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TITLE: Intranasal DMTS for Acute Cyanide Poisoning

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14. ABSTRACT

The US chemical defense program has identified finding a non-intravenous, safe antidote for acute cyanide toxicity a high priority. Current antidotes require intravenous infusion making their utility in a mass casualty poisoning limited. Evidence shows intranasal administration of medications in the prehospital setting is a safe, effective, and convenient route compared to traditional routes of drug delivery. Dimethyl trisulfide has been shown to be an effective antidote when administered via intranasal administration in a rodent model of cyanide poising. We have demonstrated that DMTS can be successfully administered via the intranasal route in swine resulting in systemic absorption of DMTS. We have also demonstrated intranasal administration of DMTS rescues from lethal cyanide poisoning.

15. SUBJECT TERMS
Cyanide, DMTS, Intranasal, Swine

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1. INTRODUCTION:

The Department of Homeland Security and the US chemical defense program in the DoD Combat Casualty Care Research Program have identified finding a non-intravenous, safe antidote for acute cyanide toxicity a high priority. Current antidotes require intravenous infusion making their utility in a mass casualty poisoning limited. Evidence shows intranasal administration of medications in the prehospital setting is a safe, effective, and convenient route compared to traditional routes of drug delivery. Dimethyl trisulfide has been shown to be an effective antidote when administered via intranasal administration in a rodent model of cyanide poising. We hypothesize that DMTS can be successfully administered via the intranasal route in swine resulting in systemic absorption of DMTS and ultimately be able to rescue from cyanide toxicity.

2. KEYWORDS: .

Cyanide, Dimethyl Trisulfide, Swine, Apnea, Intranasal

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The major goals of this project are outlined in the specific aims below.

Specific Aim 1: Optimize intranasal administration of DMTS in Yorkshire cross swine in consultation with colleagues at SipNose and USAMRICD.

Specific Aim 2: Evaluate pharmacokinetics and brain/systemic absorption of DMTS following intranasal administration in Yorkshire cross swine.

Specific Aim 3: Demonstrate efficacy of intranasal administration of DMTS following a lethal, acute cyanide exposure.

Specific Aim 4: Demonstrate improved long-term survival in swine treated with intranasal DMTS compared to untreated controls.

What was accomplished under these goals?

Major Activities

Intranasal Administration Optimization

Studies to determine the optimal administration technique for intranasal DMTS delivery to swine were conducted in consultation with our colleagues at SipNose. Swine between 45-50 kg were used for these studies. Anesthesia was induced with intramuscular ketamine and sedation maintained with isoflurane via nosecone (see detailed procedures below). Following induction, endotracheal intubation was performed and sedation maintained with a mixture of oxygen (FiO2 0.21-0.40) and isoflurane (1-3%). Vital signs were monitored during all procedures. Once the animal was adequately sedated and breathing spontaneously without ventilator support. Evans blue dye was administered using the SipNose intranasal delivery device and 90 minutes following administration and gross necropsy was performed to visualize deposition.

Intranasal DMTS Systemic Absorption and Pharmacokinetics

Following intranasal administration optimization neat DMTS was administered intranasally to evaluate pharmacokinetics and systemic absorption. Swine were anesthetized and sedated as described above. Baseline blood samples were collected prior to intranasal DMTS administration. Following DMTS administration, blood samples were also collected at 1, 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, and 90 minutes after administration. Following euthanasia at the end of the study, necropsy was performed, and tissue samples collected for DMTS evaluation.

Efficacy of intranasal administration of DMTS following a lethal, acute cyanide exposure

The efficacy of intranasal DMTS following cyanide poisoning was evaluated using our well-established swine model of potassium cyanide poisoning (Figure 1). Swine were sedated and anesthetized as described above. Following acclimation swine were exposed to potassium cyanide via intravenous infusion (0.2 mg/kg/min) until apnea occurs. At 6 minutes post apnea intranasal DMTS is administered, and the cyanide infusion stopped. Animals were observed for 90 minutes post treatment.

