This strategy allowed for an in-depth characterization of respiratory and cardiovascular changes in NHPs upon cyanide challenge, and the efficacy of SIAN therapy on survival and recovery.

2. Materials and methods

2.1. Ethics statement

All experimental procedures were performed in accordance with Institutional Animal Care and Use Committee (IACUC) and the Canadian Council on Animal Care (CCAC) guidelines for use of experimental animals. The procedures were also approved by the Office of Laboratory Animal Welfare/Vertebrate Animal Section (OLAW/VAS). The Charles River Laval (formally known as Citoxlab North America) facility is AAALAC accredited and all protocols, including humane euthanasia criteria were reviewed and approved by the IACUC prior to conduct. The study was approved by the Office of Laboratory Animal Welfare (OLAW). All procedures were conducted as per Standard Operating Procedures (SOPs) in place.

2.2. Test and control items

The synthesis of isoamyl nitrite is based on synthesis of alkyl nitrites as previously described (Noyes, 1936). Briefly, nitrous acid is generated from an aqueous mixture of sodium nitrite and sulfuric acid in the presence of isoamyl alcohol. The resulting isoamyl nitrite is mixed with Epoxidized Linseed Oil (ELSO) to reduce the risk of chemical degradation. SIAN was manufactured in accordance with current Good Manufacturing Practices (cGMP) by Southwest Research Institute (SwRI), San Antonio, TX. SIAN was supplied in 2 mL ampoules filled to approximately 1.2 mL and stored at 2–8 °C, protected from light, and with no further formulation preparation. Ampoule contents (one ampoule per animal) were transferred to a labeled glass vial right before dosing using a glass transfer pipette, and the SIAN was drawn immediately using the delivery device.

Delivery Device: The intranasal (IN) spray delivery devices used to deliver SIAN were produced and supplied by Southwest Research Institute and consisted of a nozzle tip (modified from an off-the-shelf IN atomization device to fit onto a variable volume syringe) and a modified gas-tight glass syringe. Delivery devices were prepared under the flow hood cabinet.

Potassium cyanide (KCN) (manufactured by Sigma-Aldrich) was stored at room temperature. The KCN vehicle was 0.9% sodium chloride for injection USP (saline) (Baxter Healthcare Corporation, Deerfield, IL) purchased commercially and stored at room temperature. The KCN solution was freshly prepared on the day of dosing by dissolving of KCN in the appropriate volume of saline to make a 1 mg/mL (target) solution. The solution was filtered through a 0.22 μm filter into a non-pyrogenic sterile container. The solution was kept at room temperature prior to administration. Samples of the KCN solution were analyzed by potentiometric titration at initiation and completion of the dosing period and the concentrations were within acceptance criteria (1.02 and 0.97 mg/mL, respectively).

2.3. General animal handling

A total of 22 (11 males/11 females) rhesus monkey, aged 2.0–5.3 years, and weighing 3.4–5.4 kg, were included in the study. The animal room environment was maintained at a temperature of 21 ± 3 °C with a relative humidity of 50 ± 20%, a light dark cycle of 12 h light/12 h dark, and 10–15 air changes per hour. Temperature and relative humidity were monitored continuously.

NHPs were fed a standard certified commercial chow (ENVIGO Teklad Certified Hi-Fiber Primate Diet #7195C in form of cookies) twice daily. Treats or fruits/vegetables were provided as part of the animal enrichment program. Water, which had been exposed to ultraviolet light and purified by reverse osmosis, was provided to the animals *ad libitum*.

2.4. Surgical instrumentation

All animals underwent surgical instrumentation for cardiovascular monitoring of the arterial blood pressure, left ventricular pressure, respiration, electrocardiogram, body temperature and locomotor activity.

General anesthesia and surgical preparation. The animals were fasted overnight prior to the surgical procedures. The animals were sedated using a mixture of Ketamine (9.09 mg/kg IM) and Acepromazine (0.9 mg/kg IM) followed by intubation. Lidocaine spray (10% w/w) was administered onto the glottis prior to intubation as needed. An ophthalmic ointment was applied to both eyes to prevent drying of the cornea prior and after anesthesia. Anesthesia was maintained with isoflurane by inhalation with an oxygen flow at approximately 200 mL/kg/min or as needed, and animals were mechanically ventilated, as needed, at a rate of 8–20 breaths/minute with inspiratory pressure of 18–25 cmH_2O. Intravenous fluid therapy was given throughout the anesthesia using Sterile Lactated Ringer's solution at a rate of 10 mL/kg/hr.

Animals were placed on a heating pad set to maintain the animal's body temperature at approximately 37 °C.

Surgical procedure. Two telemetry transmitters were implanted into abdominal cavity of the animals (Data Science International, Model D70-PCTR and M10). The pressure catheter was inserted into the femoral artery and the electrocardiography (ECG) biopotential leads were placed in a Lead II configuration. The negative lead was inserted via the jugular vein and was advanced to reach above the heart. The positive lead was sutured to the diaphragm at the level of the apex of the heart.

The left ventricular pressure catheter was placed in the apex of the heart, inside of the left ventricle chamber. The respiratory impedance leads were placed after the ECG biopotential leads to avoid poor signal quality and were placed both cranially and caudally to the seventh rib of the animal on each side. Lead positioning was confirmed by fluoroscopy or via the telemetry acquisition system through waveform recognition. A permanent catheter was also inserted into a femoral artery (not the one used for pressure monitoring by telemetry) for arterial blood collections.

The surgical sites were flushed with warm saline and the incisions were closed with absorbable suture material using simple continuous sutures. The skin was closed with discontinuous buried sutures using absorbable suture material. Animals were placed in a quiet post-operative room to recover from anesthesia. Surgical sites were cleaned and appropriate medication (topical and systemic) was provided.

Post-surgical recovery. Rectal body temperature was monitored in the post-operative period, and each animal was returned to its cage once the body temperature reached the physiological range. Food and nutritional support were provided to the animals after return to their cage.

For analgesia an injection of Buprenorphine (0.3 mg/mL) was given IM twice a day for 3 days starting at least 30 min prior to surgery. Cefazolin (334 mg/mL) was administered IM three times a day for 3 days and at least 30 min pre-operatively.

2.5. Experimental design

The study was designed with reference to the following test methods and/or guidelines: U.S. FDA Redbook 2000: General Guidelines for Designing and Conducting Toxicity Studies; ICH S3A: Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies; U.S. FDA Guidance for Industry October 2015: Product Development under the Animal Rule Guidance for Industry; ICH S7A: Safety Pharmacology Studies for Human Pharmaceuticals; and ICH S7B: The Non-Clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals.

Animal assignment. Male and female animals were separately assigned to dose groups by a randomized stratification system based on body weights. A total of 22 NHPs (11 males/11 females) were randomized to the vehicle and SIAN treatment groups: 3 NHPs (3 M) were assigned to the untreated or saline treated group (Group 1); 5 NHPs (3M/2F) to the very low dose SIAN group (Group 2; 3–4.5 μL/kg); 1 NHP (1F) to the low dose SIAN group (Group 3; 11.4 μL/kg); 4 NHPs (2M/2F) to the mid dose SIAN group (Group 4; 13.5 μL/kg); 4 NHPs (1M/3F) to the mid-high dose SIAN group (Group 5; 20 μL/kg); 3 NHPs (2M/1F) to the high dose SIAN group (Group 6; 23–27 μL/kg); and 2 NHPs (1M/1F) to the high dose SIAN group with delayed administration (Group 7; 23.5–26.5 μL/kg). A summary of the study design is presented in Table 1 below.

KCN challenge. All animals received an IV infusion via a previously placed temporary venous catheter, which was inserted in a saphenous vein, of a 1 mg/mL (target) KCN solution at a dose of 3.25 mg/kg for 15 min. The infusion rate was determined based on the intended dose, 15 min infusion period, formulation concentration, and weight (measured the day prior to KCN infusion) of the NHPs. The infusion rate (mL/min) was calculated as follows: (dose (mg/kg) x weight (kg)/formulation concentration (mg/mL))/infusion period (15 min).

SIAN treatment and route of administration. SIAN was administered by IN spray (i.e. using the delivery device as produced and supplied by SwRI) comprising of 50, 100 or 250 μL gas tight syringe (Hamilton Company, Reno, NV) equipped with modified Aptar tip (Aptar Pharma, Le Neubourg, France) in the left nare by a trained technician at 10 min after start of KCN challenge for Groups 2 through 6, and at 12 min after start of KCN challenge for Group 7. Animals were placed on a sling at least 30 min prior the administration of KCN to ensure the cardiovascular and respiratory functions were stable prior the challenge. Animals were kept on the sling up to 20 min post SIAN administration or later depending on the animal's condition.

Table 1
Animal assignment and study design.

Treatment Group	SIAN Dose volume (μL/kg)	Time of SIAN dosing post start of KCN (3.25 mg/kg) infusion (min)	Number of Animals	
			Males	Females
1	0	none or 12	3	–
2	3–4.5	10	3	2
3	11.4	10	–	1
4	13.5	10	2	2
5	20	10	1	3
6	23–27	10	2	1
7	23.5–26.5	12	1	1

The delivery devices were filled not more than 25 min prior to dosing to prevent loss of material as SIAN is relatively volatile. The delivery devices that were not used within 25 min were discarded and a new dose was prepared. One ampoule (containing 1.2 mL of SIAN) was used per animal. The dose volume was set according to the group identification in the *Animal assignment* section.

