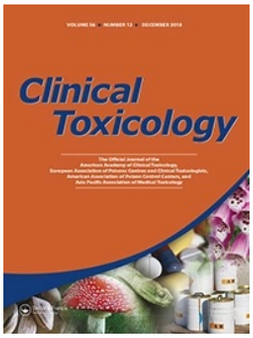

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BASIC RESEARCH

Can isosorbide dinitrate oral spray serve as an immediate bridging therapy for a mass cyanide poisoning?

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ABSTRACT

Objective: In this proof-of-concept study, the aim was to evaluate the short-term clinical effectiveness of isosorbide dinitrate (ISDN) oral spray in non-anaesthetized cyanide-poisoned swine.

Methods: A comparative study was conducted using domestic swine. Animals were intravenously poisoned with potassium cyanide (KCN), either 2 mg/kg or 4 mg/kg dose. Two control groups (one for each cyanide dose) were not further treated. Two other groups (one for each cyanide dose) were treated within 1 min after poisoning with ISDN oral spray: 3 spray actuations (averaging a total of 3.75 mg) after the lower cyanide dose and 4 spray actuations (averaging a total of 5.0 mg) after the higher dose. The study outcomes were clinical score, time to death, and blood tests including pH, lactate, and methemoglobin levels.

Results: All the animals started to convulse within 20 to 30 sec after KCN poisoning, then became unresponsive and hemodynamically depressed after another 20 to 30 sec. After the KCN 2 mg/kg dose, 3 of 4 control animals survived, while all treated animals survived. Compared with control animals, ISDN-treated animals displayed significantly better clinical scores starting 5 min after KCN poisoning. Acidosis was significantly more pronounced in the untreated animals. After the KCN 4 mg/kg dose, similar survival rates were observed for control and ISDN-treated groups (1/4), but treated animals had longer time to death and better pH and lactate levels.

Conclusion: ISDN oral spray administration following KCN poisoning in this porcine model did not result in statistically significant increased survival. However, based on clinical scores and clinical laboratory values, ISDN may benefit as a bridging countermeasure until currently-available specific cyanide antidotes can be administered. Further research is warranted to better characterize this potential role of ISDN in cyanide poisoning.

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Cyanide; poisoning; isosorbide dinitrate; oral spray; efficacy; swine

Introduction

Cyanide poisoning continues to impose a troubling threat in numerous scenarios. Industry workers may be exposed to cyanide during accidents and malpractice. Cyanide is abundantly present in smoke and can be inhaled in toxic concentrations. Deliberate terror attacks using cyanide is a major concern in many countries. Mass cyanide poisoning is a major challenge that necessitates optimal preparedness and effective immediate response.

The current on-site therapeutic response to cyanide poisoning is severely inadequate, and requires urgent improvement. Available antidotes are either slow-acting, safety-impaired, usability-challenged (e.g. on-site reconstitution, slow administration) and/or expensive [1]. Current FDA-approved cyanide countermeasures (Cyanokit® and Nithiodote®) each require intravenous administration. The main obstacle of antidote efficiency in out-of-hospital cyanide poisoning, especially mass casualty incidents, is, therefore, the lack of immediate intravenous access to deliver the current antidotes [2]. Intra-osseous administration is an acceptable alternative, but it requires special equipment and the presence of qualified experienced professionals.

In a recently published porcine study of cyanide poisoning, pre-treatment with hydroxocobalamin or dicobalt edetate was found to have only modest and variable efficacy at lethal doses of cyanide [3]. The authors concluded that in clinical poisoning scenarios, with delayed administration, approved antidotes are likely to be less effective and new antidotes are required.

Isosorbide dinitrate (ISDN) is approved for the treatment of ischemic heart disease and heart failure [4,5]. It is an inexpensive, relatively safe medication, stable and available in several formulations including an easy to use oral spray. ISDN was previously shown to improve survival in cyanide poisoned mice and rabbits [1,6–8]. Thus, it is potentially a good candidate to serve as a countermeasure against cyanide poisoning, mainly in mass incidents and especially in its oral spray formulation.

