Received: 2 August 2021 | Revised: 7 October 2021 | Accepted: 12 October 2021

DOI: 10.1002/epi4.12552

Epilepsia Open® Open Access

FULL-LENGTH ORIGINAL RESEARCH

Combination of antiseizure medications phenobarbital, ketamine, and midazolam reduces soman-induced epileptogenesis and brain pathology in rats

Lucille A. Lumley[1] | **Brenda Marrero-Rosado**[1] | **Franco Rossetti**[2] | **Caroline R. Schultz**[1] | **Michael F. Stone**[1] | **Jerome Niquet**[3,4] | **Claude G. Wasterlain**[3,4]

[1]Neuroscience Department, U.S. Army Medical Research Institute of Chemical Defense (USAMRICD), Aberdeen Proving Ground, Maryland, USA

[2]Military Psychiatry and Neuroscience Department, Walter Reed Army Institute of Research, Silver Spring, Maryland, USA

[3]Department of Neurology, David Geffen School of Medicine at UCLA, Los Angeles, California, USA

[4]Epilepsy Research Laboratory (151), Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, California, USA

Correspondence
Lucille A. Lumley, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5400, USA.
Email: lucille.a.lange.civ@mail.mil

Funding information
National Institute of Neurological Disorders and Stroke, Grant/Award Number: U01 NS074926; U.S. Department of Veterans Affairs, Grant/Award Number: I01 BX000273-07

Abstract

Objective: Cholinergic-induced status epilepticus (SE) is associated with a loss of synaptic gamma-aminobutyric acid A receptors ($GABA_AR$) and an increase in N-methyl-D-aspartate receptors (NMDAR) and amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) that may contribute to pharmacoresistance when treatment with benzodiazepine antiseizure medication is delayed. The barbiturate phenobarbital enhances inhibitory neurotransmission by binding to a specific site in the $GABA_AR$ to increase the open state of the channel, decrease neuronal excitability, and reduce glutamate-induced currents through AMPA/kainate receptors. We hypothesized that phenobarbital as an adjunct to midazolam would augment the amelioration of soman-induced SE and associated neuropathological changes and that further protection would be provided by the addition of an NMDAR antagonist.

Methods: We investigated the efficacy of combining antiseizure medications to include a benzodiazepine and a barbiturate allosteric $GABA_AR$ modulator (midazolam and phenobarbital, respectively) to correct loss of inhibition, and ketamine to reduce excitation caused by increased synaptic localization of NMDAR and AMPAR, which are NMDA-dependent. Rats implanted with transmitters to record electroencephalographic (EEG) activity were exposed to soman and treated with atropine sulfate and HI-6 one min after exposure and with antiseizure medication(s) 40 minutes after seizure onset.

Results: The triple therapy combination of phenobarbital, midazolam, and ketamine administered at 40 minutes after seizure onset effectively prevented soman-induced epileptogenesis and reduced neurodegeneration. In addition, dual therapy with phenobarbital and midazolam or ketamine was more effective than

The views expressed in this manuscript are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the US Government.

monotherapy (midazolam or phenobarbital) in reducing cholinergic-induced toxicity.

Significance: Benzodiazepine efficacy is drastically reduced with time after seizure onset and inversely related to seizure duration. To overcome pharmacoresistance in severe benzodiazepine-refractory cholinergic-induced SE, simultaneous drug combination to include drugs that target both the loss of inhibition (eg, midazolam, phenobarbital) and the increased excitatory response (eg, ketamine) is more effective than benzodiazepine or barbiturate monotherapy.

