Intravenous Ganaxolone Attenuates Sarin Nerve Agent Induced Seizures

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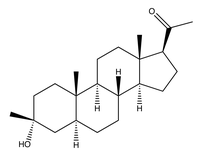
Background

- Sarin is an organophosphate neurotoxin and, along with other similar nerve agents, is classified as a chemical weapon that poses a threat to civilian populations in terrorist attacks[1]
- These nerve agents are irreversible inhibitors of acetylcholinesterase and exposure can cause serious symptoms, including status epilepticus[1]
- Medical countermeasures for nerve agent exposure include immediate treatment with atropine, pralidoxime, and midazolam[2]
- Up to 40% of exposed subjects fail to respond to these first-line treatments and progress to status epilepticus[2]
- Thus, there is a requirement for a treatment that can be administered to exposed subjects who do not respond to these standard drugs[2]

Ganaxolone

- Ganaxolone (GNX) is a neuroactive steroid and positive allosteric modulator of synaptic and extrasynaptic $GABA_A$ receptors (**Figure 1**)

Figure 1. Chemical Structure of Ganaxolone



- GNX is approved for the treatment of seizures associated with CDKL5 Deficiency Disorder in patients 2 years of age and older
- GNX is an antiepileptic in animal models of benzodiazepine-resistant status epilepticus[3,4]
- An IV formulation of GNX is currently in Phase 3 clinical trials for the treatment of refractory status epilepticus
- The preclinical studies presented here show the efficacy of IV-infused GNX in an animal model of sarin exposure and establish a pharmacokinetic/pharmacodynamic (PK/PD) relationship between GNX and anti-epileptic efficacy

METHODS

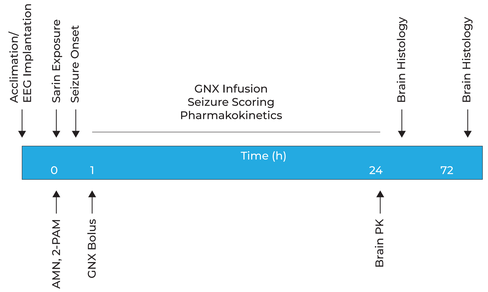
Subjects

- Adult rats were exposed to sarin and then within 2 minutes treated with atropine methyl nitrate (AMN) and pralidoxime chloride (2-PAM)
- Forty minutes after seizure onset, rats were treated with an IV bolus (0.5, 1, 2 or 4 mg/kg; 20 seconds) followed by a continuous IV infusion of GNX (0.5, 1, 2 or 4 mg/kg/h, respectively) for 24 hours
- Video-EEG was collected over 24 hours via implanted telemetry and analyzed post-collection. The severity of seizures or status epilepticus were scored based on EEG and behaviors in 10-minute intervals over the duration of each collection using a Likert scale (**Figure 2**)
- GNX plasma and brain levels were measured in a parallel group of animals
- Histopathological analyses of post-mortem brains were assessed to determine effects of GNX on neurodegeneration

Figure 2. Likert Scale of Seizure Severity

7	Continuous, severe, and high-powered status epilepticus (SE) with continuous convulsions during the scored interval. The EEG power of delta and theta >100 uV with the implant used in this study (continuous convulsion; severe SE)
	Step from 7 to 6: from severe SE with continuous convulsion to moderately powered SE with intermittent convulsions
6	Continuous, moderately powered spike and wave activity (delta and theta 50-100 uV with the MRIGlobal implant); strong yet intermittent convulsions (the presence of at least one severe convulsion in the interval)
	Step from 6 to 5: switch to low powered SE; strong convulsions subside, mild myoclonus or stereotypy can remain (considered largely non-convulsive SE)
5	Continuous low powered SE (delta and theta <50 uV with MRIGlobal implant). Mild myoclonus, tremors or stereotypy can be present, yet large convulsions subsided (considered largely as nonconvulsive SE). No exploring behaviors observed. No discernable normal background. (Step from convulsion to less severe stereotypy; low amplitude SE)
	Step from 5 to 4 (key step): SE ends, switch to intermittent seizure activity; largely non-convulsive, yet occasional short, self-terminating paroxysms can occur
4	Break of SE; high incidence of abnormalities remain (less than 50% of the interval) yet with no severe high-powered paroxysms; mild myoclonus or short self-terminating convulsive activity can occur occasionally; some normal EEG background returns and occasional exploring can occur
	Step from 4 to 3: from relatively high incidence of epileptiform activity to low incidence; all convulsions and myoclonus subside
3	Low severity epileptiform activity is present (spikes, sharp waves) occasionally with no EEG paroxysms; no convulsions, no myoclonus, etc. Active periods are present with near normal behavior. Sedation EEG with near complete lack of EEG discharges is categorized here
	Step from 3 to 2: near normal EEG, infrequent spikes, no convulsive behavior of any type, and no sedation
2	Sporadic, infrequent epileptiform abnormalities of low severity (spikes, sharp waves); no EEG paroxysms; no convulsions, myoclonus, etc; considered within the range of normal EEG; all behaviors are normal (walking, grooming, etc.) and animal is not sedated
	Transition to completely normal EEG and behavior
1	Completely normal EEG and behavior relative to the animal's state with no prodromal events (sharp waves, spikes, slowing, etc) or seizures