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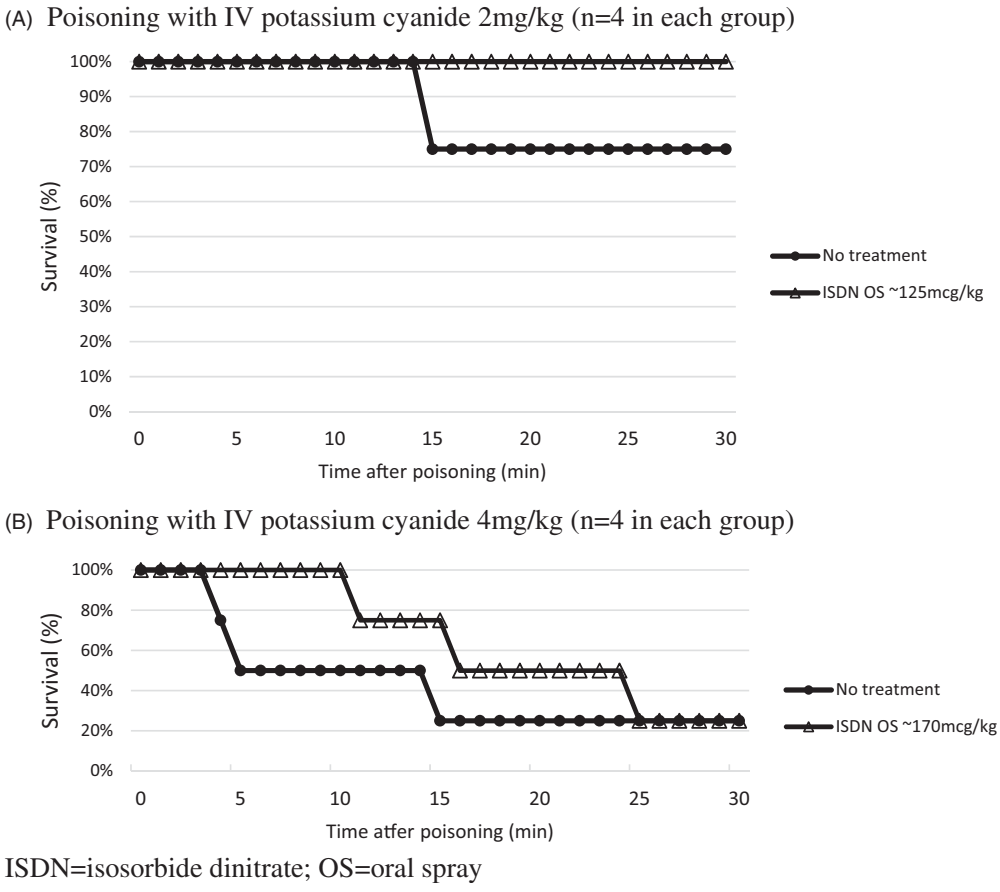

Figure 1. Kaplan–Meier Survival Plot.

The objective of this study was to perform a proof-of-concept evaluation of ISDN oral spray to improve short term clinical outcomes in a non-anesthetized swine model of cyanide poisoning.

Methods

This was a comparative study conducted using male domestic swine, purchased from Lahav Animal Research Institute, Israel, and weighing 30(±3) kg. Swine are an acceptable model for both cyanide poisoning and evaluation of nitric oxide and its inducers [9–13]. Thus, animals' type and size were appropriate for this proof-of-concept study. The swine were housed separately, under standard laboratory conditions. Arterial and venous vascular catheters were surgically inserted under sedation into the carotid and jugular vessels, respectively, of each animal. Heparin lock was used to prevent vessel occlusion. The swine recovered for at least 2 h before any further intervention; no residual effect of anaesthetic medications was expected after this recovery period. Before exposure to cyanide, the animals were individually placed in a spacious open cart (2 × 2 meters), padded inside with soft mattresses, where they could move freely and safely.