KEYWORDS

barbiturate, benzodiazepine, ketamine, organophosphorus nerve agent, refractory status epilepticus

1 | INTRODUCTION

Organophosphorus nerve agents (OPNAs) bind to and inactivate the enzyme acetylcholinesterase, thereby increasing acetylcholine in cholinergic synapses and neuromuscular junctions, leading to overt toxic signs. In cases of severe OPNA poisoning, seizure may be triggered as a result of the hyperactivation of glutamatergic synapses. Currently approved treatments in the United States include the oxime 2-pralidoxime chloride (2-PAM Cl) for the reactivation of acetylcholinesterase, the muscarinic anticholinergic drug atropine for the amelioration of peripheral effects of OPNA exposure, and the benzodiazepine diazepam as the first-line antiseizure medication. With the FDA approval of injectable midazolam to treat seizure, midazolam is anticipated to replace diazepam once an autoinjector form is approved (reviewed in Ref.1). However, in a scenario of mass casualties and/or attacks to unprepared populations, treatment with antiseizure medication may be delayed, giving rise to a prolonged self-sustaining seizure (ie, status epilepticus, SE) that becomes resistant to benzodiazepine treatment.[2–4] Since the time spent in seizure correlates to neuronal cell damage,[5] promptly controlling seizure activity is crucial. Yet, optimal treatments for cholinergic-induced refractory SE are lacking. Novel therapeutic approaches to control SE following exposure to OPNAs must take into consideration the molecular maladaptive changes that underlie its pharmacoresistance.

One of the consequences of prolonged SE is the internalization of γ-aminobutyric acid type A receptors ($GABA_AR$), which may explain the loss of efficacy of benzodiazepines.[6] Concomitantly, an increase in the number of N-methyl-D-aspartate receptors (NMDAR) at neuronal synapses drives glutamatergic excitation and promotes excitotoxicity.[7] In line with this theory, targeting NMDAR with antagonists, such as ketamine, is a highly efficient way of controlling SE. Preclinical studies demonstrated the synergistic properties of combining diazepam and ketamine[8] or midazolam and ketamine[9] to decrease seizure duration following cholinergic-induced SE. In the rodent models of soman-induced SE, a treatment composed of midazolam and ketamine reduced seizure severity, ameliorated impairment in the Morris water maze, and reduced neuronal loss.[10–13] However, the protection afforded by the combination of ketamine and midazolam was incomplete, suggesting a need for an additional adjunct medical countermeasure.

Key Points

- Pharmacoresistance is an unmet therapeutic challenge against cholinergic-induced status epilepticus (SE).
- Efficacy of phenobarbital alone or in combination with midazolam and ketamine was evaluated against soman-induced SE.
- Phenobarbital, midazolam, and ketamine triple therapy reduced time in seizure and prevented epileptogenesis in soman-exposed rats.
- Triple antiseizure medication following soman-induced SE also reduced neurodegeneration and neuronal loss in seizure-sensitive brain regions.
- Antiseizure medication combination to increase $GABA_AR$ function and reduce glutamate activity may be an effective approach against benzodiazepine-refractory SE.

Modulators of GABA receptors are administered to control seizure activity. The barbiturate phenobarbital, a second-line drug used to treat SE, enhances inhibitory neurotransmission by binding to a specific site in the $GABA_AR$ to increase the open state of the channel and, thus, decrease neuronal excitability.[14] Additionally, phenobarbital may reduce glutamate-induced currents through AMPA/kainate receptors.[15] The barbiturate pentobarbital (75 mg/kg) had moderate efficacy at reducing soman-induced seizure in rats, with treatment at 5 minutes after seizure onset being more efficacious than a 40-min delay. In midazolam-treated rats exposed to soman, phenobarbital dose-dependently reduced seizure activity, albeit with a delay in seizure termination and without a long-lasting effect.[16] Similar dose-response effects of phenobarbital as an adjunct to midazolam were observed following exposure to the pesticide diisopropyl fluorophosphate (DFP).[17,18] The latter studies, however, did not investigate the effect of phenobarbital adjunct treatment on epileptogenesis and neuronal cell death at an endpoint longer than 24 or 72 hours. The antiseizure effects of phenobarbital occur in other seizure models, including maximal electroshock, picrotoxin, and electrical hippocampal stimulation.[19,20] Less is known about the effects of a subanesthetic dose of midazolam in combination with other antiseizure medications.

