Hydrogen Sulfide Poisoning Antidote Development for Industrial Poisoning Exposures

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Program Goal

1. To develop an **effective antidote to treat and/or prevent injury from H$_2$S exposures** in oil and gas industry

2. Seeking help from the industry to support our team’s research and development efforts.
Hydrogen Sulfide Antidote Development

1. Discuss our research and development progress to date
2. Define our strategies to answer unmet hydrogen sulfide needs in oil & gas industry
3. Outline development plans and funding needs
**H₂S Review**

- Highly toxic, flammable, colorless gas, “rotten eggs” odor
- Naturally occurring or byproduct of industrial processes
- Respiratory irritation (50-100ppm), olfactory nerve paralysis (100-150ppm), pulmonary edema (300-500ppm), death (600-1000ppm).
- Rapid “knockdown”, residual cognitive impairment risks
- Highly lethal gas, oil & gas industry, potential terrorist attacks
- No specific antidotes, only supportive treatments (amyl nitrite, O2)
- Challenge: Development of rapid acting, easily administered antidote
  - Acute exposure
  - Post exposure residua
Background

Cobinamide as an Antidote for Cyanide and H$_2$S

-Cyanide Antidote Development-

• Cyanide and Hydrogen Sulfide have many similar biological properties and potential to respond to the same antidote
  – Cyanide antidote studies ongoing for 7 years
  – Highly effective by intravenous (IV), intramuscular (IM), intraosseous (IO) and by nebulization into the lung
  – Testing in fruit flies, mice, rabbits, pigs
  – Multiple formulations tested
  – Close to readiness for “GLP” FDA testing for an IM formulation for CN poisoning
  – Possible use in H$_2$S poisoning?
HCN and H$_2$S - Similar Mechanism of Induction of Toxicity at Cellular Level
Cyanide Antidote Delivery

What we currently have for CN: Hydroxocobalamin IV for CN

*What we currently have for H₂S: Nothing!!!

What we need: < 5ml, IM, Potent, Safe
**Nitrocobinamide as an Antidote for Cyanide and H₂S**

- More soluble, much more concentrated, IM
- Much tighter binding of ligands (CN, H₂S)
- Binds 2 ligands  pK 10⁻²²
- Many ligand groups could be bound (nitrite shown here, could be OH, sulfides, etc…)
- Muscle transport, matrix binding, side effects
- Sulfide does not bind as tightly as CN
Cyanide and sulfide bind to ligand on cytochrome-c oxidase causing copper-A to stay in reduced state.

Unique Technology: Diffuse Optical Spectroscopy (DOS) is capable of quantitatively detecting changes in concentration of both reduced and oxidized CUA as well as hemoglobin oxygenation.
Oxygenated and De-oxygenated Blood in Tissues

Cyanide or $H_2S$ Poisoning: Non-invasive Optical-Measurements

Normal Baseline

Input Source Fiber

Output Source Fiber

Skin

Body

CN Toxicity

Input Source Fiber

Output Fiber

Skin

Body
Methods

Animal model
- New-Zealand white rabbits (3.5-5.5 Kg)
- Anesthetized with Ketamine/Xylazine
- Intubated and ventilated
- NaCN is infused IV
- Physiologic parameters monitored via pulse oximetry, arterial and venous lines.
- DOS measurements
- CWNIRS measurements on the brain region.
Intravenous Cobinamide Rapidly Reverses Cyanide Poisoning

Low Dose Cyanide Alone (controls)

Low Dose Cyanide Followed by Cobinamide
Scenarios and Modes of Administration For CN and H₂S Antidotes

- Intravenous
  - Skill, time, blood pressure, individual administration
  - Very rapid effects

- Intraosseous
  - Blood pressure required

- Intramuscular
  - Skill, individual administration
  - Very rapid effects

- Inhaled (nebulized)
  - Rapid, low skill, self administration
Ultrafast Drug Nebulizer Development

Chen Tsai, PhD

Jason Eichenholtz
Blood plasma from transpulmonary nebulized treatment animal.
From baseline to 90 min post Cobinamide
Transpulmonary Cobinamide
Nitrocobinamide Intramuscular Injection in CN Exposed Rabbits

Survival Summary:

Control CN IM: 14%
Nitrocobinamide IM: 80%

Scales to < 1 cc IM injection in adults
Addition of Nitrite Speeds Cobinamide Absorption

Absorption of Nitrocobinamide (4:1 Nitrite to Cobinamide) vs Nitroaquocobinamide (2:1 Nitrite to Cobinamide) after IM Injection

4:1 Nitrite:Cobinamide
Potential for Cobinamide in H$_2$S Poisoning

- Small grant for pilot studies
- Sequence of studies
  - Chemical combination/spectrophotometry
  - Cell cultures
  - Fruit flies
  - Mice
  - Rabbits
  - Pigs
Cobinamide Binds H$_2$S
Cobinamide Binding Affinity for Sulfide
Cobinamide Restarts H$_2$S Blocked Oxygen Pathways