Clinical observations and body weight. Cage-side clinical signs were recorded on all animals at least twice daily throughout the study and on 8 occasions (i.e. pre-dose, 8, 12, 15, 30 min, 1, 2 and 6 h post start of KCN administration) after each dose. The time of initiation of dyspnea, apnea and mortality, if applicable, were also recorded. A detailed clinical examination (DCE) was performed on each animal at animal transfer and prior to animal assignment, and body weights recorded for all animals at animal transfer, prior to animal assignment and on the day prior to dosing.

Cardiovascular and respiratory evaluation by telemetry recordings. Cardiovascular and respiratory evaluations were performed on all conscious animals. Arterial blood pressures (BP) were obtained from the transmitter catheter inserted into the femoral artery. Electrocardiograms (ECG) were obtained from the intravascular/diaphragm biopotential leads, and from the telemetry transmitter, in a Lead II configuration. Physical activity was calculated by measurement of the variability of the signal from the telemetry transmitter as a result of changes in distance relative to the receiver. Body temperature was obtained from the sensor included in the telemetry transmitter. Respiratory function (tidal volume, respiratory rate and minute volume) was obtained by impedance via the electrodes implanted subcutaneously at the mid thoracic level. Ventricular contractility was measured using dp/dtmax (maximum rate of rise of LVP), Vmax (maximum velocity at no load) and contractility index ((dP/dtmax/LVP), via the catheter inserted in the left ventricle. All parameters were recorded in conscious animals.

To ensure suitability of the animals used for the cardiovascular monitoring, arterial BP, left ventricular pressure (LVP), respiratory and electrocardiogram data was verified once during acclimation. During each treatment session, cardiovascular function (arterial BP and ECG), body temperature, physical activity and respiratory functions were recorded continuously for a period of at least 24 h following dosing for each animal.

Cardiovascular function parameters and respiratory parameters were averaged:

1. Every 5 min for the 30 min prior to the initiation of KCN infusion (when the animals were on the sling). This was used as baseline.

2. Every minute from 10 min post-test item administration up to 30 min post dosing.
3. Every 1 h from 4 h post-dosing up to 24 h post-dosing.

Corrected QT values were calculated and analyzed using Bazett's (QTcB) formulae. Electrocardiographic tracings (Lead II) were also assessed for gross changes indicative of cardiac electrical abnormalities.

The timeline for KCN challenge, SIAN treatment, clinical observations and monitoring of cardiovascular and respiratory parameters are outlined in Fig. 1.

Methemoglobin measurement. Blood samples were collected from all animals prior to KCN challenge and then at 2 min post SIAN administration (12 min post start of KCN challenge for Groups 2 to 6, and 14 min post start of KCN challenge for Group 7), and at 5, 8, 15, 20, and 60 min post SIAN administration. Each blood sample was collected from the permanent arterial catheter or from an appropriate vein (femoral, cephalic or saphenous) in tubes containing lithium heparin as anticoagulant and kept at room temperature on orbital shaker until analysis. Whole blood samples were analyzed using a co-oximeter AVOXimeter® 4000 (Instrumentation Laboratory Company, Bedford, MA) for determination of % metHb.

Arterial Blood Gas Assessment. Partial pressure of oxygen (PaO2), partial pressure of carbon dioxide (PaCO2), lactate and pH were measured in the arterial blood prior to start of KCN infusion and then 8, 12, 15, 20, 24, 27, 30 and 45 min post KCN infusion start using VetScan I-STAT Analyzer (Abaxis Inc., Union City, CA).

2.6. Statistical analysis

Statistical comparisons were performed on pooled gender and groups with less than 3 observations were excluded. The significance level was set to 5% for all tests. The pairwise comparisons of interest were each of Groups 1 and 2 versus each of the other groups. A parametric one-way analysis of variance (ANOVA) was performed for numerical data and the underlying assumptions of normality and homoscedasticity were assessed on the residuals using respectively the Shapiro-Wilk and the Levene tests.

Each group comparison was conducted via a two-sided test at the 5% significance level and the significant results were reported as either $p \leq 0.001$, $p \leq 0.01$ or $p \leq 0.05$, where p represents the observed probability.

3. Results

3.1. SIAN administration improved survival of NHPs in a dose-dependent manner after KCN challenge

Administration of SIAN was found to be effective in protecting against cyanide poisoning in a dose dependent manner starting from 20% survival at a dose of 3.0–4.5 μL/kg (Group 2) up to 100% survival rate for doses ≥ 13.5 μL/kg (Groups 4 to 6) when administered 10 min post initiation of the KCN infusion (Fig. 2). The survival benefit was statistically significant in Groups 4 (13.5 μL/kg; $p = 0.0286$) and 5 (20.0 μL/kg; $p = 0.0286$) compared to Group 1 (Untreated or saline treated). The survival benefit was also statistically significant in Groups 4 (13.5 μL/kg; $p = 0.0476$) and 5 (20.0 μL/kg; $p = 0.0476$) compared to Group 2 (3–4.5 μL/kg). Survival results are presented in Table 4 below.

3.2. SIAN administration reversed the deteriorating clinical condition caused by KCN challenge

In all animals, KCN infusion induced several clinical signs starting shortly after initiation of the challenge and subsequentially leading to a serious deteriorating condition or death. Initial symptoms started 2–3 min post KCN-infusion start and included salivation, ptosis, agitation, decreased activity level, skin pallor and emesis; then followed 3–4 min later by dyspnea, gasping, nystagmus, mydriasis, muscle twitches and/or myoclonic jerks mostly on the upper body with convulsion occurring in some animals. The administration of SIAN 10 min post KCN-start at doses ≥13.5 μL/kg was followed by a rapid reversal of the respiratory and CNS clinical signs leading to a progressive improvement of the animals' general condition until complete recovery within an hour post-dose. The SIAN non-treated animals and animals receiving a SIAN dose <13.5 μl/kg or treated 12 min post KCN infusion-start continued to deteriorate until death occurred between 10 and 15 min post KCN infusion-start. The following exceptions were observed: one Group 2 animal receiving 4.4 μl/kg of SIAN and one Group 7 animal receiving 23.8 μl/kg of SIAN at 10- and 12-min post KCN infusion-start respectively survived the challenge, but remained lying on the cage floor without any signs of recovery and were humanely euthanized 3- and 5-h post-SIAN administration, respectively.

3.3. Cyanide in plasma concentration prior and after SIAN administration correlated with survival results

No cyanide was detected in the plasma samples collected prior to initiation of the KCN infusion. Eight minutes after the start of cyanide

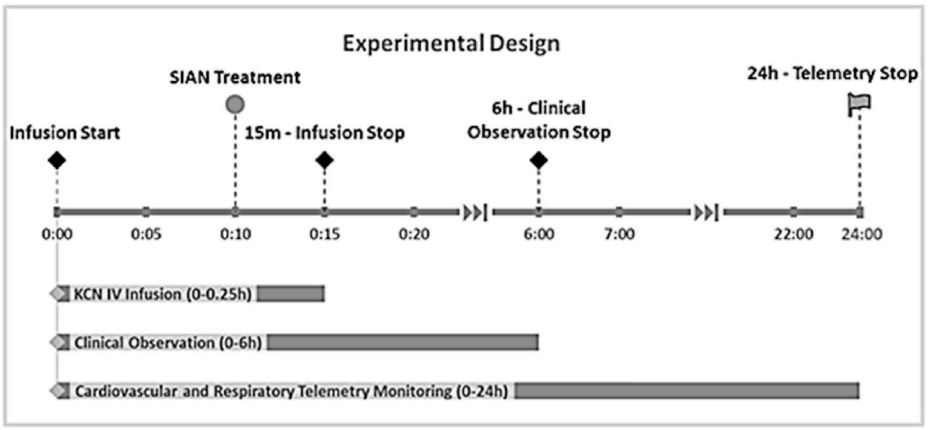

Fig. 1. Timeline for KCN challenge, SIAN treatment and monitoring. The animals received an IV infusion of KCN solution at a dose of 3.25 mg/kg for 15 min. SIAN was administered by intranasal atomization at 10 min after start of KCN challenge for Groups 2 through 6, and at 12 min after start of KCN challenge for Group 7.