When treatment of cholinergic-induced seizure is delayed to greater than 30 minutes, blocking one pathway (eg, benzodiazepine monotherapy) is insufficient, and polytherapy is needed to simultaneously activate the remaining $GABA_AR$ and block NMDAR activation.[21] Currently, we report that the combination of the antiseizure medication phenobarbital with midazolam and ketamine effectively halts acute seizure activity, reduces the incidence of epileptogenesis, and reduces neuronal cell degeneration following SE induced by soman exposure.

2 | METHODS

2.1 | Animals

Male Sprague Dawley rats (350-400 g; Charles River) were individually housed following telemetry implantation surgery, with food and water available ad libitum, on a 12-h:12-h light-dark cycle with lights on at 0600 hours. Rats were weighed daily. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the Animal Welfare Act of 1966 (PL 89e544), as amended.

2.2 | Implantation of electrodes and electroencephalographic (EEG) analysis

Electroencephalographic (EEG) telemetry transmitters were subcutaneously (SC) implanted in rats. Under anesthesia by isoflurane (3%-5% induction, 1.5%-5% maintenance), rats were secured in a Kopf stereotaxic apparatus (David Kopf Instruments; Tujunga, CA, USA). Four cortical stainless steel screw electrodes were implanted on the skull 2 mm bilaterally to the midline and 1.6 mm anterior and 4 mm posterior to bregma. Stainless steel wires from the F40-EET transmitters (Data Sciences International [DSI], Inc, St. Paul, MN) were implanted subcutaneously, wrapped around the electrodes, and secured in place using dental acrylic. Rats were administered buprenorphine (0.03 mg/kg, sc) immediately after removal from anesthesia with 1 week to recover from surgery prior to soman exposure. For EEG activity recording, an RPC-1 PhysioTel receiver from DSI was placed under each animal's home cage for continuous data collection (24 h/d) using Dataquest ART Acquisition software (DSI, Inc). EEG data were recorded continuously for at least 24 hours prior to soman exposure to day of euthanasia (14 days after exposure). EEG data were digitized at 250 Hz to evaluate seizure onset, acute seizure duration, and the development of spontaneous recurrent seizure (SRS).

2.3 | Soman-induced seizure and administration of medical countermeasures

Rats were administered 1.2 LD_{50} of soman (pinacolyl methylphosphonofluoridate; 118.1 μg/kg; 0.5 ml/kg; SC), obtained from the US Army Combat Capabilities Development Command Chemical Biological Center (Aberdeen Proving Ground, MD, USA), and 1 minute later administered intramuscularly (IM) an admix of atropine sulfate (2 mg/kg) and the oxime HI-6 dichloride salt (93.6 mg/kg). Forty minutes after seizure onset, antiseizure medication treatments were administered intraperitoneally (IP). Soman-exposed animals were assigned to one of five groups: (1) midazolam (3 mg/kg) monotherapy; (2) phenobarbital (30 mg/kg) monotherapy; (3) phenobarbital (30 mg/kg) and ketamine (30 mg/kg); (4) phenobarbital (30 mg/kg) and midazolam (3 mg/kg); and (5) phenobarbital (30 mg/kg), ketamine (30 mg/kg), and midazolam (3 mg/kg). Midazolam and ketamine were

diluted in sterile water, and phenobarbital was diluted in a vehicle containing 40% propylene glycol, 10% ethanol, 1.5% benzyl alcohol, and 48.5% distilled water. Seizure onset was on average 7 minutes, so treatment was approximately 47 minutes after exposure. Following soman exposure, EEG was monitored in real time and seizure onset was defined as the appearance of rhythmic high-amplitude spikes (>2 × baseline) that lasted at least 10 s (based on Ref.22). Rats were monitored for 6 hours following exposure, and behavioral seizures were scored using a modified Racine scale[23] composed of five stages: 1, masticatory movements; 2, head myoclonus; 3, limb clonus or tonus, body tremor; 4, forelimb clonus with rearing; and 5, rearing and falling with generalized tonic-clonic convulsions.

