Allopregnanolone Terminates Soman-induced Electrographic Status Epilepticus in Rats: Analysis Using a Novel Translationally Aware Approach Based on Standardized Clinical Criteria

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INTRODUCTION

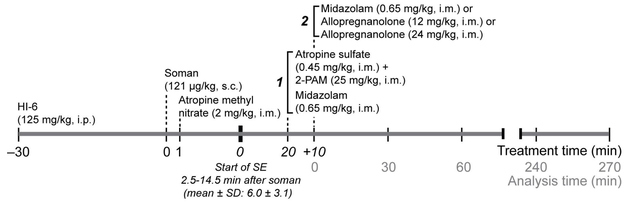
Studies of electrographic status epilepticus (ESE) therapies in rodents using conventional analysis approaches have uncertain translational relevance as they typically have assessed treatment effects using measures of electroencephalogram (EEG) power that do not identify ESE onset or termination in a manner corresponding with the approach used in critical care neurology. This study assesses the potential of allopregnanolone (ALLO), an allosteric modulator of GABA-A receptors, to terminate electrographic status epilepticus (ESE) induced by the organophosphate (OP) nerve agent soman using a new analysis approach based on the 2021 ACNS Standardized Critical Care EEG Terminology definition of electrographic seizure (ESz).

Soman is a human-made chemical warfare agent classified as a nerve agent. Exposure can occur through skin contact, eye contact, inhalation, food contamination, and water contamination. It can induce status epilepticus, leading to increased risk of severe neuropathology and long term behavioral and cognitive defects.

Allopregnanolone (ALLO) is a powerful antiseizure molecule that acts as a positive allosteric modulator of both synaptic and extrasynaptic GABA-A receptors. ALLO has demonstrated efficacy in animal models of refractory seizures.

METHODS

- Rats with chronic cortical screw EEG electrodes implanted 5-7 days before the day of the experiment received the oxime HI-6, soman, and the peripheral muscarinic acetylcholine receptor antagonist atropine methyl nitrate. Drugs were administered at the times and doses indicated in the schematic shown below. Animals were treated with HI-6 and atropine methyl nitrate to increase survival to the onset of status epilepticus (SE). Twenty min after the onset of behavioral and electrographic SE, all animals received the initial treatment (***1***) consisting of midazolam at a dose (0.65 mg/kg, i.m.) that produces peak plasma levels comparable to those obtained with the recommended human midazolam dose (10 mg), and the nerve agent antidotes atropine sulfate and 2-PAM, as in the protocol for emergency nerve agent treatment. Ten min after the initial treatment, animals received the second treatment (***2***) consisting of either a second dose of midazolam (0.65 mg/kg, i.m.) or allopregnanolone (12 or 24 mg/kg, i.m.). Analysis time values (time from the second treatment) are used to define EEG epochs.



- Our analysis method was intended to correspond to the 2021 ACNS Standardized Critical Care Criteria for EEG Terminology as show in the box below. The EEG was analyzed using Neuroscore (Data Sciences International). For each animal, a baseline EEG recording was acquired for 38-60 min prior to administration of soman. The RMS amplitude values for each 1 s interval in the baseline recording was averaged. A spike was scored when the voltage excursion in the subsequent recording exceeded 4 times the average baseline RMS amplitude value for the animal. The presence or absence of electrographic seizures (ESz) was determined from the spike timing date algorithmically using R Studio. A 10 s moving window was advanced in 1 s steps and the presence (>25 spikes) or absence (≤25 spikes) of ESz was determined. The 1 s step was assigned a value of 1 when ESz was present and a value of 0 when ESz was not present. Seizure burden was determined as the sum of the values at each step divided by the number of steps. Recordings were carried out for up to 4 h after the initial treatment administration. The following time intervals were analyzed: (1) baseline period (38-60 min), (2) period from soman injection to initial treatment, (3) 10 min period between the initial treatment and the second treatment, and (4) each 30 min period during the remainder of the recording up to 4 h.

2021 ACNS Standardized Critical Care Criteria[1]

- Electrographic spikes were defined as a "transient, clearly distinguished from background activity, with a pointed peak at a duration of 20 to 70 ms" .
- Electrographic seizure (ESz) was identified in EEG recordings as an event lasting at least 10 s in which spikes occurred at a frequency of >2.5 Hz.
- Electrographic status epilepticus (ESE) was scored as present when the ESz burden was ≥20% within the time interval analyzed.

RESULTS

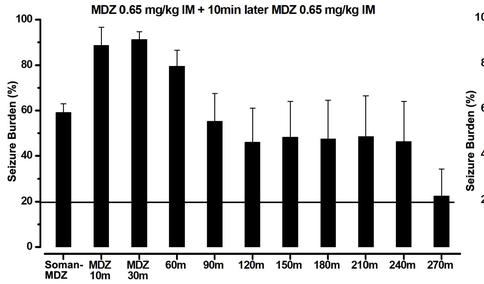


Fig. 1. Average seizure burden in animals with soman-induced status epilepticus treated with MDZ (0.65 mg/kg, IM) followed 10 min later with a second dose of MDZ. The bars indicate percentage of seizure burden within presented time window calculated as the mean ± S.E.M for n=7 rats.