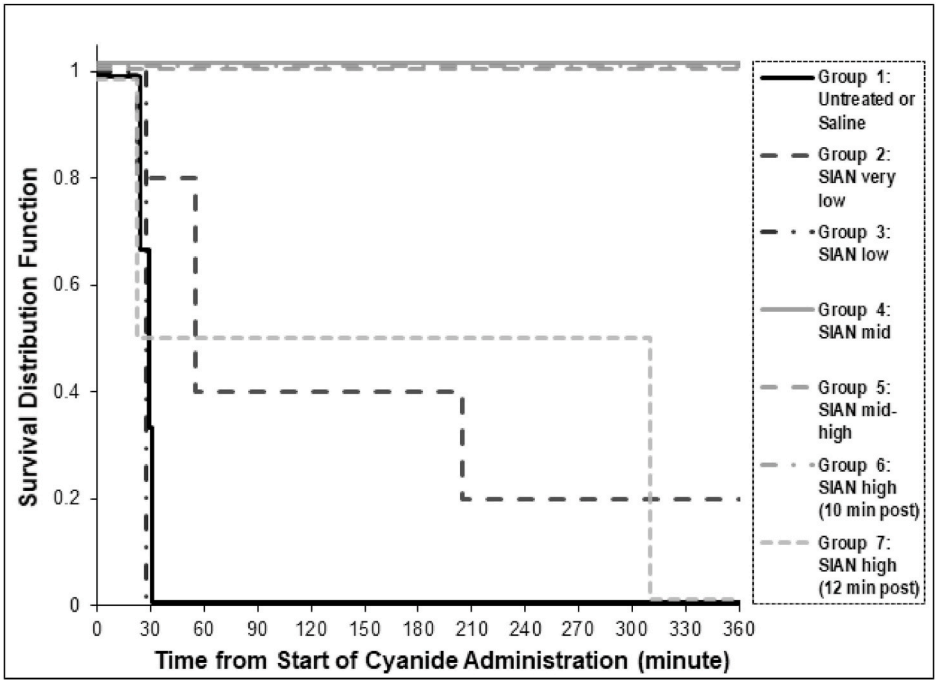

Fig. 2. Kaplan meier curves representing time to death and survival data for each group at 6 hours.

infusion, the cyanide concentration increased to an average of 1422 ng/mL in all groups. At 12 min post-start of KCN infusion (i.e. 2 min post SIAN administration except for Groups 1 and 7), the cyanide concentration was reduced by an average of more than half in all groups treated with SIAN, except for Groups 2 and 7 where cyanide concentration continued to increase in most animals. The cyanide concentration continued to decrease slowly in all other groups up to 60 min post KCN infusion. Between the approximate time of SIAN administration and 60 min post KCN infusion, the decrease in cyanide concentration showed a trend proportional to the dose of SIAN administered. Individual KCN plasma concentrations are presented in Table 2 below.

3.4. SIAN exposure when measured by isoamyl alcohol (IAA) plasma level acts as a fast-acting antidote, correlating with the rapid reversal of cyanide effects

After IN administration of SIAN, IAA T_{max} occurred as early as 0.5–2.5 min post-dose, with mean plasma terminal elimination half-lives ranging from 1.4 to 4.1 min, both with no clear relationship to dose level. When animals survived, the last quantifiable isoamyl alcohol concentrations were detected at the last blood sampling time point, (20 min post-dose), and were low when compared to C_{max}. Systemic exposure to IAA generally increased with dosage in a dose proportional manner, although this should be considered with caution due to low

Table 2
Individual KCN plasma concentration over time.

Animal ID	SIAN Dose (μL/kg)	KCN plasma concentration (ng/mL)								
		0 min	8 min	12 min	15 min	17 min	24 min	30 min	45 min	60 min
1003[a]	0	0	1340	1480	–	–	–	–	–	–
2002[a]	3.6	0	309	Absent[c]	Absent[c]	1150	520	–	–	–
2003[b]	4.4	0	1220	1310	3020	3120	1380	1090	711	636
2004[a]	3.1	0	2660	4530	5780	–	–	–	–	–
2502	3.8	0	1150	896	2650	1550	1090	590	716	499
2503[a]	3.7	0	1700	2800	2690	1530	–	–	–	–
3505[a]	11.4	0	2640	1130	3290	3040	–	–	–	–
4005	13.5	0	1530	587	1140	844	565	512	393	308
4006	13.4	0	1010	651	1080	696	490	583	533	448
4504	13.5	0	1220	535	701	686	570	497	450	399
4505	13.5	0	1080	324	754	717	247	324	366	363
5505	20	0	1420	609	684	416	405	386	304	162
5506	20	0	1380	767	555	515	436	397	371	362
5001	20	0	1080	499	441	413	354	297	196	179
5502	20	0	1480	708	502	463	535	498	381	336
6001	23.3	0	1450	643	1250	815	495	439	327	302
6002	27	0	1440	454	412	248	333	211	157	133
6501	27	0	1730	392	458	310	234	210	178	154
7001[b]	26.3	0	1340	1690	912	545	309	219	160	113
7501[a]	23.8	0	1260	1930	1120	917	–	–	–	–

-: No sampling due to death or euthanasia.
[a] Animal dead.
[b] Animal euthanized.
[c] Sample could not be collected.

number of animals per group (n = 1–3) and IAA plasma concentration variability. A summary of pharmacokinetic parameters is presented in Table 3 below.

3.5. SIAN administration increased the metHb levels in treated animals

A dose-dependent increase in absolute metHb concentration was observed 2 min after the administration of SIAN, with higher metHb levels correlating with improved survival (Fig. 3). After 2 min post SIAN administration time point, the metHb concentration generally decreased slightly and stabilized up to the last collection performed 1 h after SIAN administration (Table 4). Overall, increased doses of SIAN administration (doses of ≥20 μL/kg) appeared to be associated with elevated levels of metHb in the surviving animals up to 62 min post SIAN administration.

3.6. SIAN treatment post-KCN challenge rapidly improved respiratory parameters

Cyanide is known to directly stimulate chemoreceptors in the carotid and aortic bodies (Calvelo et al., 1970; Comroe and Mortimer, 1964), resulting in increased respiration which was observed 5 min post KCN infusion start with followed by decrease in respiratory function with continued administration of the toxicant. For animals that were rescued by SIAN, the respiratory function improved during the period following intranasal administration and a few minutes post the end of KCN challenge as detailed below.

In most animals, the respiratory rate (RR) increased for approximately 5 min post start of the KCN infusion (Fig. 4A), and decreased at up to 10 min post start of the KCN infusion. The RR was restored following SIAN administration (at 10 min post start of KCN infusion) in most animals, rapidly returning to baseline comparable values at approximately 15 min after SIAN administration. In most animals, tidal volume (TV) increased during the first 8–10 min post start of KCN administration (Fig. 4B), with substantial individual variations. For some animals, the increase in TV was slight, while for others it was more pronounced (such as in the sole animal in Group 3 dosed with 11.4 μL/kg of SIAN, whose TV more than doubled on some occasions during the first 8–10 min post start of KCN infusion). Similar to the respiratory rate, TV decreased to baseline comparable values at 10 min post start of KCN administration. Interestingly, during the first 2–3 min after SIAN treatment (doses of ≥13.5 μL/kg), TV showed signs of compensatory increase in SIAN treated groups (doses of ≥13.5 μL/kg) at 10 min post KCN infusion start but not in Group 7 animals (23.5–26.5 μL/kg) treated 12 min after KCN infusion-start. Afterward, TV showed a slow return to baseline values within approximately 30 min post SIAN administration for all surviving animals. The minute volume variations were very similar to TV variations, but the increase post KCN infusion was observed earlier (i.e. 5–7 min post start of the KCN infusion) due to the increase in respiratory rate.

Table 3
Mean plasma isoamyl alcohol (IAA) pharmacokinetic parameters after SIAN intranasal administration.

Group (SIAN dose)	No. of Animals	C_{max} (ng/min)	$AUC_{(last)}$ (min × ng/mL)	T_{max} (min)[a]	$T_{1/2}$ (min)
2 (3–4.5 μL/kg)	5	3500	5500	0.999 (0.498–2.00)	2.41
3 (11.4 μL/kg)	1	10800	27300	0.499 (0.499–0.499)	2.19
4 (13.4 μL/kg)	4	17100	20600	0.500 (0.500–0.500)	2.87
5 (20 μL/kg)	4	25700	28600	0.500 (0.500–1.00)	3.34
6 (23–27 μL/kg)	3	22700	25400	0.500 (0.500–2.50)	2.76
7 (23.5–26.5 μL/kg)	4	20700	29200	0.750 (0.500–1.00)	4.08

[a] Values represented as Median (Min-Max).

3.7. SIAN had a minimal effect on arterial blood gases, serum lactate and pH caused by KCN challenge

For most animals in the study across groups, the partial pressure of oxygen (PaO_2) increased (by approximately 15 mmHg) at 8 min post-start of the KCN infusion time point and prior to SIAN administration. During the same period, decreases between 18.3 and 24.4 mmHg in $PaCO_2$ were observed. It should be noted that animals for which the $PaCO_2$ was >60 mmHg, and/or PaO_2 was <60 mmHg, and/or pH was <7 during or after the KCN infusion succumbed post challenge. The arterial blood pH showed subtle increase at 8 min post-start KCN infusion followed by a decrease in most animals across groups from 12 min. This continued until the end of the observation period or started to increase without a return to baseline values.

After dosing with SIAN, the partial pressure of carbon dioxide ($PaCO_2$) increased very slightly and was still well below baseline at 45 min post-start of the KCN infusion with the exception of a few animals, which had elevated $PaCO_2$ levels starting at 15 min post challenge. The PaO_2 remained stable around 105 mmHg and the pH decreased slightly below baseline values and returned to baseline values (i.e. pH of circa 7.4) at 45 min post-start of the KCN infusion.

Lactate levels increased (2–10-fold) in all animals at 8 min post-start KCN infusion and peaked around the end of the 15-min KCN infusion (ranged between 14.3 and 20 mol/L). SIAN administration had no effect on lactate concentration, which continued to increase in all animals at least until 27 min post-start of KCN (17 min post SIAN), then started to decrease in some animals or remained high up to the last sampling time point at 45 min post-start of KCN in other animals, independently to dose level.

All the changes in blood gases, lactate and pH are consistent with the mechanism of action of cyanide where cellular respiration is compromised.