2.4 | EEG seizure analysis

Scoring of EEG for the full duration of the recordings was performed using a high-throughput seizure detection algorithm as previously described.[24] Briefly, seizure activity was determined in 2-s epochs with detection thresholds set by the slope of a linear robust fit applied to a fast Fourier transform and normalized power spectra (0.1-10 Hz) during 24 hours of baseline. During seizure episodes, the power spectra increase in magnitude and dominant frequencies are shifted to the left, increasing the slope of the linear robust fit. When both power spectra and slope reach threshold, the 2-s epoch is marked as a potential seizure. Detection using this method generated a list of candidate seizures that an observer blinded to the treatment groups visually inspected for confirmation of seizures and rejection of false positives arising from the detection of normal behavior (eg, sleep activity) or artifacts in the EEG signal. In the triple therapy group, one rat did not survive to 24 hours, and a second rat with poor EEG signal was excluded from the seizure data analysis.

2.5 | Brain tissue collection and immunohistochemistry

Two weeks after soman exposure, rats were injected with sodium pentobarbital (75 mg/kg, IP, Fatal Plus; Patterson Veterinary) and perfused with 0.9% heparinized saline in 0.1 M phosphate buffer (FD Neurotechnologies, Columbia, MD), followed by 4% paraformaldehyde. Brains were removed, postfixed for 6 hours in 4% paraformaldehyde, and cryoprotected in 20% sucrose. Histological sectioning and staining of brain tissue were conducted by FD Neurotechnologies. Coronal 50-μm sections were stained with FD NeuroSilver™ to identify neurodegeneration in brain regions previously shown to be affected following soman exposure (eg, Ref.25–27). Brain regions were qualitatively scored by an observer blinded to treatment, on a scale of 0-4, with 4 being the most severe. Brain regions scored included the thalamus (lateral, medial, reuniens), amygdala (basolateral, lateral, medial, central), hippocampus (CA1, CA2, CA3, dentate gyrus, radiatum, stratum lucidum, oriens layer), and piriform cortex (layers 1, 2, 3), as well as fiber tracts (cingulum, corpus callosum, external capsule, internal capsule, fimbria of hippocampus), at bregma −3.00 mm. Medians of the median score for each subregion were taken to represent the overall brain region.

In addition, coronal 30-μm sections were stained for the mature neuronal marker NeuN and the inhibitory neuronal marker glutamate decarboxylase 67 (GAD67). A monoclonal mouse anti-NeuN IgG (1:10,000; Millipore, Billerica, MA) and a monoclonal mouse anti-GAD67 IgG (1:2000; Abcam, Cambridge, MA) were used in the immunohistochemistry procedure. Subsequently, the immunoreaction product was visualized according to the avidin-biotin complex method[28] with the Vectastain Elite ABC Kit (Vector Lab., Burlingame, CA). NeuN-positive (NeuN+) and GAD67-positive (GAD67+) cell densities were determined in various brain regions using Image-Pro Plus v7.0 (Media Cybernetics Inc, Rockville, MD, USA; for additional details, see Ref.29). Areas evaluated included the thalamus, basolateral amygdala, piriform cortex, and hippocampus. For neuropathology assessments, controls (no agent) were used for comparison with soman-exposed, treated rats.

2.6 | Data analysis

Data analysis was conducted using SPSS v21 (IBM Inc, Armonk, NY, USA). Graphs were compiled using SigmaPlot 14.0 (Systat Software, Inc, San Jose, CA, USA). Repeated-measures ANOVA was used to analyze body temperature, body weight, and motor activity with repeated time points as the within-subjects variable and treatment as the between-subjects variable. If a significant interaction was found, a one-way ANOVA was conducted at each level of the within-subjects variable. For comparison of seizure duration, as well as NeuN+ and GAD67+ cell densities, a general linear model analysis was used, followed by Tukey's test. A Kaplan-Meier test was performed to compare treatment effects on median time to onset of initial SRS event. To determine the relationship between treatments and percentage of animals that showed SRS by the study endpoint, a logistic regression analysis was performed and the chi-square followed

