Intravenous Injectable Isoflurane Emulsion as an Antiseizure Treatment for Soman Nerve Agent Exposure in a Mouse Model

Noah J. Benn, Maria J. Chavez- Vazquez, Jacob G. Janssen, Julia A. Leighton, Mackenzie N. Ellis, Thomas M. McClymont, Abigail M. Methvin, & Erik A. Johnson

US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD

Introduction & Methods

Rationale

- Current countermeasures lose effectiveness after a small time window following nerve agent exposure, leading to refractory seizure, and thus extensive brain damage
  - Isoflurane adjunct treatment can lengthen the therapeutic time window
- Isoflurane (ISO) FDA-approved and effective in human use, with few to no side effects (Eger et al 1995)
  - Potent anti-convulsant – effectively halts SZ recurrence and preserves brain tissue with longer therapeutic window than midazolam (Krishnan et al 2017)
- More operationally relevant route of administration is needed - emulsion
- We hypothesize that an injectable isoflurane emulsion, in conjunction with standard MCM, can reduce seizure activity, seizure recurrence and brain tissue loss after GD exposure in a mouse model

Subjects

- Male C57BL/6J mice aged 8-10 weeks, under anesthesia, were implanted with a jugular catheter and EEG transmitters

Soman Exposure & ISO Treatment

- Mice were given a single dose of GD (110-147 µg/kg, SC) administered subcutaneously in the scruff of the neck, then observed for convulsive activity and seizure onset.
- Immediately following agent administration, HI-6 dichloride (50 mg/kg, intraperitoneal; IP in saline) and atropine sulfate (ATS, 2-4 mg/kg, SC) were given, followed by midazolam 40-50 min later (MDZ; 5-15 mg/kg, SC).
- In experimental groups, one injection of emulsified isoflurane (optimized dose from Experiment 1) or equivalent volume of vehicle (20-30% Intralipid™ solution, IV) was given with MDZ 40 minutes post seizure onset.

Data Collection

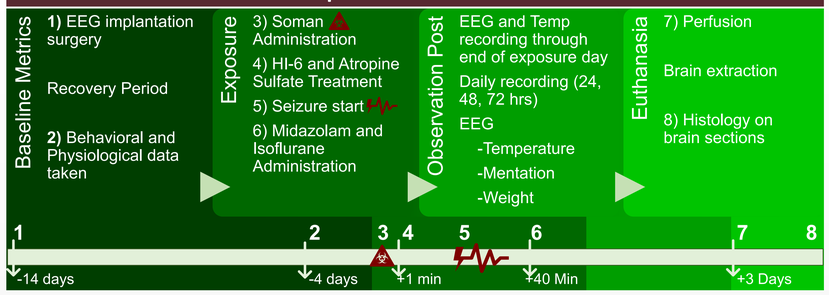
- Mice were placed on EEG measurement pads, connected to Ponemah® software on a computer to visualize EEG signals. Technicians monitored signals for a minimum 200% increase in signal intensity to classify a seizure
- Coronal cross sections, embedded in paraffin on microscope slides, were obtained following perfusion and brain extractions. These slides were deparaffinized and stained with Fluoro®-Jade B to visualize neurodegeneration

Isoflurane Administration Observations

Dose	Concentration	Side Effects
0.01 mL	10%	A few respirations, death
0.08 mL	1%	Slightly twitchy
0.005 mL	10%	Sedated for 5 seconds, reactive and wobbly for 30 seconds
0.005 mL	10%	Sedated for 15 sec, reactive and wobbly for 1 min
0.01 mL	5%	Sedated for 2-3 sec, wobbly for 45 sec, normal by 60 sec
0.015 mL	5%	Sedated, loss of righting (LOR) 10 sec, normal by 45 sec
0.02 mL	5%	Sedated, LOR 10 sec, regained righting 25 sec, normal by 75 sec
0.025 mL	5%	Anesthesia for 30 sec (LOR + no toe pinch response) normal by 60 sec
0.03 mL	5%	30 sec respiration delay, few respirations, death
0.025 mL	5%	20 sec respiration delay, weak toe pinch response, death by 20 sec
0.02 mL	5%	Sedated for 30 sec, minimal respiration delay, normal by 60 sec
0.02 mL	5%	Sedated for 15 sec, normal by 60 sec
0.02 mL	5%	30 sec respiration delay, normal by 45 sec

Table 1: Varying doses and emulsion concentrations of isoflurane, listed in experimental order, along with the corresponding observations following administration. The combination of 0.2ml and 5% emulsion concentration met the criteria previously detailed as the optimized dose.

Experimental Timeline



Results

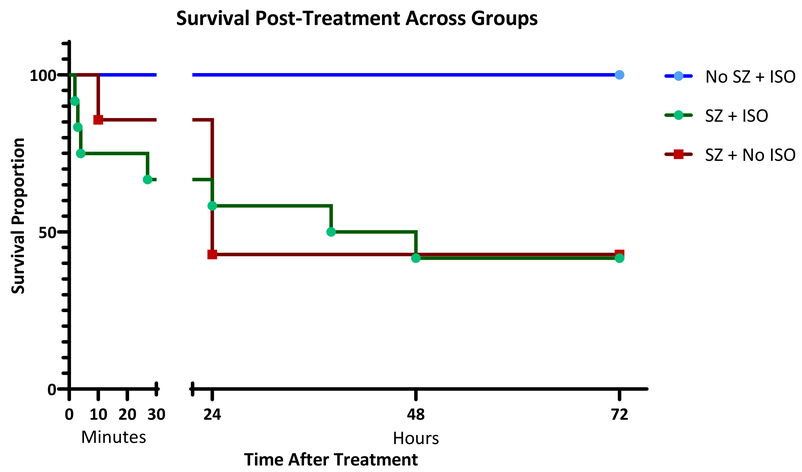


Figure 1. Proportion of surviving individuals in No SZ + ISO, SZ + ISO, and SZ+ no ISO groups in minutes, then 24-, 48-, and 72 hour checkpoints following ISO administration post-exposure. The no SZ + ISO group (n=4) had a 100% survival rate through 72 hours. The SZ + ISO group (n=12) had an initial drop in survival on exposure day but maintained a slightly higher survival rate compared to the SZ + no ISO group (n=7), whose survival dropped to 41.67% by 24 hours. Survival in the SZ + ISO group eventually dropped to the same rate as the SZ + no ISO group by 72 hours but had a better survival rate within the first 48 hours after exposure.