3.8. KCN challenge and/or SIAN treatment had limited effects on clinical pathology parameters

No effect on hematology and coagulation parameters were observed. Clinical chemistry changes were limited to a significant increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in the majority of the animals that survived the KCN challenge (euthanized approximately 24 h post-KCN challenge), with no similar effect on alkaline phosphatase (ALP). Higher AST and ALT levels in the survived animals suggests a liver damage but could also be attributed to muscle injuries due to manipulation of the animals and hyperactivity on the sling during the KCN infusion. Absence of similar changes in AST and ALT in the dead animals at termination was considered normal given the expected delayed expression in the serum after the effect. Mean AST, ALT and ALP results are presented in Table 5.

3.9. Mid to high doses of SIAN administration at 10 min post KCN infusion-start effectively reversed the histotoxic and hypoxic effects induced by KCN via improvements in cardiovascular parameters

The majority of cardiovascular variations associated with KCN administration were related to changes in heart rate. After an initial progressive increase in heart rate to approximately 4 min after the start of KCN challenge, heart rate progressively and variably decreased in individual animals until SIAN administration or until death. In control animals (Group 1) and animals receiving low doses of SIAN (Groups 2 and 3; 3–4.5 μL/kg and 11.4 μL/kg), no recovery of the heart rate was observed and all animals exhibited cardiovascular collapse progressing to death within 30 min with the exception of two animals from Group 2

Table 4
Individual MetHb level (%) over time.

Animal ID	SIAN (μL/kg)	Time SIAN Dose Post KCN- start (min)	MeHb Level (%)							Survival
			Pre-SIAN	2 min Post-SIAN	5 min Post-SIAN	8 min Post-SAIN	15 min Post-SIAN	20 min Post-SIAN	60 min Post-SIAN	
2002	3.6	10	2.9	Absent[b]	Absent[b]	2.3	2	–	–	died
2003	4.4	10	1.6	1.7	1.6	1.5	1.3	1.6	1.5	euthanized[a]
2004	3.1	10	2.4	2.2	2.2	–	–	–	–	died
2502	3.8	10	2.2	3.1	2.5	2.8	2.5	2.8	2.3	survived
2503	3.7	10	1.8	1.8	1.7	–	–	–	–	died
3505	11.4	10	1.6	1.7	1.9	2.1	–	–	–	died
4005	13.5	10	1.1	2.7	2.2	2.4	2.1	1.6	1.8	survived
4006	13.4	10	1.7	2.3	2.1	2	2.5	2	2.2.	survived
4504	13.5	10	1.4	2.9	2.3	2.1	2.3	2	1.7	survived
4505	13.5	10	1.9	3.1	1.8	2.1	1.8	2	2.1	survived
5001	20	10	3.5	5.6	4.8	4.4	absent	4.4	4.5	survived
5502	20	10	1.6	3.3	2.3	2.9	2.7	2.2	2.7	survived
5505	20	10	4.4	5.1	5	5.8	4.5	5.2	5.4	survived
5506	20	10	1.6	3.8	3.4	3.4	2.9	3.3	2.7	survived
6001	23.3	10	2.2	3.1	3.2	3.1	1.7	3.4	2.3	survived
6002	27	10	4.4	7.8	6.7	6.4	5.4	5.4	5	survived
6501	27	10	2.9	6.6	5.6	5.7	5.7	4.9	4.4	survived
7001	26.3	12	1.5	2.8	3.1	3.3	3.3	3.8	3.5	euthanized[a]
7501	23.8	12	1.4	1.6	1.8	–	–	–	–	died

-: No sampling due to death.
[a] Euthanasia within 3–5 h due to no recovery.
[b] Sample could not be collected.

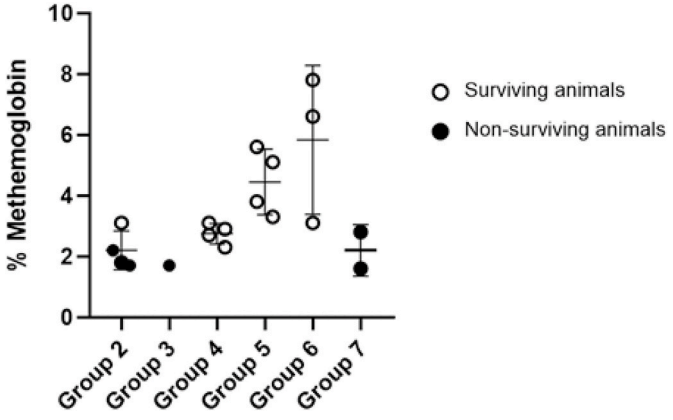

Fig. 3. Methemoglobin levels in NHPs at 2 min post SIAN administration.

(Fig. 5A). For all SIAN treated groups that received a mid to high dosage (≥13.54 μL/kg), recovery of heart rate occurred rapidly after SIAN administration (i.e. usually within 2 min after the administration) with the exception of Group 7, where recovery occurred much later for the single surviving animal (Fig. 5A). An apparent second phase of decrease in heart rate, initiated approximately around 1 h and 45 min post start of KCN challenge was observed in all surviving animals from Groups 4, 5 and 6 (Fig. 5B).

Progressive increases were also observed in QRS complex durations from 15 to 25 min post start of KCN challenge, especially in animals from Groups 1, 2 and 3 (untreated or saline, 3–4.5 μL/kg, and 11.4 μL/kg, respectively) (Fig. 5C). The QRS complex duration returned to pre challenge levels in the surviving animals at 25 min post challenge – except for the surviving animal in Group 2, which had lowered QRS durations at up to 24 h post KCN challenge.

Cardiac contractility changes paralleled the heart rate effects. An initial slight transient increase in contractility (i.e. maximum rate of left ventricular pressure decrease (max dP/dt)) was noted, peaking with heart rate changes at approximately 2–4 min post initiation of KCN infusion followed by a rapid decline, which continued until death for untreated, or saline treated animals, as well as for most of SIAN treated animals at doses < 13.4 μL/kg or treated 12 min post KCN infusion (Fig. 6A). For animals treated with SIAN at 10 min post KCN infusion start, ventricular contractility showed signs of recovery with a trend towards dose dependent effects (Fig. 6B). Signs of cardiac contractility recovery were noted before SIAN administration for Group 5 (20 μL/kg) and Group 7 (23.5–26.5 μL/kg) (Fig. 6C). Early recovery of ventricular contractility may be due to compensatory mechanisms that were activated by KCN induced hypotension. Contractility values remained slightly lower than baseline immediately after SIAN treatment in experimental groups. This may be a result of slightly higher baseline contractility values in the animals, which were likely due to stress from restraining procedures.

A progressive fall in arterial BP occurred at about 7–10 min post start of KCN challenge. This was observed in all animals, and was likely the direct result of histotoxic hypoxia, progressive myocardial failure, and changes in heart rate induced by KCN exposure. SIAN treated Groups 4, 5 and 6 showed uniform, rapid improvements in arterial blood pressure parameters (i.e. systolic arterial pressure (SABP), mean arterial pressure (MABP), diastolic arterial pressure (DABP), and pulse arterial pressure (PABP)) immediately after SIAN administration which appeared to persist at least until approximately 2 h after SIAN administration (Fig. 7). While arterial blood pressure values remained lower than the baseline (confirmed by the percentage change from baseline values) after rescue, the range was considered within normal physiological values for conscious restrained rhesus monkeys. Stress from restraining required for dosing likely resulted in higher blood pressure values prior to cyanide infusion, and the post-SIAN values were considered normal for conscious animals. A second phase of progressive decrease for all arterial blood pressure parameters appeared to be somewhat dose dependent. The highest SIAN dose showed the least decrease (approximately 20% lower in all surviving animals at 24 h and 10 min post start of KCN challenge for all arterial blood pressure parameters, except for PABP. This second phase of decrease in arterial blood pressure corresponded with decreases in heart rate.

This observation also confirmed the outcome associated with the dose level of KCN used in the challenge. Lethality was confirmed in untreated or saline treated animals, as well as animals in Groups 2, 3, and 7 who received SIAN at low dose levels, or at high dose levels but at a slightly delayed time (i.e. 12 min post start of KCN challenge). Animals from Groups 3 to 6 that received mid to high doses of SIAN (≥13.54 μL/kg) showed some recovery of normal arterial BP parameters after IN