The animals, without anaesthesia, were intravenously poisoned with potassium cyanide (Sigma-Aldrich Co. LLC, Germany), 2 mg/kg or 4 mg/kg. Poison solution was freshly prepared using a previously described method [2]. Cyanide was injected within 5 s through an extension tube to the venous access followed by a 10 ml push of isotonic saline. Animals were randomly designated to 4 groups, 4 animals in each. Groups 1 and 2 (one for each cyanide dose) were not further treated and served as controls. Groups 3 (2 mg/kg KCN) and 4 (4 mg/kg KCN) were treated within 1 min after initial clinical signs of poisoning (e.g. convulsions, loss of consciousness, collapse) with ISDN oral spray: 3 spray actuations (3.75 mg; ∼125 μg/kg) or 4 spray actuations (5 mg; ∼170 μg/kg), respectively. A commercial off-the-shelf ISDN oral spray formulation (Schwarz Pharma Production GmbH, Germany) was used for treatment. The ISDN dose used to treat the animals poisoned with the lower cyanide dose was based on human doses given to cardiac patients during acute coronary event [4,5]. During preliminary testing of the study model, we have noticed no effect with this ISDN dose after doubling the poison dose. It was decided to use a 33% higher dose (4 actuations instead of 3) for the 4 mg/kg KCN dose arm to provide a better chance to show effect in this proof-of-concept study, without a significant deviation from the equivalent human dosing range. ISDN oral spray

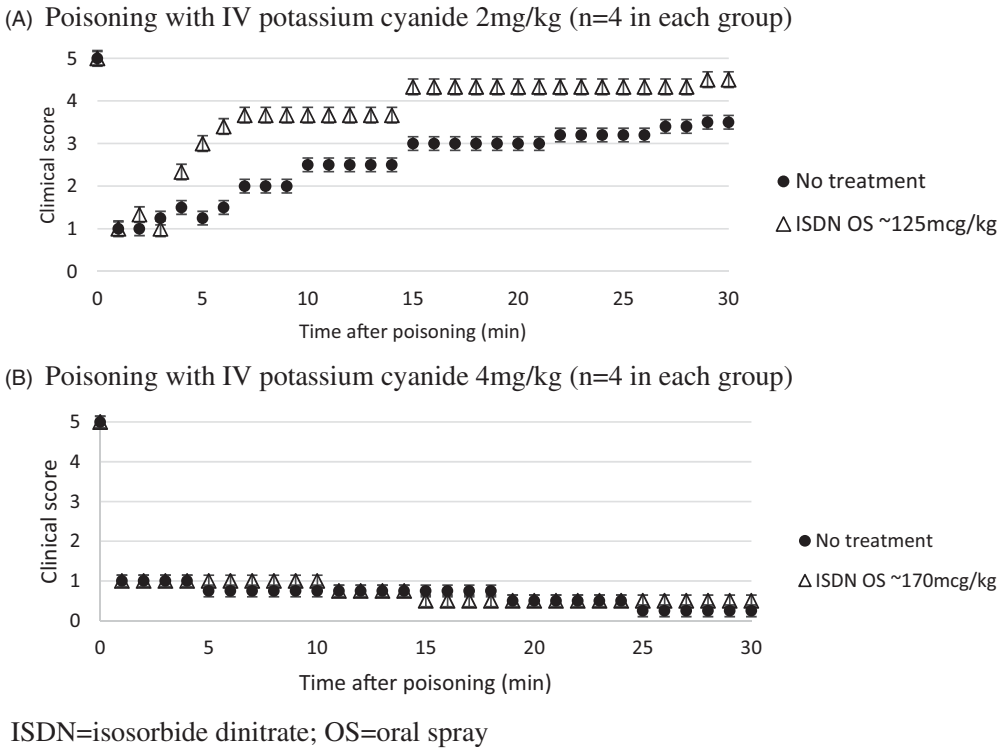

Figure 2. Average clinical scores.

actuations were delivered after opening the unresponsive animal's mouth and directed to the buccal tissue. The timing of treatment (1 min after initial clinical signs of poisoning) was regarded as appropriate for a proof-of-concept study.