by Fisher's exact test was conducted to account for group comparisons. For number of SRS, a one-way ANOVA was used. When homogeneity of variance failed, the Kruskal-Wallis test was conducted, followed by multiple comparisons and adjusted significance to account for multiple comparisons. For comparisons of toxic signs and silver score, a Kruskal-Wallis test was used. Post hoc comparisons were performed using a Mann-Whitney *U* test. Data are displayed as mean ± standard deviation or median and interquartile range (IQR).

3 | RESULTS

3.1 | Phenobarbital combination therapies reduced acute seizure activity and incidence of epileptogenesis following soman exposure

Subcutaneous exposure to soman resulted in severe and prolonged seizure activity in rats, with average (± SD) seizure onset of 7 minutes (± 4.7 minutes). A general linear model analysis detected a main effect of treatment on acute seizure duration. Therapies that included phenobarbital led to a lower average seizure duration during the first 24 hours following soman exposure compared with midazolam monotherapy (Figure 1A). The average (±SD) seizure duration in the first 24 hours after soman exposure was 542.6 ± 285.7, 235.6 ± 100.1, 161.8 ± 134.2, 156.7 ± 113.2, and 71.2 ± 69.6 minutes for midazolam monotherapy, phenobarbital monotherapy, phenobarbital and ketamine, phenobarbital and midazolam, and phenobarbital, ketamine, and midazolam combination therapy, respectively. Triple therapy also reduced the behavioral seizure, scored using a modified Racine scale, with significantly lower seizure severity score than in rats treated with midazolam or phenobarbital monotherapy or with phenobarbital and midazolam dual therapy by 30 minutes after treatment (Figure 1B). Rats treated with midazolam monotherapy continued to display behavioral seizure for over 5 hours after treatment with significantly higher toxic sign scores compared with triple therapy–treated rats for this duration.

In the days following soman-induced SE, EEG activity was continuously recorded and subsequently analyzed for the development of SRS. A logistic regression analysis detected a main effect of treatment on the percentage of animals showing SRS by the study endpoint. The group of animals receiving a combination therapy of phenobarbital, ketamine, and midazolam did not