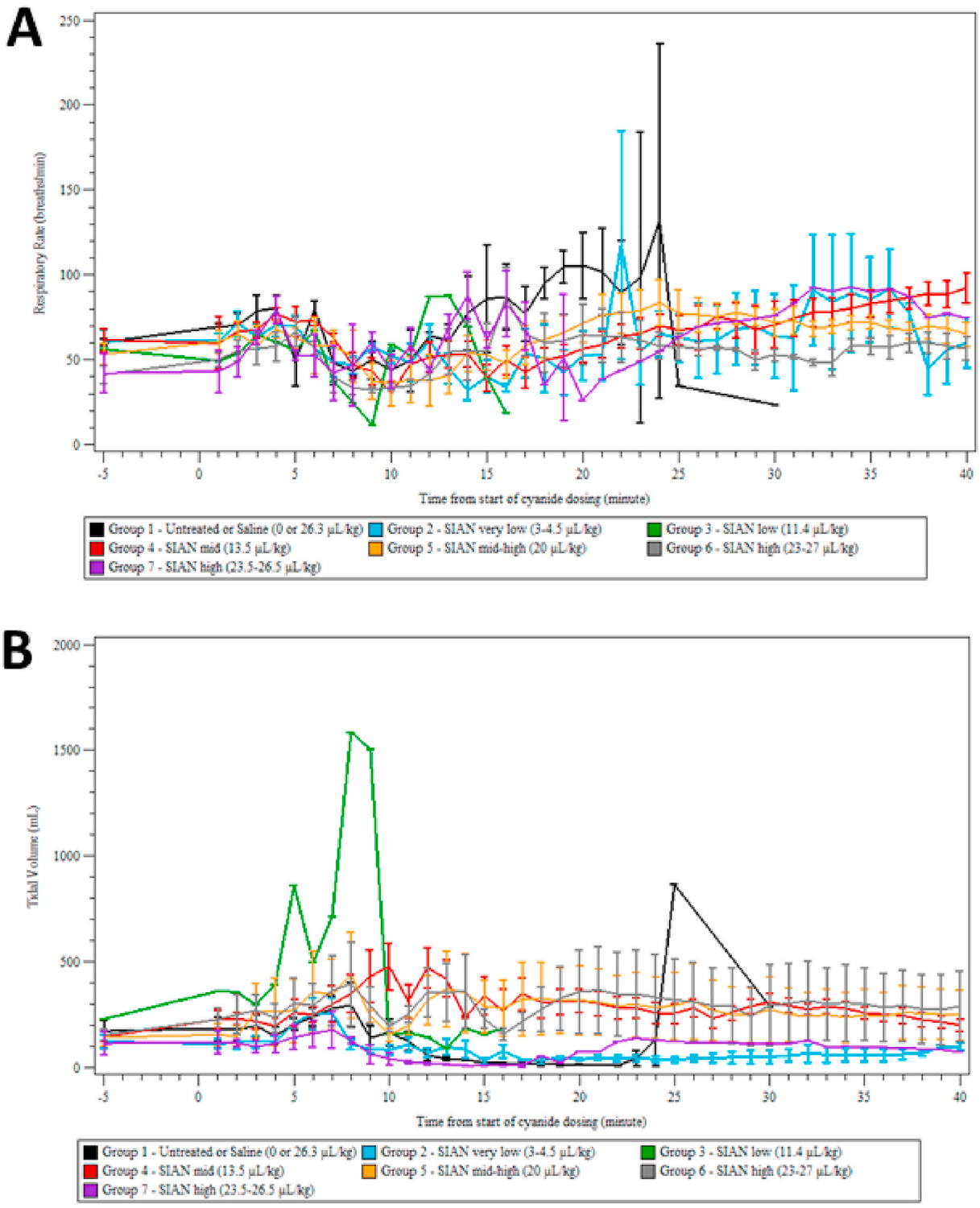

Fig. 4. Absolute changes in respiratory rate and tidal volume of SIAN-treated NHPs up to 40 min post start of KCN challenge. **A.** Respiratory rate, **B**. tidal volume of NHPs at up to 40 min post start of KCN challenge. SIAN was administered at dosages indicated to groups at 10 min post start of KCN challenge, except for Group 7 animals, which were treated at 12 min post start of KCN challenge. Shown are the treatment means (±SEM).

administration of SIAN (Fig. 7). Group 2 animals, which received a very low dose of SIAN (3–4.5 µL/kg), however, continued to show progressive decreases in SABP until the nadir was reached at 15 min and 45 s post start of KCN challenge (−52.4%) (Fig. 7A). This was followed by a sudden reversal of hypotension by 16 min and 30 s post challenge, where only 3 out of 5 animals in the group survived. Subsequently, SABP values fluctuated and remained low and unstable. The sole animal in Group 3 showed a rapid initial improvement in SABP post SIAN administration, but deteriorated at 3 min post SIAN administration, became apneic by 17 min, and died. When high dose level of SIAN (23.5–26.5 µL/kg) was administered 12 min post start of KCN challenge instead of 10 min (Group 7), the arterial blood pressure parameters showed a greater decrease. Interestingly, the single surviving Group 7 animal also exhibited the best recovery of arterial blood pressure parameters after

Table 5
Liver enzymes levels in survivors and decedents.

KCN Challenge Outcome	Time point	AST (U/L) (Mean values)	ALT (U/L) (Mean values)	ALP (U/L)
Survivors	Baseline	39.2	51.1	388.2
	At termination (24h post-KCN challenge)	254.9	114.1	393.4
	% change from baseline	***+549.9***	***+123.0***	***+1.3***
Decedents	Baseline	48.8	46.5	456.5
	At termination (immediately before death)	23.3	8.5	370.3
	% change from baseline	***−52.3***	***−81.7***	***−18.9***

SIAN administration compared to all treated animals, which began at 18 min post KCN challenge, returning to baseline values at some time points.

In all groups, administration of KCN resulted in similar, uniform decrease in systolic myocardial performance (i.e. left ventricular systolic pressure (LVSP)) (Fig. 8A). Histotoxic hypoxia of the myocytes had negative effects on inotropy, which resulted in lethal cardiovascular collapse and death as described previously. In Groups 2 and 3 (doses of ≤11.4 μL/kg), systolic and diastolic function recovered in only one animal from Group 2. However, systemic consequences of KCN administration still resulted in morbidity and justified subsequent euthanasia of this animal. In Groups 4 to 6 (doses of ≥13.5 μL/kg), there was an apparent dose related, progressive improvement in both systolic and diastolic (i.e. left ventricular diastolic pressure (LVDP)) functions as early as 1–2 min after the administration of SIAN (Fig. 8B). Even though complete recovery of systolic and diastolic function was not reached, all animals of these three groups survived to the end of the study.

4. Discussion and conclusions

While amyl nitrite is used as a cyanide antidote for decades, its efficacy and safety in both animals and human requires further investigation due to the shortage of data. There is also a lack of studies in well characterized primate models of cyanide poisoning. The information regarding effects of isoamyl nitrite on both respiratory and hemodynamic parameters are also limited, and whether there is a dose-response correlation between isoamyl nitrite treatment and recovery of these parameters remains unknown. As such, we developed a model using telemeterized NHPs to assess the effects of SIAN treatment after cyanide exposure.

This showed that mid to mid-high doses of SIAN treatment (13.5, 20, and 23–27 μL/kg) significantly enhanced survival of animals post lethal dose cyanide challenge ($p = 0.0286$ for Group 4 and 5 compared to control Group 1; Group 6 comparisons with controls were not statistically significant, but all animals survived post challenge). Low doses of SIAN (≤13.5 μL/kg) and delayed SIAN treatment after cyanide exposure, even at the highest dose, still resulted in death. Increased survival was also demonstrated with isosorbide dinitrate in rabbits (Lavon et al., 2017). In the cyanide-poisoned non-anaesthetized swine model treatment with isosorbide dinitrate oral spray led to a longer time to death and better clinical scores and clinical laboratory parameters presumably due to rapid increase of NO levels in the body (Lavon et al., 2020).

There were several mechanisms of action suggested for nitrites antidotal potential. The antidotal properties of amyl nitrite were historically attributed to induction of metHb and later to NO mediated vasodilation (Lavon and Bentur, 2010). The nitrites oxidize iron from ferrous to the ferric state in Hb molecule, converting it into metHb. When cyanide binds metHb, it produces much less toxic cyanmethemoglobin, freeing cytochrome *c* oxidase from cyanide (Reade et al., 2012). Alternatively, generation of NO may directly antagonize cyanide inhibition of cytochrome *c* oxidase (Leavesley et al., 2010). In addition, it was shown that nitrite acts as an effective cyanide antidote when methylene blue administered to prevent metHb formation (Nelson et al., 2011). There is a possibility that nitrites could reverse circulatory effects caused by cyanide via vasodilatory activity of NO rather than neutralization of cyanide by metHb. Even marginally increased levels of methemoglobin have been previously associated with improved survival rates in human cases of cyanide toxicity (Hall et al., 1987; Johnson et al., 1989). This was also observed in the current study, where a dose dependent increase in both absolute and percent change of metHb concentration occurred 2 min after the administration of SIAN, with higher metHb levels correlating with the rapid exposure of SIAN when measured by IAA plasma level, reversing cyanide effects and improving survival.

Cyanide poisoning affect the respiratory and cardiovascular systems via its direct effect on vascular smooth muscle and likely on the central nervous system (Cope and Abramowitz, 1960; Kulig, 1991; Salkowski and Penney, 1995). In the current study, both respiratory and cardiovascular parameters were rapidly improved by mid to high doses of SIAN administration, which were also associated with elevated levels of metHb at up to 62 min post SIAN administration.

The data presented herein show that rapid administration of SIAN post cyanide challenge significantly improved survival and reversed the cardiopulmonary depression caused by cyanide poisoning in a well characterized NHP model. There are indeed some drawbacks to the use of SIAN as a cyanide antidote, such as nitrite-induced methemoglobinemia and abuse (Balster, 1998; Haverkos and Dougherty, 1988; Maickel, 1988). However, its swift antidotal action and ease of use shows that SIAN may still be considered a rapid first line response against acute cyanide exposure.

