Inhaled isoflurane and benzodiazepines prevent neuronal damage following nerve agent/traumatic brain injury polytrauma in mice.

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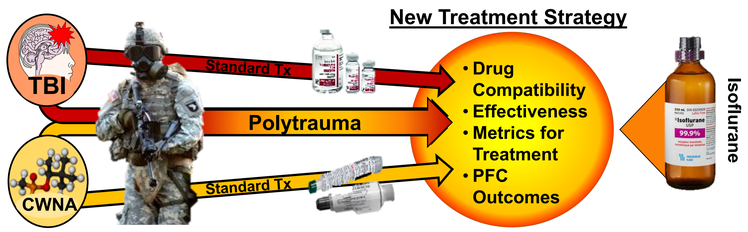
Introduction

- **Problem**: The shift to **prolonged field care (PFC)** in future battle scenarios places more treatment burden on role of care (ROC) 1 & 2 personnel with limited medications.
- **Polytrauma** (*i.e.,* multiple injuries) during combat is common and injuries are often synergistic, increasing morbidity and mortality and further complicating treatment strategies. **Traumatic brain injury (TBI)** is the most common battlefield injury for active military personnel.
- Current countermeasures to **chemical warfare nerve agents (CWNA)** are effective when given rapidly but delayed treatment, likely during a mass casualty response, promotes benzodiazepine-resistant refractory seizure and brain damage.
- **Research Objective**: Develop agnostic new treatment strategies to mitigate polytrauma injuries and increase the treatment window for CWNA exposure to sustain casualties for delayed evacuation.
- **Proposed Solution**: Use the inhaled anesthetic **isoflurane (ISO)** as an adjunct for seizure control after CWNA exposure/ TBI polytrauma.
  - Acts on multiple receptor subtypes implicated in the initiation, maintenance and recurrence of seizure activity.
  - It is safe and effective for human use, has a wide therapeutic index and recovery after anesthesia is quick with few side effects, not contraindicated for standard CWNA countermeasures or TBI treatments.
  - Required equipment and personnel are already located within ROC 2+ units.



Methods

Animal: male mice, ~14 weeks old; strain C57BL/6-Ces1ctm1.1LocAChEtm1.1Loc/J; (*aka* KIKO)

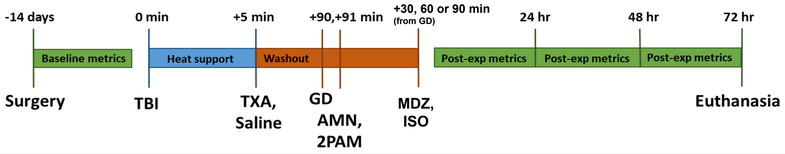
Surgery: Mice were anesthetized and implanted with DSI ETA F-10 wireless EEG transmitter followed by a 4.0mm craniectomy for TBI

Polytrauma Injury: 2.0mm TBI via controlled cortical impact and seizurogenic dose of soman (GD) SC

Standard Medical Countermeasures (MCM):
TBI MCM: tranexamic acid (TXA), 10 mg/kg IP; saline support SC
CWNA MCM: atropine methylnitrate (AMN), 6 mg/kg IP; pralidoxime (2PAM), 25 mg/kg IP; midazolam (MDZ), 5 mg/kg SC

Isoflurane Treatment: 2% for 2 min then 4% for 3 min

Immunohistochemistry: Antibodies – astrocytes (GFAP), microglia (iba1)