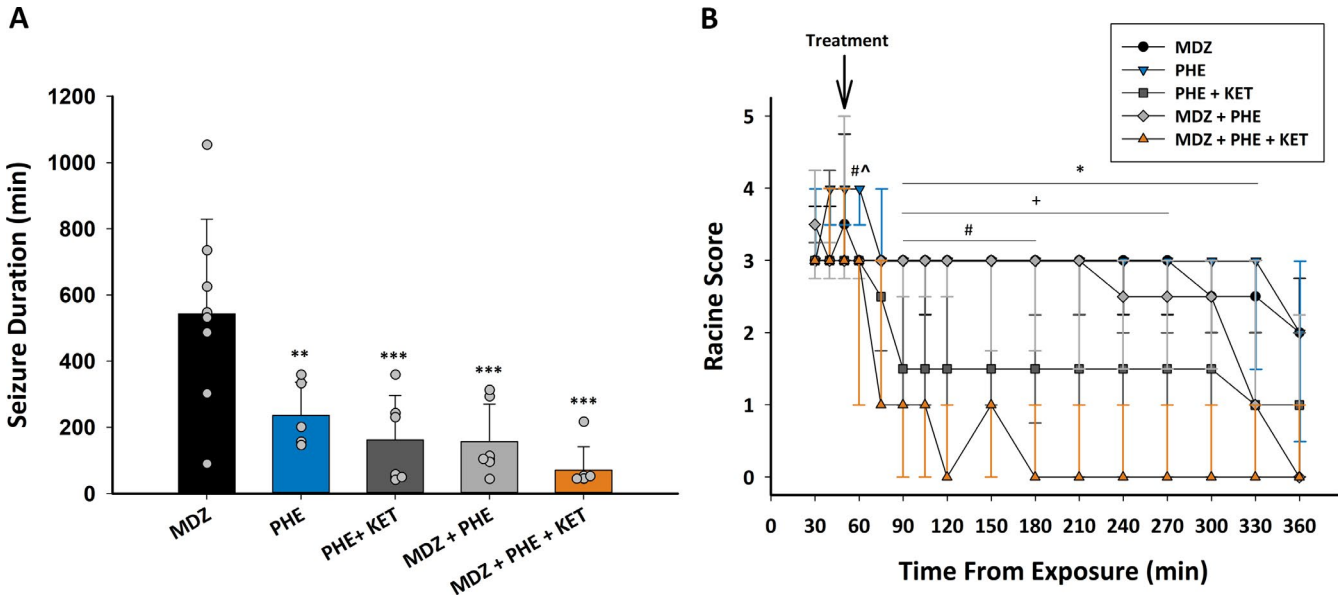

FIGURE 1 Effect of delayed treatment with antiseizure medications on seizure duration and activity following soman (GD) exposure in rats: benefit of simultaneous administration of triple antiseizure medication therapy. Midazolam (MDZ) administered at 40 min after seizure onset induced by GD resulted in prolonged initial seizure activity (status epilepticus). A, Rats that received phenobarbital monotherapy (PHE), dual therapy of phenobarbital with ketamine (PHE + KET) or midazolam (PHE + MDZ) or triple antiseizure medication therapy of midazolam, phenobarbital, and ketamine (MDZ + PHE + KET) had a reduced time in initial seizure compared with MDZ-treated rats. Seizure duration is shown as mean ± SD. $^{**}P < .01$ and $^{***}P < .001$ compared with midazolam. B, Monotherapy with MDZ or PHE was ineffective at terminating behavioral seizure activity, whereas treatment with triple therapy (MDZ + PHE + KET) reduced the Racine score by 30 min after treatment (postexposure time 60 min) compared with MDZ ($^{*}P < .05$) or PHE ($+P < .05$) monotherapy. Triple therapy also significantly reduced the toxic sign score compared with midazolam and phenobarbital dual therapy ($\#P < .05$). Racine score is shown as median ± IQR