This study clearly demonstrated the survival and therapeutic benefits of SIAN treatment of lethal cyanide exposure. Nevertheless, follow up investigations are warranted to address some gaps and limitations. First, the manipulation and restraining of the conscious animals led to stress-related changes in the baseline values of some cardiovascular and/or respiratory parameters add some challenges to interpret the results. Second, the group size and gender ratio should be optimized to insure proper statistical analysis and evaluation of the drug exposure-response relationships.

In conclusion, intranasal administration of SIAN 10 min following initiation of a lethal KCN infusion was found to be an effective antidote with 100% survival at doses ≥13.5 μL/kg and improved clinical outcomes in NHP model of lethal cyanide intoxication. This study will serve as a model for further pivotal efficacy studies for cyanide poisoning.

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Author agreement

All authors have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

CRediT authorship contribution statement

Yifei Zhong: Writing – original draft. **Robert E. Raulli:** Conceptualization, Methodology. **Samir Abtout:** Investigation, Methodology, Writing – review & editing. **Simon Authier:** Writing – review & editing. **Alexis Ascah:** Investigation, Methodology. **Daniel Lambert:** Methodology. **Gloria Gutierrez:** Methodology, Writing – review & editing. **Norma L. Cantú:** Methodology, Writing – review & editing. **Nadean**

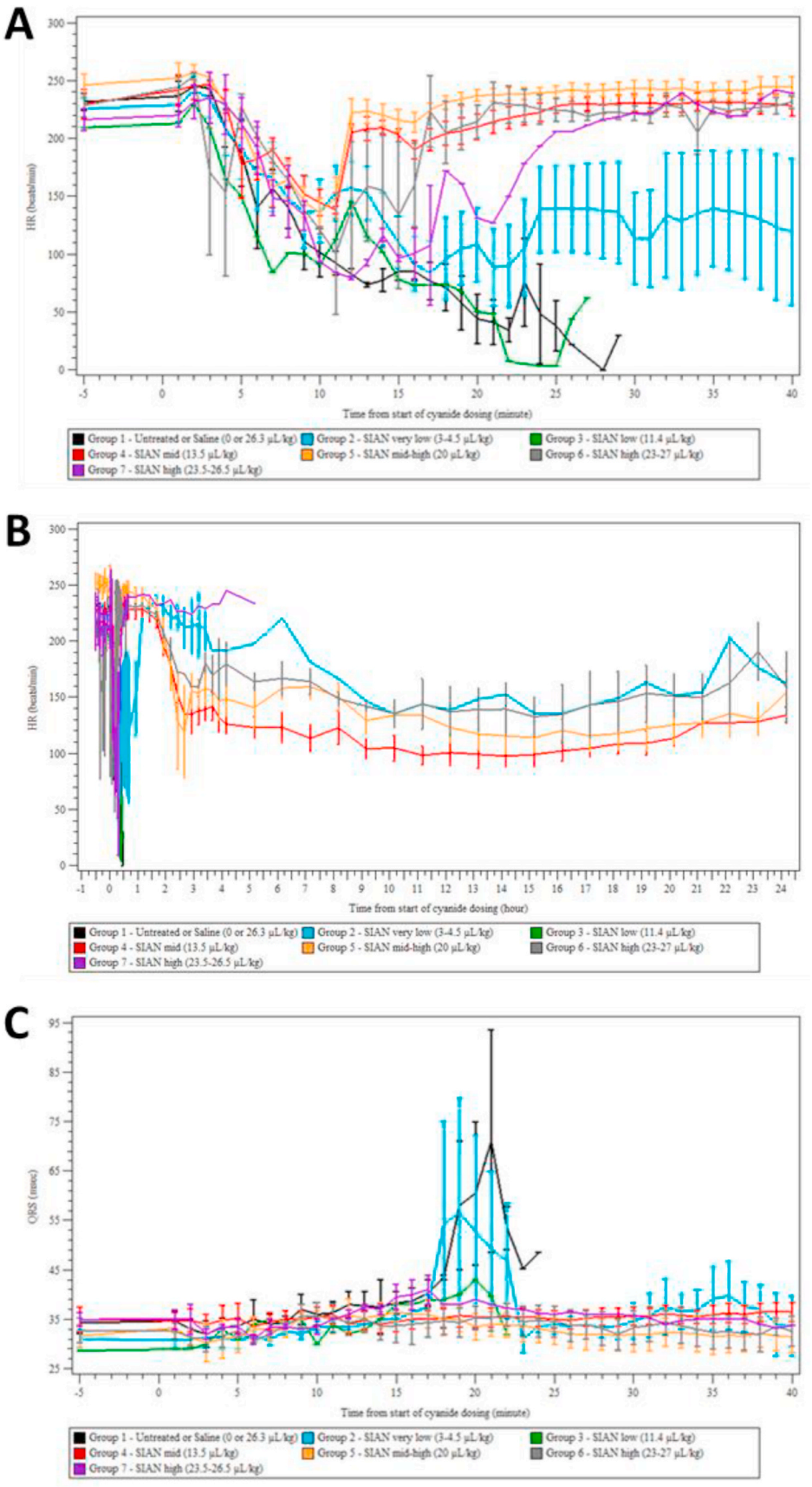

Fig. 5. Absolute changes in heart rate and QRS interval of SIAN-treated NHPs up to 40 min and 24 h (HR) post start of KCN challenge. Heart rate of NHPs at **A.** 40 min and **B.** 24 h post start of KCN challenge **C.** QRS intervals of NHPs 40 min post start of KCN challenge. SIAN was administered at dosages indicated to groups at 10 min post start of KCN challenge, except for Group 7 animals, which were treated at 12 min post start of KCN challenge. Shown are the treatment means (±SEM).

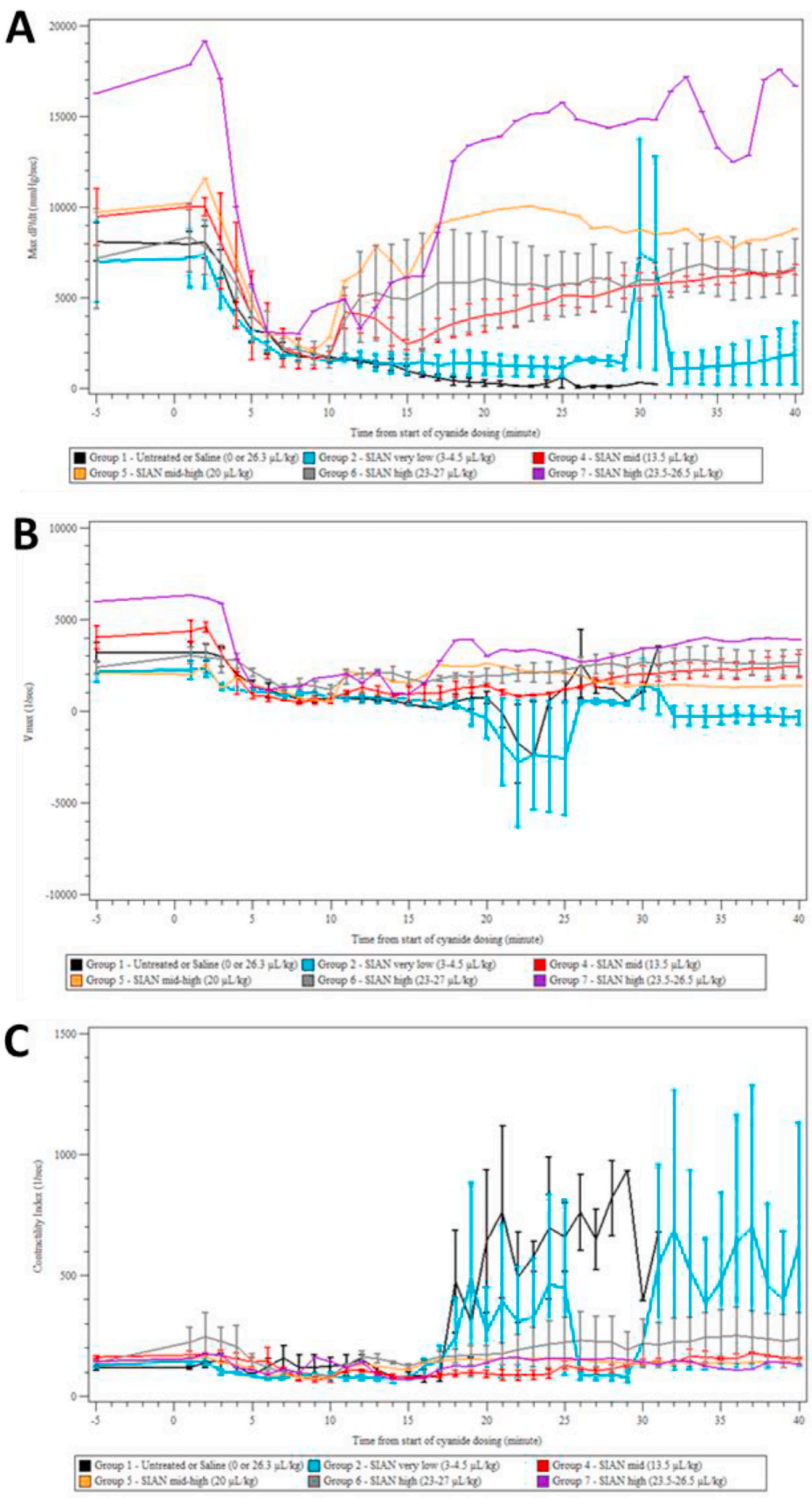

Fig. 6. Absolute changes in max dP/dt, Vmax, and contractility index of SIAN-treated NHPs up to 40 min post start of KCN challenge. A. Max dP/dt of NHPs at 40 min post start of KCN challenge. **B.** Vmax of NHPs at 40 min post start of KCN challenge. Contractility index of NHPs at **C.** Contractility index of NHPs at 40 min. SIAN was administered at dosages indicated to groups at 10 min post start of KCN challenge, except for Group 7 animals, which were treated at 12 min post start of KCN challenge. Shown are the treatment means (±SEM).