Results

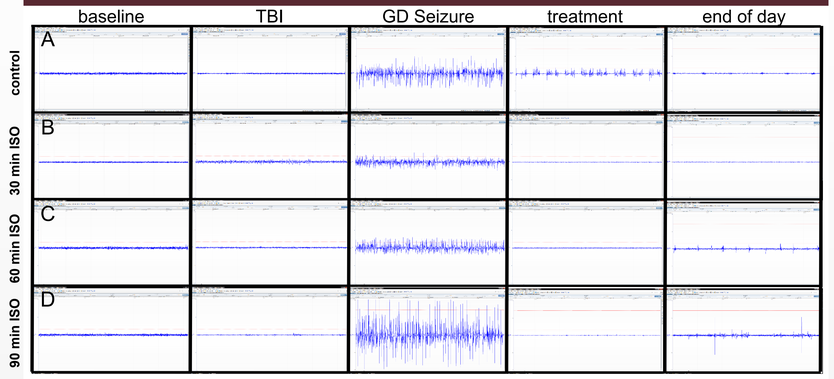
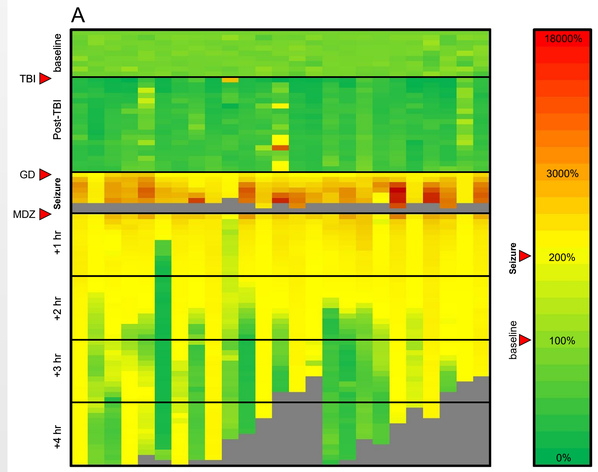


Figure 1: Representative 30 sec EEG tracings from mice receiving standard MCM and standard MCM with ISO at either 30, 60 or 90 min after GD exposure. A) Tracings from standard MCM-treated mice often show refractory seizure developing after treatment followed by post-ictal abnormal spiking activity. B) Tracings from standard MCM-treated mice with ISO at 30 min consistently afford rapid and lasting seizure control throughout the study. Similarly, C) though rapid, seizure control was less persistent at 60 min and D) 90 min.



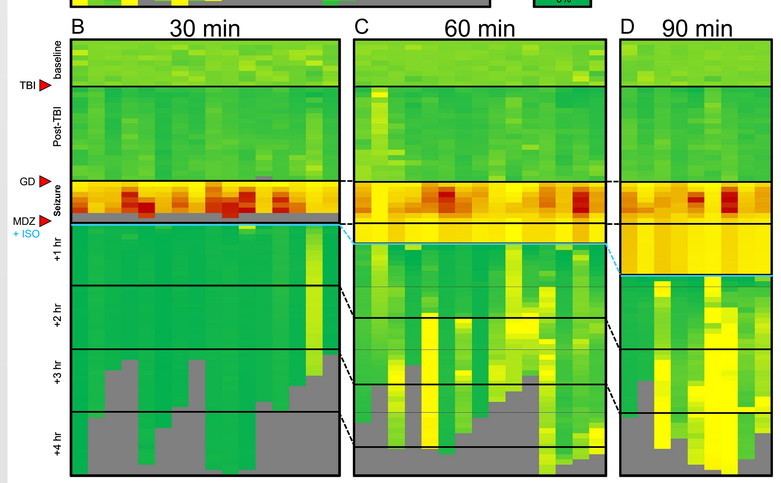


Figure 2: Heat map showing seizure incidence, seizure power and seizure on/off times from mice receiving standard treatment and standard treatment with ISO at 30, 60 or 90 min after injury. A) Standard treatment (atropine, oxime, MDZ) at 30 min did not stop seizure activity quickly or consistently. stopped seizures rapidly and consistently. B) The addition of ISO at 30 min rapidly halted seizure activity for up to 4 hr. C) Increasing ISO treatment time to 60 min was still effective for most though recurrent seizure was evident prior to end of day. D) Giving ISO treatment time at 90 min was less effective, demonstrating a clear dose/time response. However, even at 90 min, ISO was still more effective than standard treatment alone.