The swine were closely and continuously observed for up to 30 min after poisoning. The duration of observation was chosen to represent the relevant clinical scenario of toxicological mass casualty incident in an urban setting. The animals were connected to an arterial line and a monitor (Surgivet, Smiths Medical PM, Inc., Waukesha, WI, USA) that recorded pulse and blood pressure (BP). The study outcomes were clinical score, time to death, pulse, BP, and blood tests including pH, lactate, and methemoglobin levels. The clinical score was recorded before poisoning (time 0) and every minute post exposure. Its metrics were 0 for death, 1 for unresponsive, 2 for moving only one limb or the head, 3 for lying with a raised head, 4 for sitting, and 5 for standing and moving freely. This clinical score was adapted from previously published animal studies and adjusted to swine [2,6]. Arterial blood was sampled before poisoning (time 0) and 2, 5, 10, 15 and 30 min postexposure. The blood samples were immediately and in equal times put in ice and transferred to a certified clinical laboratory, where they were analysed for pH, lactate and methemoglobin using a calibrated chemical blood-gas and CO-oximeter analyser (GEM® Premier 4000, Ilex Medical Ltd., USA). The total sampled blood volume was 6 ml per animal, which is less than 1% of its total body blood volume.

A power analysis (using the G power software, version 3.1.9.4; University of Dusseldorf, Germany) indicated that a total sample of 8 animals (4 in the control group and 4 in the treatment group) for each intervention was sufficient to detect a clinically significant increase of the clinical score of at least 1 at each time point with 80% power and an alpha of 0.05. Recorded data were statistically analysed using a designated software (SPSS Statistics version 22, IBM®, Armonk, New York, USA). Unpaired t-test was performed to compare time to death, clinical scores, and blood pH and lactate levels between each treatment group to its corresponding control group.

The study was approved by the Rappaport Faculty of Medicine Institutional Animal Care and Use Committee, Technion-Israel Institute of Technology, Haifa, Israel. Approval number IL0030116.

Results

All the animals started to convulse within 20 to 30 s after poisoning, then became unconscious and unresponsive after another 20 to 30 s (clinical score = 1) with profound bradycardia and low BP.

Following exposure to the KCN 2 mg/kg dose, 3 of 4 control animals survived, while all (4/4) ISDN-treated animals survived. Following exposure to the KCN 4 mg/kg dose, similar survival rates were observed for both the control group and the ISDN-treated group (1/4); only one animal survived to the end of the observation period in each group. The time to death trended longer in treated *vs.* control animals, with the time to death in control animals averaging 8 min (4 min, 5 min and 15 min), and that in the ISDN-treated animals averaging 17.33 min (11 min, 16 min and 25 min). Although clinically different, it did not reach statistical significance (unpaired t-test, $p = 0.15$). Survival Rates plotted as Kaplan-