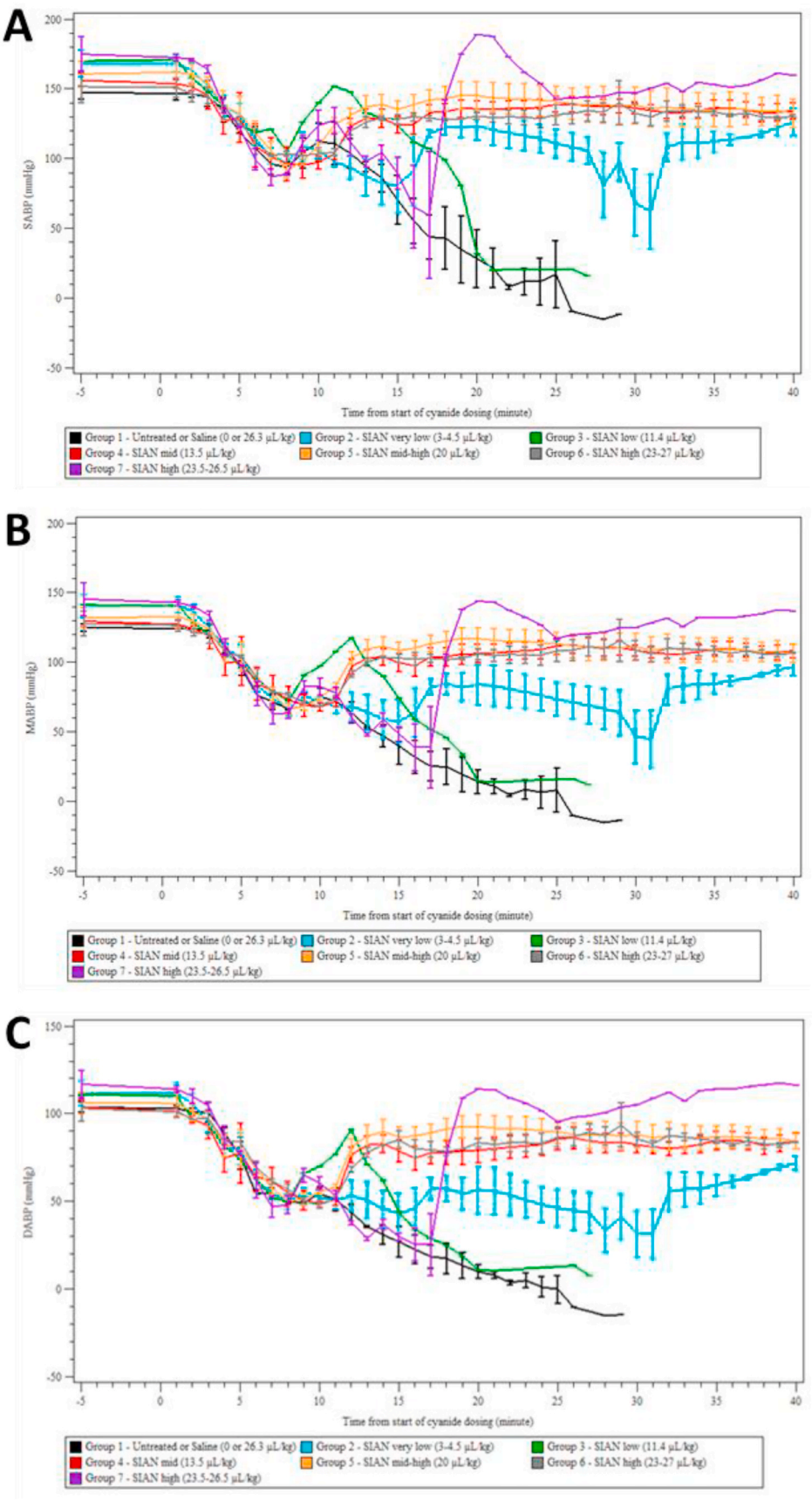

Fig. 7. Absolute changes in SABP, MABP and DABP of SIAN-treated NHPs up to 40 min post start of KCN challenge. **A.** SABP of NHPs at 40 min post start of KCN challenge. **B.** MABP of NHPs at 40 min post start of KCN challenge. **C.** DABP of NHPs at 40 min post start of KCN challenge. SIAN was administered at dosages indicated to groups at 10 min post start of KCN challenge, except for Group 7 animals, which were treated at 12 min post start of KCN challenge. Shown are the treatment means (±SEM).

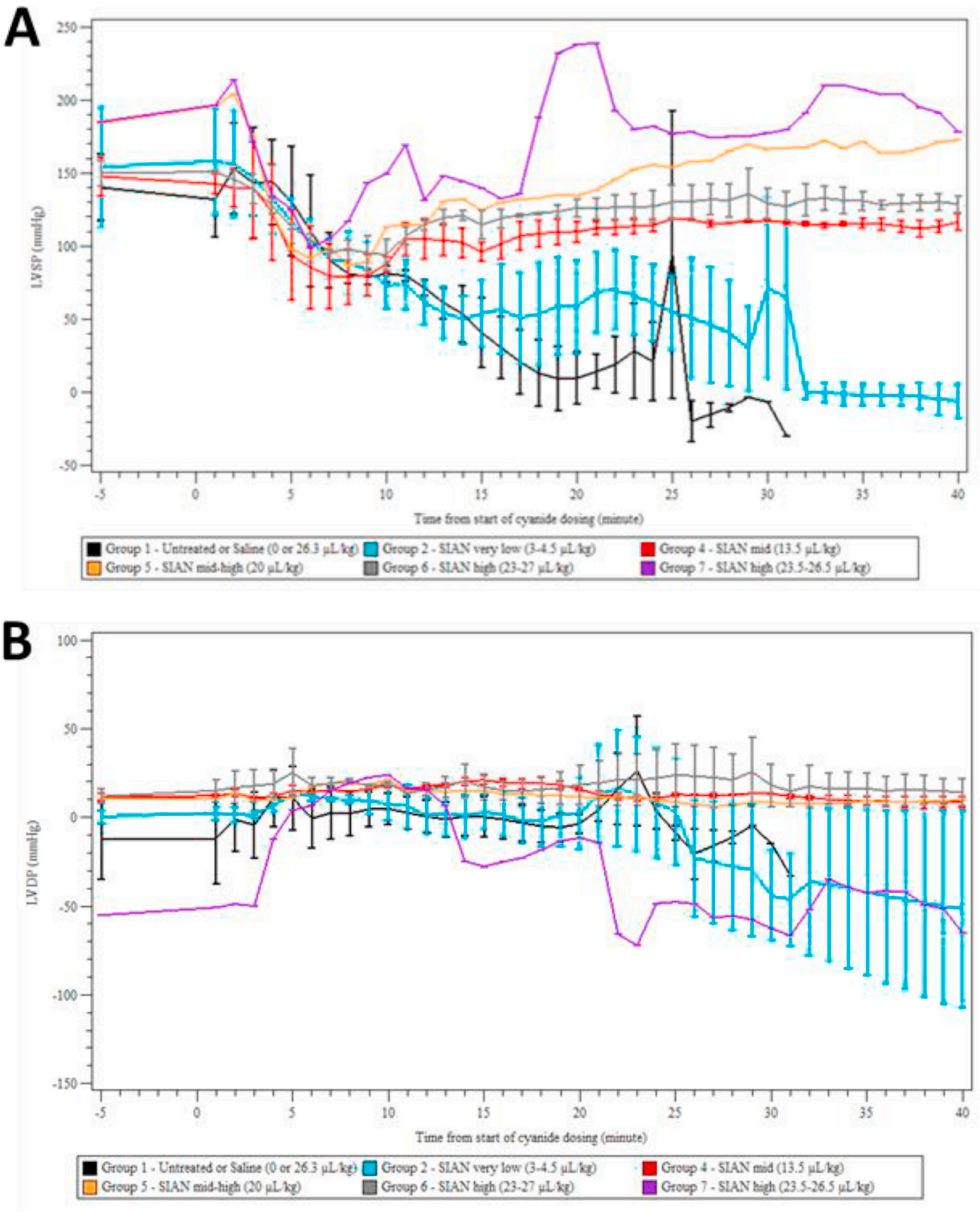

Fig. 8. Absolute changes in LVSP and LVDP of SIAN-treated NHPs up to 40 min post start of KCN challenge. **A.** LVSP of NHPs at 40 min post start of KCN challenge. **B.** LVDP of NHPs at 40 min post start of KCN challenge. SIAN was administered at dosages indicated to groups at 10 min post start of KCN challenge, except for Group 7 animals, which were treated at 12 min post start of KCN challenge. Shown are the treatment means (±SEM).

