Inflammatory Biomarkers of Hydrogen Sulfide Induced Neurotoxicity and Neurodegeneration

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Abstract

Hydrogen sulfide (H_2S) has dual actions in the human body as an internally produced signaling molecule and a toxin, with intoxication leading to short and long term adverse neurological symptoms. However, the underlying mechanisms are still unknown. Our hypothesis is that neuroinflammation, characterized by an inflammatory response from agitated support neurons, can cause cell death by invoking the production of many damaging chemicals, called cytokines. To test this hypothesis we used immunostaining techniques to visualize the affected tissue; then using cytokine assays, measured the chemicals of interest in the brain tissue and blood of mice exposed to H_2S by inhalation. Results show increased reactivity of neurons, starting around day 3 post exposure. Understanding the basic mechanisms underlying neuroinflammation contributes to our long term objective of discovering countermeasures against and treatment of H_2S induced neurodegeneration.

Introduction

- H_2S is colorless, with a characteristic rotten egg odor
- An occupational hazard in several industries including intensive swine confinement operations, petroleum and oil production; natural sources include volcanic gas
- At high concentration (1000-2000 ppm) death is instantaneous.
- Acute exposure → short and long term neurological sequelae: impaired motor function, memory loss and cognition disorders, seizures, hearing impairment, etc.
- H_2S is a noncompetitive inhibitor of cytochrome c oxidase, an enzyme used in mitochondrial ATP generation
- No field treatment available for use by first responders
- Neuroinflammation is a mechanism of degeneration in related disorders such as Alzheimer's and Parkinson's diseases

❖ Objective:

To characterize inflammatory biomarkers of toxicity that can be targeted for treatment of H_2S induced neurodegeneration

Methods

Treatment Paradigm

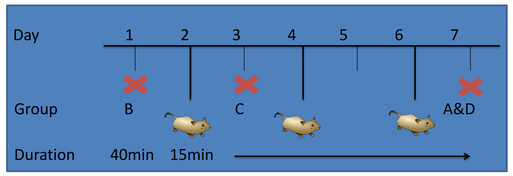

Fig. 1. Diagram of exposure day, duration of exposure, euthanization days (red x) and animal behavior days (mouse). Group A: Control (Breathing air), Groups B – D (H_2S) .

Proposed Schematic

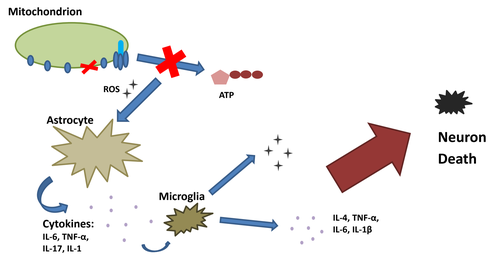

Fig. 2. Hypothetical neuroinflammatory scheme showing signaling cascade after mitochondrial injury caused by H_2S toxicity. Reactive astrocytes release cytokines, propagating an intense inflammatory response

Cytokine Quantification

- 5 mice/group (4 groups total) exposed to H_2S + breathing air via inhalation; euthanization 2 hours post exposure on last day of treatment
- Upon euthanization, brain tissue collected via decapitation and blood collected for serum

Luminex

- **For Brain:** Tissue samples homogenized, lysates analyzed
- **For Serum**: Samples added to a multiplex assay plate containing *beads* conjugated with capture antibody → quantify analyte concentration by light emission

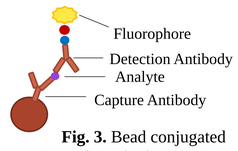

Fig. 3. Bead conjugated with antibody

Meso Scale Discovery (MSD)

- **For Brain and Serum**: Samples added to a multiplex *high binding carbon* assay plate → electrical stimulation cause light emission from electrochemiluminescent tag → quantify analyte concentration by light intensity

Results

Clinical Observations - Behavior

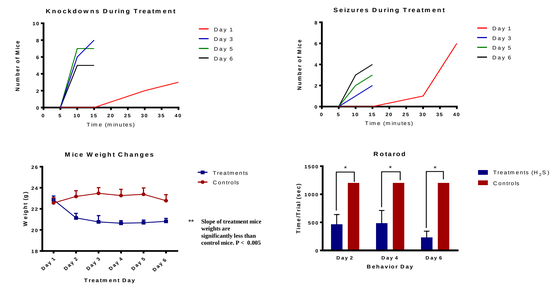

Fig. 4. Behavior changes in control mice vs. H_2S-treated mice. H_2S treated mice show a significant reduction in weight over the course of 7 days, impaired motor activity, and increasing sensitivity to H_2S exposure with subsequent treatments.

Histopathology

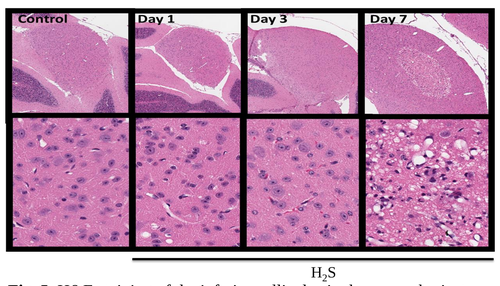

Fig. 5. H&E staining of the inferior colliculus in the mouse brainstem. Gross lesions observable by day 7.

Cytokine Expression Quantified using Luminex

Serum

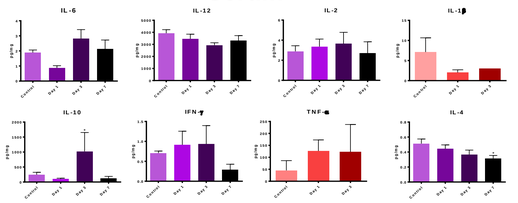

Fig. 6. Cytokines expressed in serum of mice samples. Purple graphs indicate an n > 3. Red graphs indicate an < 3 values used in calculation. * indicates p<.05.

Brain

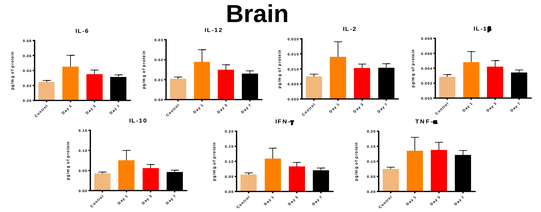

Fig. 7. Cytokines expressed in brain tissue homogenate of mice samples from central inferior colliculus.

Results

Cytokine Expression Quantified using MSD

Serum

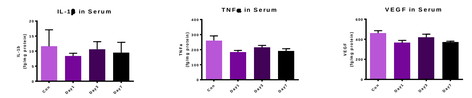

Fig. 9. Cytokine expression in the serum of mice samples.

Brain

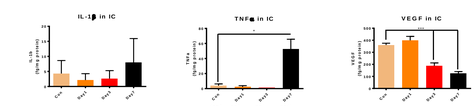

Fig. 8. Cytokine expression in the central inferior colliculus of mice samples. * indicates $p < 0.05$ *** indicates $p < 0.0005$

Conclusions

- There is damage occurring in the brains of H_2S-treated mice, with the most sensitive area being the inferior colliculus
- Mice exposed to H_2S show an increase in sensitivity to the gas suggesting cumulative effects
- This damage in mice leads to adverse effects similar to those seen in humans exposed to the gas, such as neurodegeneration and motor impairment
- Profiles of cytokine expression were not greatly altered in serum.
- Exposure to H_2S induces upregulation of IL-1β and TNF-α, biomarkers for neuroinflammation, while downregulating VEGF, which is important for its protective role against stress in the brain
- Future directions include investigating activation of the inflammasome and neuroprotective effects of therapies

References

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