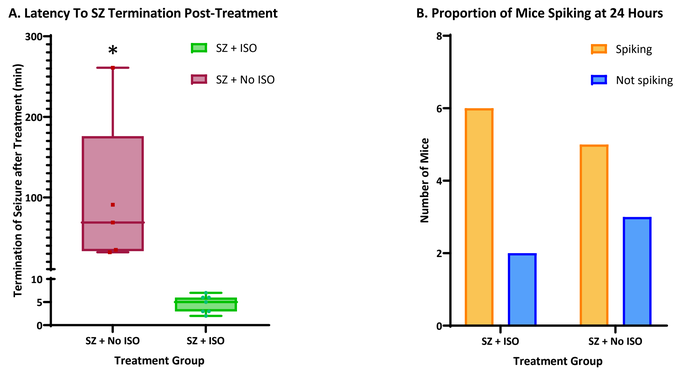


Figure 2. **A.** Comparing time to seizure termination following treatment administration (ISO in the experimental group and MDZ in experimental group), the experimental group saw rapid seizure termination, in just minutes. The control group that didn't receive ISO took much longer to turn seizures off. **B.** Comparing the proportion of mice spiking and not spiking 24-hours post exposure, 75% of mice in the experimental group were still spiking, while roughly 63% were spiking in the control group.

Histological Results: Immunohistochemistry of Fluro-jade B

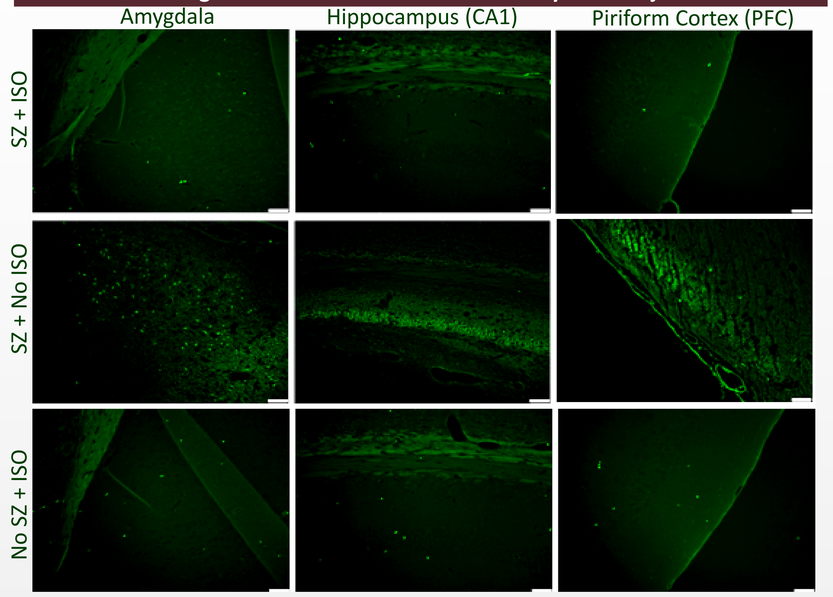


Figure 3. Neurodegeneration staining using FluoroJade-B in Amygdala, Hippocampus, and Piriform Cortex.

Discussion

Mortality

Both groups that seized and did not seize received the ISO treatment, but survival dropped steadily throughout 72 hours in the group that seized, while survival remained at 100% through 72 hours for those that did not seize. This finding makes ISO appear to be a safe treatment for individuals displaying seizure symptoms, but not achieving full seizure, which is likely in an environment without advanced medical care/monitoring.

EEG/Seizure

24-hr spiking data between both groups that did and didn't receive ISO yielded similar results. While 75% of mice in the ISO-treatment group were still spiking at 24 hours, only roughly 63% were spiking at 24 hours in the group that didn't receive ISO. This area needs future exploration to see if results stay similar here or show significant differences, after testing on more subjects.

Immunohistochemistry

SZ + ISO

Little positive staining was seen in the brain regions of interest from animals in this treatment group, meaning little neurodegeneration post-exposure was potentially observed.

SZ + no ISO

More positive staining was observed in these brain regions, possibly due to more neurodegeneration post-exposure in mice that did not receive the ISO emulsion treatment.

No SZ + ISO

Like observations in the SZ + ISO treatment group, very little positive staining was recorded in brains from this group, possibly due to little neurodegeneration post-exposure.

Without quantitative analyses between these images, true differences in neurodegeneration post-exposure cannot be made, but these pictures suggest a potential difference. Future tests to incorporate more mice and include quantitative measurements of positive staining and neurodegeneration will be done.

References

1. Krishnan, J.K.S., et al., *Brief isoflurane administration as a post-exposure treatment for organophosphate poisoning.* Neurotoxicology, 2017. 63: p. 84-89.
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3. Eger, R.P. and B.A. MacLeod, *Anaesthesia by intravenous emulsified isoflurane in mice.* Can J Anaesth, 1995. 42(2): p. 173-6.

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