Figure 3. Study Timeline



AMN, atropine methyl nitrate; GNX, ganaxolone, 2-PAM, pralidoxime chloride; PK, pharmacokinetics.

Figure 4. Ganaxolone Decreases Seizure Severity in a Dose-Dependent Manner

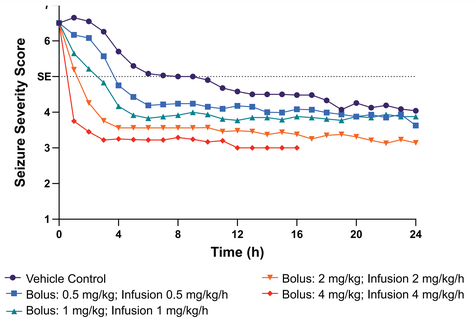
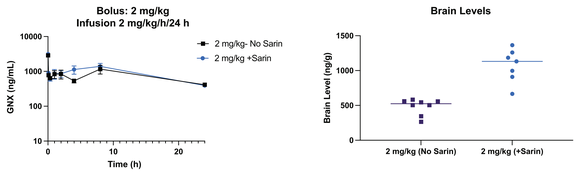


Figure 5. Sarin Does Not Alter GNX Plasma PK, but Increases GNX Brain Penetrance



Steady-State Plasma Level (+Sarin): 1159 ng/mL
Steady-State Plasma Level (No Sarin): 1101 ng/mL

GNX brain levels after 24 h of IV infusion

Conclusions

- **Sarin evoked status epilepticus within 40 minutes after exposure**
- **Administration of GNX (bolus followed by infusion) produced a dose-dependent reduction in seizure severity; this timing reflects Refractory Status Epileptic response**
- **A dose of 2 mg/kg (bolus) followed by 2 mg/kg/h (infusion) produced a maximally effective response with no adverse activities**
- **Corresponding PK showed that steady-state plasma levels of GNX between 500 and 1100 ng/mL were efficacious**
- **Sarin exposure did not alter GNX plasma PK, but increased GNX brain penetrance 2-fold**
- **GNX showed evidence of neuroprotection in specific brain regions at 24 and 72 hours after sarin exposure**

Figure 6. Histopathology Scores Decreased With Higher Doses

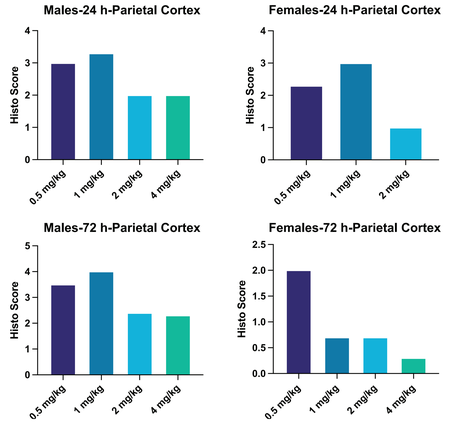
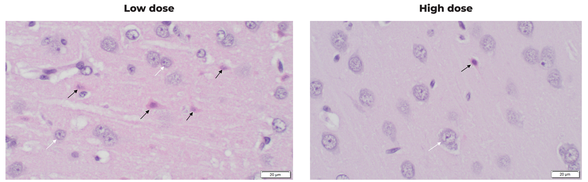


Figure 7. Histopathology: Parietal Cortex - Low Dose vs. High Dose at 72-h termination



High dose shows just a few necrotic neurons (black arrows) mixed among a majority of unaffected neurons (white arrows)

References

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Disclosures

PA, KB, AH, JM, and PB are employees of MRI Global.
MM is an employee of Preclinical Electrophysiology Consulting.
MS is an employee of Marinus.

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MARINUS SCIENTIFIC AFFAIRS

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