Reversal of H$_2$S Toxicity in Cells: Oxygen Consumption and pH Changes

Human iPSc Derived Cortical Neurons

Cos 7 Kidney Cells
H₂S “Knockdown” Model in Fruit Flies

- Fruit flies hatch and are grown with usual food or food containing cobalamin or cobinamide (pretreatment model)
- Filter paper soaked in HCl and dried placed in glass vials
- 50 flies added to vials, and a drop of NaSH placed on acidified filter paper
- H₂S gas created causes “knockdown” of all flies within seconds
- Flies remain exposed for 2 minutes
- Flies monitored for recovery over 1 hour
Cobinamide Reversal of H$_2$S Toxicity in Fruit Flies

Improved Survival
Inhalational H$_2$S Exposure Mouse Model

- Mice are exposed to H$_2$S gas for 15’, injected IM with cobinamide, and re-exposed for 25’ to H$_2$S gas
- Simulates real-life scenario of H$_2$S released in closed spaces with ~ 15’ for EMTs to arrive and 25’ to treat and evacuate
- H$_2$S gas produced by acidifying NaSH with HCl
- 1550 ppm exposure
- Limitation: Animals are in an enclosed chamber and cannot be monitored closely
Cobinamide Reversal of H$_2$S Inhalation Toxicity in Mice: Improved Survival
Rabbit Model of $\text{H}_2\text{S}$ Poisoning

- New-Zealand white rabbits (3.5-5.5 Kg)
- Anesthetized with Ketamine/Xylazine
- Intubated and ventilated
- NaHS is infused IV for 90 min
- Physiologic parameters monitored via pulse oximetry, arterial and venous lines (blood gasses, blood pressure, heart rate).
- DOS measurements on the right leg recorded continuously
- CWNIRS measurements on the skull recorded continuously.
Cobinamide Reverses H$_2$S Poisoning in the Rabbit Model

*Optical (CWNIRS) Monitoring*

Effects of NaHS on brain Oxy, Deoxy and Total Hemoglobin with and without treatment as measured by CWNIRS.
Cobinamide Formulations With Most Effectiveness in Blocking H2S Poisoning

Total Tolerated NaSH with Antidote Treatments

- Control group received no antidote.
- Survival increased in all treatment groups, greater amount of NaHS tolerated.
- All cobinamide treatment groups tolerated greater amounts of NaHS.
- Highest amount of NaHS was tolerated in the IV Aquohydroxocobinamide and IM Nitrocobinamide group.
Cobinamide Reversal of $H_2S$ Toxicity in Rabbits: Formulations with Most Improved Survival

- Animals receiving intravenous aquohydroxocobinamide and intramuscular “tetra”nitrocobinamide all survived NaSH doses of over 200 mg.
- Animals receiving intramuscular dinitrocobinamide had over 50% survival at 200 mg doses (able to tolerate more than $2 \text{LD}_{50}$ of NaHS).
Comparison of Cytochrome c oxidase redox state recovery/return after H2S exposure in animals treated with saline, left, or tetranitrocobinamide IV, 2 or 20 minutes after the end of exposure. Note the greater rise in cytochrome c oxidase, black line with cobinamide than in saline-treated animal, and marked response to cobinamide after 20 minute “recovery”.

Cobinamide Reversal of H2S Toxicity in Rabbits: Cytochrome c Oxidase Redox State Recovery
Conclusions & Current Status

- Early studies indicate efficacy of cobinamide as an H$_2$S antidote
- Intramuscular formulations could provide ease of administration
- Possibility for prophylaxis for first responders
  - IM or inhaled self-administration
  - Incorporated into sulfide-specific gas masks
- Highly stable compound with good shelf life
- Patents filed by U. California to protect IP for use by future corporate partners
H$_2$S Antidote Development Plans

- Best formulation of cobinamide needs to be determined
- Pig model under development-closer dose scaling to humans (collaborator: Vik Bebarta, MD, USAF)
- Testing of inhaled antidote for responder prophylaxis
- Construction and testing of gas mask filter incorporation of cobinamide
- Finalization of animal models and antidote configuration for FDA required testing under two animal rule.

We need your help to provide funding and guidance for the continued development of H$_2$S antidote cobinamide!
FUNDING

- Estimated 5 years to FDA-testing readiness, timeline and goals established
- Estimated $500-600K/year direct research/development cost
- CDC NIOSH grant funding pending review, could provide up to 40% of necessary funds
- Seeking research funding support from oil and gas industry, partnering for worker safety
  - 5-10 industry leader partners providing $50-100k each/year
  - In form of donations to our program to the Regents of the University of California, 501(c)(3).
  - Industry contact information

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