Long-term efficacy of intranasal DMTS following a lethal acute, cyanide exposure

To evaluate the long-term efficacy of intranasal DMTS swine were survived for 7 days post rescue. Observations were made twice daily to assess behavioral outcomes and blood samples were collected at 1, 5, and 7 days post treatment for the assessment of renal and hepatic function Swine treated with DMTS showed improved long-term survival and showed no adverse outcomes. Furthermore, there was no observed adverse effects on renal or hepatic function with treatment (Figure 3)

Figure 1. Cyanide Exposure Model.

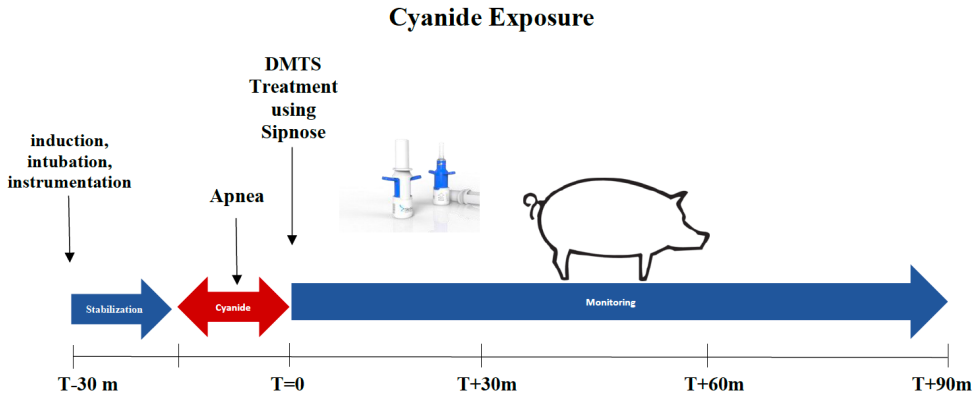


Figure 1. Potassium cyanide is infused at a rate of 0.2 mg/kg/min until apnea occurs. Six minutes after apnea the cyanide infusion is stopped and intranasal DMTS is administered.

Specific Objectives

- Optimization of intranasal administration using the SipNose device in swine.
- Obtain pharmacokinetic data in swine following intranasal administration in the absence of cyanide poisoning.
- Evaluate DMTS tissue concentrations following intranasal administration.
- Evaluate intranasal DMTS efficacy in an acute model of cyanide poisoning
- Assess long-term outcomes following rescue with intranasal DMTS following cyanide poisoning.

Significant Results

We determined that we can successfully administer up to 1 ml of DMTS using the SipNose device into one nostril of sedated swine. Furthermore, administration of evans blue dye demonstrated significant deposition in the olfactory tissue.

We have also determined we can successfully administer neat DMTS without adverse response to sedated swine. Blood concentrations following intranasal administration of DMTS results in a rapid C_{max}, 5 minutes post administration (figure 2).

Figure 2 Pharmacokinetics of DMTS following intranasal administration

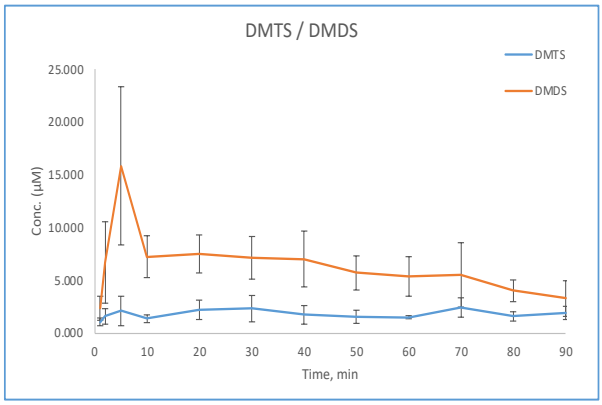


Figure 2. Three swine received intranasal administration of DMTS a C_{max} was achieved 5 minutes post administration. Data shows both DMTS and its metabolite DMDS.

Following optimization of intranasal DMTS administration we evaluated whether it would be able to rescue from a lethal cyanide exposure. Initial studies evaluated efficacy for 90 minutes post treatment. We found intranasal DMTS significantly improves survival (p=0.0098) compared to control (figure 3). Additionally, we found survival is improved long-term in animals rescued with intranasal DMTS and there are no adverse renal or hepatic effects with treatment (Figure 4).