Gutierrez: Methodology, Writing – review & editing. **Larry Cabell:** Conceptualization, Methodology, Writing – review & editing. **Joe McDonough:** Conceptualization, Methodology, Writing – review & editing. **Kelly C. Cakouros:** Project administration. **Pablo Gurman:** Conceptualization, Writing – review & editing. **Vladimir Savransky:** Conceptualization, Writing – review & editing. **John Barry:** Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Authier, S., et al., 2007. A cardiovascular monitoring system used in conscious cynomolgus monkeys for regulatory safety pharmacology. Part 2: pharmacological validation. J. Pharmacol. Toxicol. Methods 56, 122–130. https://doi.org/10.1016/j.vascn.2007.03.011.

Balster, R.L., 1998. Neural basis of inhalant abuse. Drug Alcohol Depend. 51, 207–214. https://doi.org/10.1016/s0376-8716(98)00078-7.

Barillo, D.J., 2009. Diagnosis and treatment of cyanide toxicity. J. Burn Care Res. 30, 148–152. https://doi.org/10.1097/BCR.0b013e3181923b91.

Benardeau, A., et al., 2000. Effects of the T-type Ca(2+) channel blocker mibefradil on repolarization of Guinea pig, rabbit, dog, monkey, and human cardiac tissue. J. Pharmacol. Exp. Therapeut. 292, 561–575.

Butler, A.R., Feelisch, M., 2008. Therapeutic uses of inorganic nitrite and nitrate: from the past to the future. Circulation 117, 2151–2159. https://doi.org/10.1161/CIRCULATIONAHA.107.753814.

Calvelo, M.G., et al., 1970. Reflex vascular responses to stimulation of chemoreceptors with nicotine and cyanide. Activation of adrenergic constriction in muscle and noncholinergic dilatation in dog's paw. Circ. Res. 27, 259–276. https://doi.org/10.1161/01.res.27.2.259.

Chen, K.K., Rose, C.L., 1952. Nitrite and thiosulfate therapy in cyanide poisoning. J. Am. Med. Assoc. 149, 113–119. https://doi.org/10.1001/jama.1952.02930190015004.

Chen, K.K., et al., 1933. Methylene blue, nitrites, and sodium thiosulphate against cyanide poisoning. PSEBM (Proc. Soc. Exp. Biol. Med.) 31, 250–251. https://doi.org/10.3181/00379727-31-7079p.

Comroe Jr., J.H., Mortimer, L., 1964. The respiratory and cardiovascular responses of temporally separated aortic and carotid bodies to cyanide, nicotine, phenyldiguanide and serotonin. J. Pharmacol. Exp. Therapeut. 146, 33–41.

Cooling, L., 2014. The RBC as a physiological object. In: McManus, L.M., Mitchell, R.N. (Eds.), Pathobiology of Human Disease. Academic Press, San Diego, pp. 3049–3067. https://doi.org/10.1016/B978-0-12-386456-7.06202-X.

Cope, C., Abramowitz, S., 1960. Respiratory responses to intravenous sodium cyanide, a function of the oxygen-cyanide relationship. Am. Rev. Respir. Dis. 81, 321–328. https://doi.org/10.1164/arrd.1960.81.3.321.

Eckstein, M., 2004. Cyanide as a chemical terrorism weapon. J. Emerg. Med. Serv. 29, suppl 22–31.

Gauvin, D.V., et al., 2006. Electrocardiogram, hemodynamics, and core body temperatures of the normal freely moving cynomolgus monkey by remote radiotelemetry. J. Pharmacol. Toxicol. Methods 53, 140–151. https://doi.org/10.1016/j.vascn.2005.07.004.

Hall, A.H., et al., 2007. Sodium thiosulfate or hydroxocobalamin for the empiric treatment of cyanide poisoning? Ann. Emerg. Med. 49, 806–813. https://doi.org/10.1016/j.annemergmed.2006.09.021.

Hall, A.H., et al., 1987. Nitrite/thiosulfate treated acute cyanide poisoning: estimated kinetics after antidote. J. Toxicol. Clin. Toxicol. 25, 121–133. https://doi.org/10.3109/15563658708992618.

Hamel, J., 2011. A review of acute cyanide poisoning with a treatment update. Crit. Care Nurse 31, 72–81. https://doi.org/10.4037/ccn2011799 quiz 82.

Hassimoto, M., Harada, T., 2003. Use of a telemetry system to examine recovery of the cardiovascular system after excitement induced by handling stress in a conscious cynomolgus monkey (Macaca fascicularis). J. Med. Primatol. 32, 346–352. https://doi.org/10.1046/j.1600-0684.2003.00036.x.

Haverkos, H.W., Dougherty, J., 1988. Health hazards of nitrite inhalants. Am. J. Med. 84, 479–482. https://doi.org/10.1016/0002-9343(88)90269-0.

Jandorf, B.J., Bodansky, O., 1946. Therapeutic and prophylactic effect of methemoglobinemia in inhalation poisoning by hydrogen cyanide and cyanogen chloride. J. Ind. Hyg. Toxicol. 28, 125–132.

Johnson, W.S., et al., 1989. Cyanide poisoning successfully treated without 'therapeutic methemoglobin levels. Am. J. Emerg. Med. 7, 437–440. https://doi.org/10.1016/0735-6757(89)90057-0.

Keim, M.E., 2006. Terrorism involving cyanide: the prospect of improving preparedness in the prehospital setting. Prehospital Disaster Med. 21, s56–60. https://doi.org/10.1017/s1049023x00015910.

Kerns, W., Kirk, M., 2006. Cyanide and hydrogen sulfide. In: Flomenbaum, N., et al. (Eds.), Goldfrank's Toxicologic Emergencies. McGraw-Hill, New York, pp. 1498–1514.

Kirk, R.L., Stenhouse, N.S., 1953. Ability to smell solutions of potassium cyanide. Nature 171, 698–699. https://doi.org/10.1038/171698b0.

Klimmek, R., Krettek, C., 1988. Effects of amyl nitrite on circulation, respiration and blood homoeostasis in cyanide poisoning. Arch. Toxicol. 62, 161–166. https://doi.org/10.1007/BF00570134.

Kulig, K., 1991. Cyanide antidotes and fire toxicology. N. Engl. J. Med. 325, 1801–1802. https://doi.org/10.1056/NEJM199112193252508.

Lavon, O., et al., 2017. Effectiveness of isosorbide dinitrate in cyanide poisoning as a function of the administration timing. BMC Pharmacol Toxicol 18, 13. https://doi.org/10.1186/s40360-017-0122-0.

Lavon, O., Bentur, Y., 2010. Does amyl nitrite have a role in the management of pre-hospital mass casualty cyanide poisoning? Clin. Toxicol. 48, 477–484. https://doi.org/10.3109/15563650.2010.505573.

Lavon, O., et al., 2020. Can isosorbide dinitrate oral spray serve as an immediate bridging therapy for a mass cyanide poisoning? Clin. Toxicol. 1–7 https://doi.org/10.1080/15563650.2020.1856382.

Leavesley, H.B., et al., 2010. Nitrite-mediated antagonism of cyanide inhibition of cytochrome c oxidase in dopamine neurons. Toxicol. Sci. 115, 569–576. https://doi.org/10.1093/toxsci/kfq084.

Maickel, R.P., 1988. The fate and toxicity of butyl nitrites. NIDA Res. Monogr. 83, 15–27.

Megarbane, B., et al., 2003. Antidotal treatment of cyanide poisoning. J. Chin. Med. Assoc. 66, 193–203.

Musshoff, F., et al., 2002. Cyanide fatalities: case studies of four suicides and one homicide. Am. J. Forensic Med. Pathol 23, 315–320. https://doi.org/10.1097/01.PAF.0000043071.33751.12.

Nelson, L., et al., 2011. Goldfrank's Toxicologic Emergencies. McGraw-Hill, New York, pp. 1689–1691.

Noyes, W., 1936. n-Butyl Nitrite, vol. 16. Organic Syntheses, p. 7. https://doi.org/10.15227/orgsyn.016.0007.

Petrikovics, I., et al., 2015. Past, present and future of cyanide antagonism research: from the early remedies to the current therapies. World J. Methodol. 5, 88–100. https://doi.org/10.5662/wjm.v5.i2.88.

Reade, M.C., et al., 2012. Review article: management of cyanide poisoning. Emerg. Med. Australasia (EMA) 24, 225–238. https://doi.org/10.1111/j.1742-6723.2012.01538.x.

Salkowski, A.A., Penney, D.G., 1995. Metabolic, cardiovascular, and neurologic aspects of acute cyanide poisoning in the rat. Toxicol. Lett. 75, 19–27. https://doi.org/10.1016/0378-4274(94)03152-w.

Shepherd, G., Velez, L.I., 2008. Role of hydroxocobalamin in acute cyanide poisoning. Ann. Pharmacother. 42, 661–669. https://doi.org/10.1345/aph.1K559.

Vick, J.A., Froehlich, H., 1991. Treatment of cyanide poisoning. Mil. Med. 156, 330–339.

Vick, J.A., Froehlich, H.L., 1985. Studies of cyanide poisoning. Arch. Int. Pharmacodyn. Ther. 273, 314–322.

Way, J.L., et al., 1984. Recent perspectives on the toxicodynamic basis of cyanide antagonism. Fund. Appl. Toxicol. 4, S231–S239. https://doi.org/10.1016/0272-0590(84)90157-x.