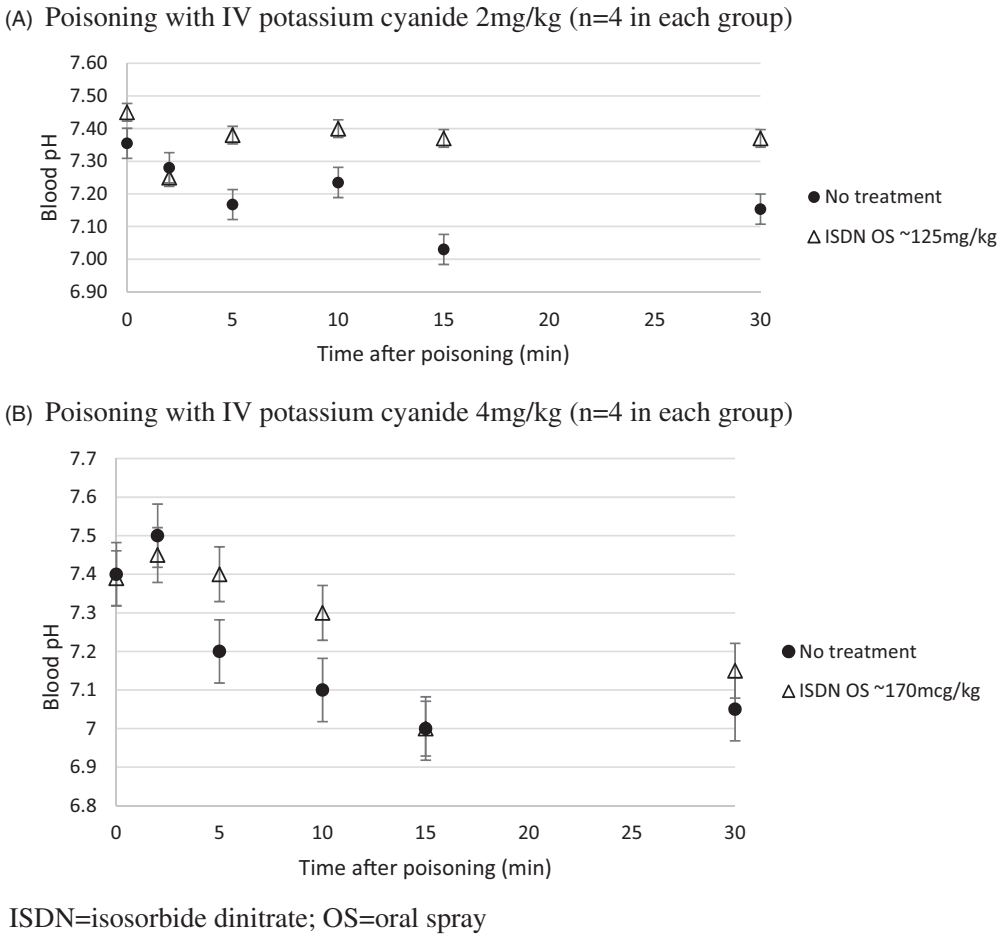

Figure 3. Average blood pH. ISDN: isosorbide dinitrate; OS: oral spray.

Meier curves (Figure 1) show graphically better survival of the treated animals within the first 25 min after poisoning.

Following exposure to the lower poisoning dose of KCN, treated animals had significantly better clinical scores starting 5 min after poisoning and at each timepoint thereafter, compared to control animals (Figure 2). At 5 min, the average clinical score was 3 for treated animals *vs.* 1.25 for control (unpaired t-test, $p < 0.05$). At the end of the observation, the average clinical score was 4.66 for treated animals *vs.* 3 for control (unpaired t-test, $p = 0.05$). After the higher dose of KCN, the single surviving treated animal had better clinical score at 30 min post challenge compared to the single surviving control animal (score 2 *vs.* 1).

Following exposure to the lower poisoning dose of KCN, acidosis (pH and lactate levels) was significantly worse in the untreated animal, starting 5 min after poisoning and at each timepoint thereafter (unpaired t-test, $p < 0.01$). At the higher poison dose treated animals had marginally better pH at the 5 min and 10 min time points. Figures 3 and 4 present pH and lactate results, respectively.

Methemoglobin levels were below 1% at each time point in all animals.

Discussion

The study results demonstrate benefit of ISDN after exposure to the lower cyanide dose; treated animals had better clinical scores and laboratory results. At this dose of KCN, mortality was low to none, regardless of treatment. Thus, ISDN impact on survival could not be clearly evaluated. After the higher dose of KCN, no statistically significant improvement was observed with ISDN treatment regarding overall survival, clinical score or laboratory measures. There was a statistically non-significant but clinically evident trend for a delayed time of death for the animals treated with higher ISDN doses. This is clinically important, as in a real cyanide mass casualty event, even a small improvement could provide meaningful benefits. This could result in fewer victims requiring intubation and allowing for a more timely evacuation of victims to hospitals for definitive care.