Results

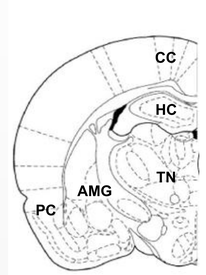
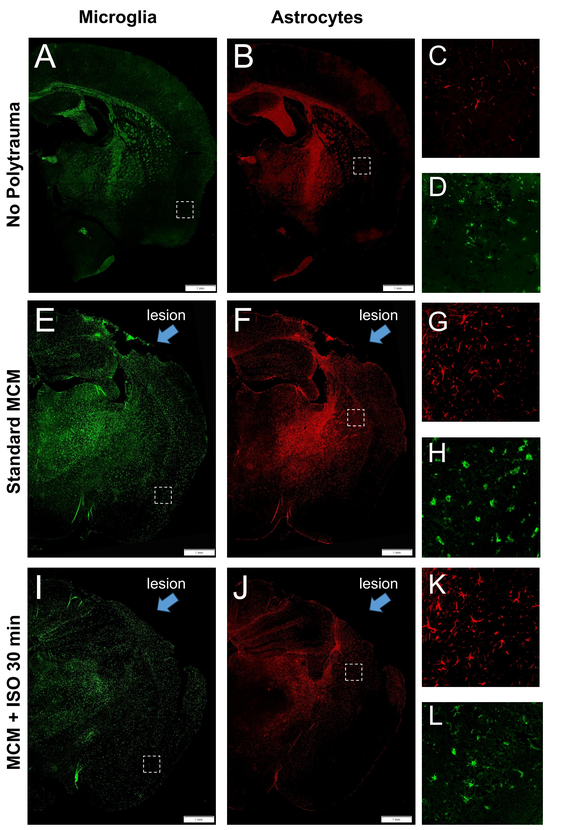


Figure 3: Representative IHC pictures from a no polytrauma control, polytrauma with only standard treatment and polytrauma with standard treatment and ISO at 40 min labeled for **microglia** (green) or **astrocytes** (red). No polytrauma controls have sparse and quiescent microglia (A and D) along with non-activated astrocytes (B and C). Polytrauma with standard MCM tissues have extensive microglial activation (E), many with a changed ameboid morphology indicating activation and neuroinflammation (H). Astrocytes are activated and hypertrophic also indicating inflammation (F and G). Adding ISO to standard MCM reduces the extent of microglial activation (I) and many remain activated but not phagocytic (L). Astrocyte activation is reduced throughout the tissue (J) though do remain hypertrophic and reactive (K).



Conclusions

- The addition of briefly administered ISO increased the seizure off rate, even when seizure had become refractory to benzodiazepines alone. While ISO and benzodiazepines both rely on $GABA_A$ signaling pathway antagonism for their inhibitory functions, ISO additionally acts on multiple other signaling pathways to reduce neural activity (*i.e.*, TREK-1 K+ channels, glutamate & glycine receptors), potentially allowing for a longer therapeutic window.
- ISO can rapidly (<5 min) halt seizure activity during a polytrauma event, typically faster than benzodiazepines alone. This rapid action, in addition to multiple pathway targeting, may also be from rapid uptake and diffusion directly to the brain as metabolism is not required for activity.
- ISO increased the recurrence time for seizure after treatment compared to standard MCM alone though became less effective as time went on. As more NT systems are recruited by the seizure activity and become active, seizures become more difficult to control.
- ISO treatment reduced neuroinflammation, likely through primary interactions with the TLR4 inflammation pathway and secondary to seizure control.
- **Take Home Message**: The addition of brief, sedative exposure to ISO up to 90 min after injury can stop seizure activity and reduce morbidity from TBI/CWNA exposure during PFC conditions. Increased sustainment can lead to more casualties available for evacuation to higher tier medical facilities.

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