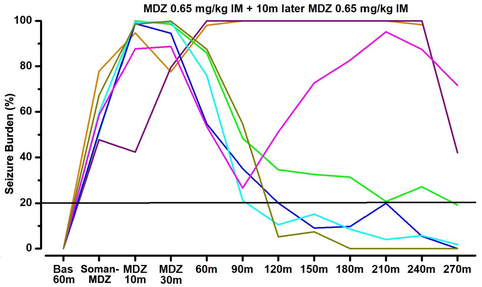


Fig. 2. Seizure burden for individual animals with soman-induced status epilepticus treated with MDZ (0.65 mg/kg, IM) followed 10 min later with a second dose of MDZ.

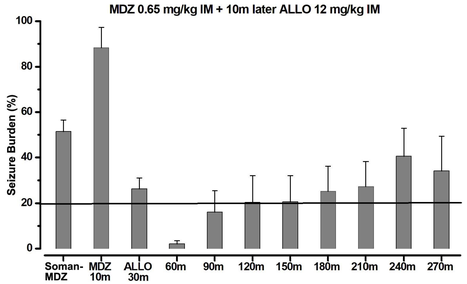


Fig. 3. Average seizure burden in animals with soman-induced status epilepticus treated with MDZ (0.65 mg/kg, IM) followed 10 min later with ALLO (12mg/kg IM). The bars indicate percentage of seizure burden within presented time window calculated as the mean ± S.E.M for n=11 rats.

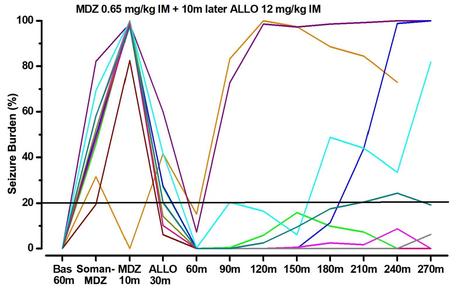


Fig. 4. Seizure burden for individual animals with soman-induced status epilepticus treated with MDZ (0.65 mg/kg, IM) followed 10 min later with ALLO (12mg/kg IM).

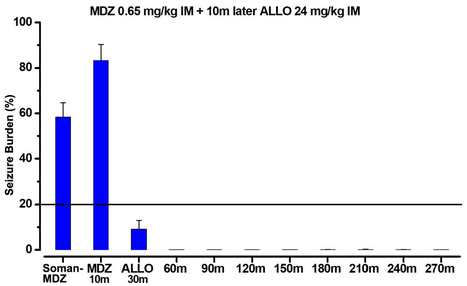


Fig. 5. Average seizure burden in animals with soman-induced status epilepticus treated with MDZ (0.65 mg/kg, IM) followed 10 min later with ALLO (24mg/kg IM). The bars indicate percentage of seizure burden within presented time window calculated as the mean ± S.E.M for n=12 rats.

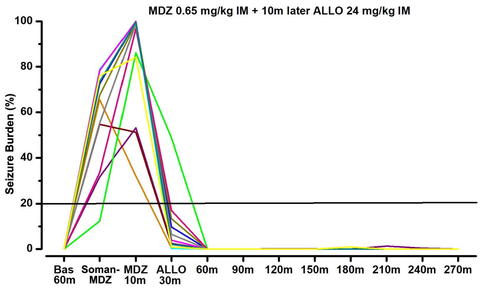


Fig. 6. Seizure burden for individual animals with soman-induced status epilepticus treated with MDZ (0.65 mg/kg, IM) followed 10 min later with ALLO (24mg/kg IM).

DISCUSSION OF RESULTS

- Responses to 2 doses of MDZ were variable. In 3 animals, the seizure burden dropped below 20%. Thus, according to the predefined definition, there was transitory suppression of electrographic status epilepticus (ESE). The other animals failed to show suppression. Overall, the mean seizure burden values remained above 20% during the entire observation period.
- All animals that received 12 mg/kg ALLO demonstrated transitory suppression of ESE but the suppression did not persist in 4 of 8 animals. The mean seizure burden values were below 20% during the first two 30 min epochs but, based on average seizure burden values, status epilepticus suppression did not persist after the second 30 min epoch.
- All animals that received 24 mg/kg ALLO demonstrated rapid termination of ESE without recurrence for the 4 h observation period. The mean seizure burden was not suppressed during the period after initial treatment with midazolam but was suppressed during the period after ALLO treatment and remained suppressed during the entire recording period.

CONCLUSIONS

- A new analysis approach has been developed that identifies ESE according to currently accepted clinical criteria. Using this method, MDZ failed to consistently terminate ESE whereas ALLO at a high dose was effective, supporting the potential utility of high dose ALLO in the treatment of benzodiazepine-refractory status epilepticus.
- The novel analysis method may have application broadly in the study of treatments for status epilepticus.

REFERENCES

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