In previous studies of ISDN treatment in cyanide-poisoned rabbits, ISDN dramatically improved survival after lethal cyanide poisoning [2,6]. Species differences could explain the different results observed in the current study. Mechanisms of cyanide poisoning and ISDN interaction and pharmacokinetic properties may be diverse and expressed differently across animal species. Absorption of ISDN after oral and sublingual dosing is nearly complete, but bioavailability is variable [5]. Effective ISDN doses per weight for cyanide poisoning in swine could be higher than in rabbits, and this should be further explored. Longer observation periods and larger group sizes may also better characterize possible advantages of ISDN on survival and recovery in a large animal model, such as swine.

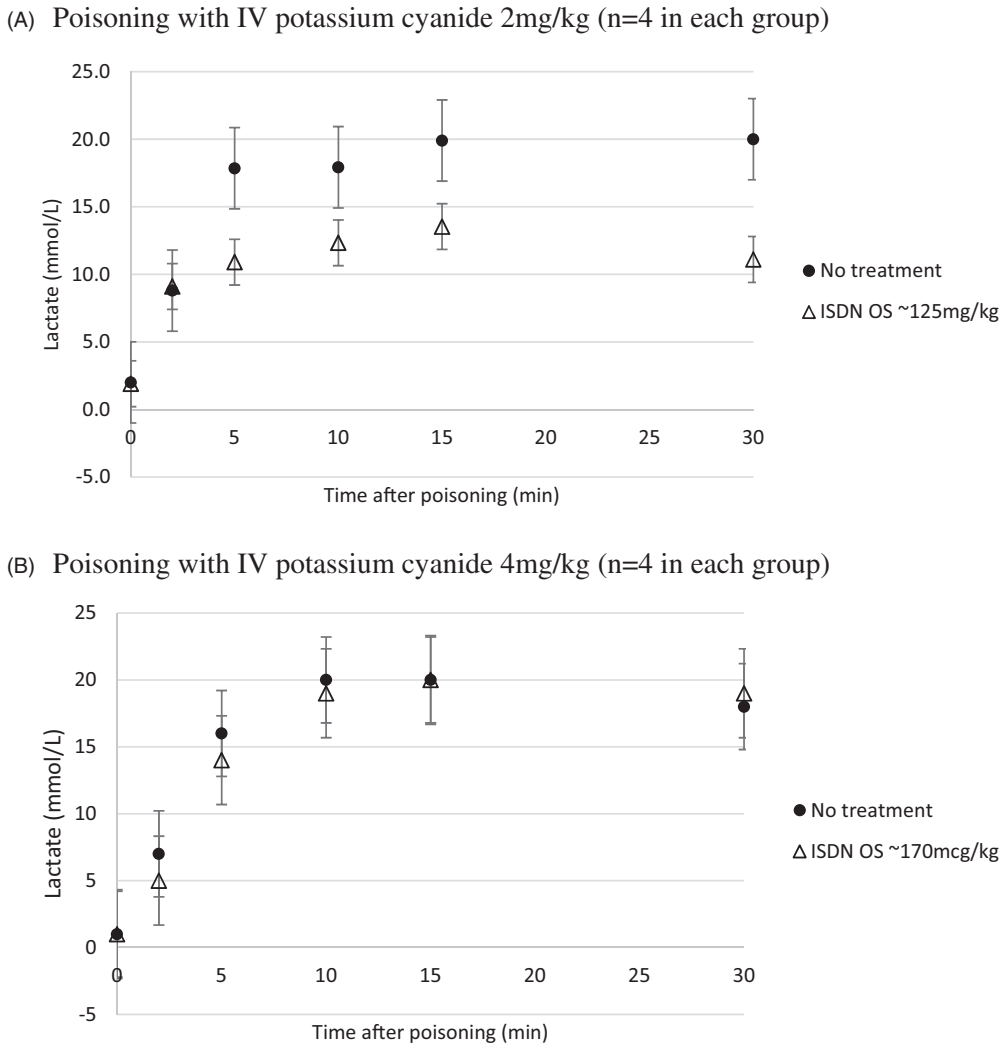

Figure 4. Average blood lactate.