Figure 3. Survival and Blood Lactate

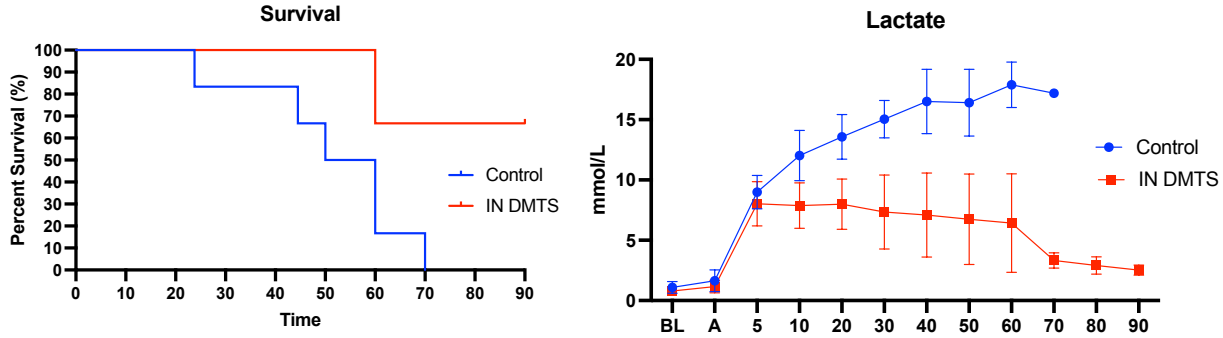
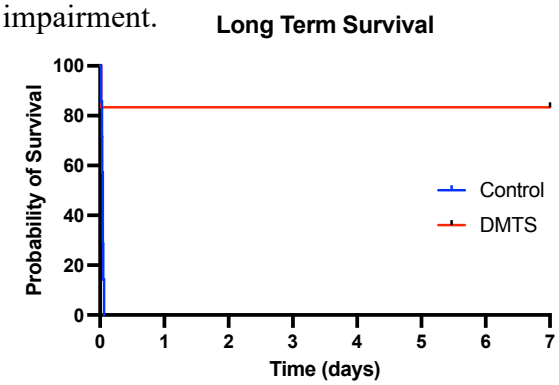


Figure 3. Survival and blood lactate concentration is improved in animals treated with DMTS.

Figure 4. Long-term survival and renal and hepatic function following DMTS treatment. Swine treated with intranasal DMTS showed improved survival for 7 days post treatment without renal or hepatic function impairment.



Laboratory Parameter	Reference Range	Baseline	Day 1	Day 5	Day 7
BUN	2-13	7.7	10.9	7.2	9.1
Creatinine	0.6-1.8	1.4	1.5	1.4	1.4
Phosphorus	5.8-10	8.7	9.1	9.5	9.9
ALT	27-55	38	48	46	46
ALP	40-224	120	126	108	119
Total Bilirubin	0.2-0.5	0.1	0.1	0.2	0.2

Conclusion

The major goals of our specific aims were accomplished in this study period. We are currently preparing a manuscript to report our findings.

What opportunities for training and professional development has the project provided?

Nothing to report

Describe how the results were disseminated to communities of interest.

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

Goals and objectives have been accomplished and we are currently preparing a manuscript.

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?

Determining the most effective method of intranasal DMTS administration, pharmacokinetics and drug deposition, and efficacy is an important step toward FDA approval. Data from these studies demonstrate intranasal DMTS is a rapid acting, easy to administer, safe cyanide antidote applicable to mass casualty incidents.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to report.

Changes that had a significant impact on expenditures

During year 1 our grants office experienced significant delays in billing and payments. Additionally, the COVID-19 pandemic resulted in a hiring freeze and delays in hiring staff.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animal

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

  Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: *Vikhyat Bebarta, MD*
Project Role: *PI*

Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *5*

Contribution to Project: *Dr. Bebarta has contributed to the design and execution of experiments.*

Name: *Tara Hendry-Hofer, BSN, RN*
Project Role: *PM*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *5*

Contribution to Project: *Ms. Hendry-Hofer has contributed to the design and execution of experiments.*

Name: *Carter Severance, BS*
Project Role: *PRA*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *5*

Contribution to Project: *Carter Severance has contributed to the execution of experiments.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.