The potential mechanism of antidotal action of ISDN is not totally clear. It is evident that ISDN is not a methemoglobin former; its administration does not result in any significant rise in methemoglobin levels, either in swine, rabbits or humans [2,6–8,14]. ISDN in the body is rapidly reduced to nitric oxide (NO) through a NO synthase-independent pathway [15]. It was previously shown that NO carries antidotal properties against cyanide, specifically in the mitochondrial electron transport chain. NO competes with cyanide for binding to cytochrome C oxidase [15,16]. Both direct or non-direct (through sodium nitrite) NO administration mitigated the inhibition of cytochrome C oxidase by cyanide [16–18]. In addition, NO is neuroprotective mainly by reducing hyperactivity of NMDA receptors in the brain [7,19–21]. This may prevent or counteract the effects of cyanide on the central nervous system, clinically expressed as coma, seizures and respiratory depression.

The multiple reports demonstrating effects of NO following cyanide exposure should not be overlooked. ISDN or any other nitrate administration is a safe and easy way to rapidly increase NO levels in the body. The limited efficacy of ISDN in severely cyanide poisoned swine observed in the present study does not eliminate the possibility of ISDN to be used as a countermeasure in cyanide poisoning. ISDN may serve as an initial or add-on antidote to postpone or mitigate some of the acute insults of cyanide, before a more definitive treatment can be administered. This study's results point to improved clinical score and reduced acidosis in moderately poisoned animals treated with ISDN, and to a delayed time to death when lethally poisoned swine were treated with ISDN. These potential advantages are further emphasized when considering the use of the oral spray formulation. The easy-to-use, available, and affordable medication offer 300 actuations of 1.25 mg ISDN in a single spray bottle. First responders could rapidly treat dozens of cyanide-poisoned patients on scene using ISDN oral spray. Field applicability of oral spray is also based on its efficient pharmacokinetics with high C_{max} and short t_{max}, observed within 3-4 min after administration [22].

Even limited efficacy of ISDN could provide profound benefit in such a mass casualty event. The risk-benefit balance of ISDN as a bridging antidote in cyanide poisoning is positive due to its documented safety and simple immediate administration. Until more effective and safe antidotes for

cyanide mass poisoning are developed, repurposing approved medications for this indication may help to improve, even modestly, the outcome of lethal and catastrophic incidents. ISDN is a "low-hanging fruit" that should be picked and utilized. Combination cyanide antidotes studies involving ISDN seem warranted.

This study has limitations. Sample size was small, and the observation period was short. Poisoning method and doses were distinct. The route of poisoning was intravenous; an inhalation model would of course be most relevant. These limitations are balanced with the use of a clinically relevant model. Non-anesthetized animal model eliminates the confounding of sedative medications, and enables observation of the full clinical manifestations of poisoning. Sample size was determined based on power analysis to detect a clinically significant increase of the clinical score, as described in the Methods section. Relatively similar sample sizes were used in previously reported swine studies of acute cyanide poisoning [10,11]. The acute poisoning with a bolus of cyanide simulates the most serious potential exposure (acute inhalation), and provides challenging conditions for the evaluation of antidotes. Different protocols of cyanide poisoning were used in other studies using swine, and included slow infusions of cyanide solutions in sedated and ventilated animals [10,11]. While these models allow better experimental control, they do not provide improved simulation of immediate massive cyanide inhalation, which is the feared scenario for mass poisoning.

In the current study, cyanide levels were not evaluated. However, as ISDN is not a direct cyanide antidote it is less important in such a proof of concept study to measure cyanide levels. In future safety and efficacy studies it will be relevant and important to look also at cyanide blood levels.

In conclusion, ISDN oral spray administration following cyanide poisoning in this porcine model did not result in statistically significant increased survival. However, based on clinical scores and clinical laboratory values, ISDN may be found as a fit bridging countermeasure until other treatment measures can be administered. Further research is warranted.

Disclosure statement

The authors declare no conflict of interest.

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