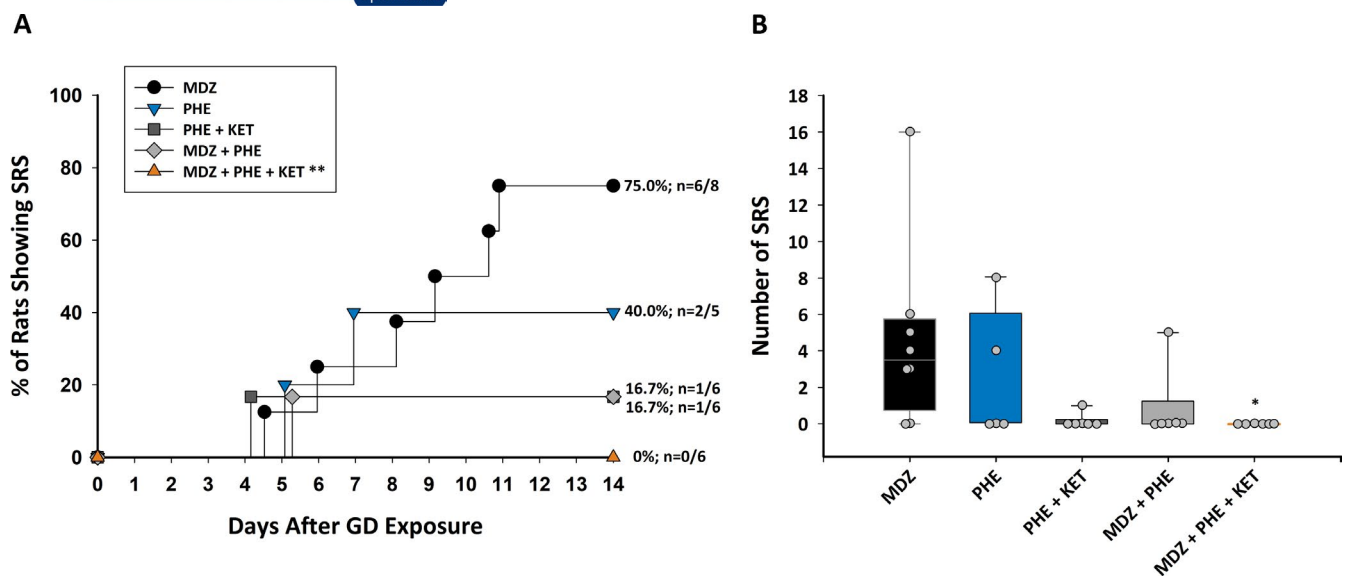

FIGURE 2 Effect of delayed treatment with antiseizure medications on epileptogenesis following soman (GD) exposure in rats: benefit of simultaneous administration of triple antiseizure medications. Midazolam (MDZ) administered at 40 min after seizure onset induced by GD resulted in epileptogenesis in the weeks after exposure. A, Rats that received phenobarbital triple antiseizure medication therapy of midazolam, phenobarbital, and ketamine (MDZ + PHE + KET) had reduced incidence of spontaneous recurrent seizure (SRS) compared to those treated with midazolam (MDZ) monotherapy, while those that received phenobarbital monotherapy (PHE) or dual therapy of phenobarbital with ketamine (PHE + KET) or midazolam (PHE + MDZ) were not different from MDZ monotherapy. B, In addition, rats that received triple therapy (MDZ + PHE + KET) had reduced number of SRS following GD exposure compared with MDZ monotherapy. Number of SRS is shown as median ± IQR. *$P < .05$; **$P < .01$

present SRS, and a chi-square analysis with Fisher's exact test revealed that this percentage was significantly reduced compared with the percentage of animals developing SRS in the midazolam monotherapy group (Figure 2A). A general linear model analysis detected a main effect of treatment on the average number of SRS events occurring up until study endpoint, with phenobarbital triple therapy showing a significantly fewer average number of SRS (Figure 2B).

3.2 | Soman-induced transient reduction in body temperature and body weight

A reduction in body temperature was observed in all soman-exposed groups (Figure S1). However, soman-exposed rats treated with phenobarbital and ketamine dual therapy had the shortest decline in temperature, with temperature returning to baseline by 10 hours after soman exposure. Compared with midazolam monotherapy, rats treated with phenobarbital and ketamine dual therapy, midazolam and phenobarbital dual therapy, or phenobarbital, ketamine, and midazolam triple therapy had greater temperature by 10, 16, and 20 hours, respectively, after GD exposure. All soman-exposed groups had significant loss of body weight compared with baseline by 24 hours after exposure, with body weight returning to baseline within a week of exposure. No significant differences in body weight loss were observed among groups.

3.3 | Combination therapy of midazolam, phenobarbital, and ketamine prevented the development of soman-induced hyperactivity

Gross motor activity measured using DSI telemetry transmitters revealed that hyperactivity occurred during the dark cycle (when rats are most active) by postexposure day 3 in soman-exposed rats treated with midazolam monotherapy at 40 minutes after seizure onset; this hyperactivity continued throughout the study (Figure 3). Activity was also greater in midazolam-treated rats during the light cycle on postexposure day 13, but not at other times as activity is low in general during the light cycle. Development of hyperactivity in soman-exposed rats was reduced in rats that received triple therapy of phenobarbital, ketamine, and midazolam. Comparisons of each group on each day revealed that triple therapy (phenobarbital, ketamine, and midazolam) significantly reduced activity compared with midazolam monotherapy on postexposure days 9 through 13. Those groups treated with dual therapy tended to have reduced activity compared with the midazolam monotherapy group, but it was not significant.

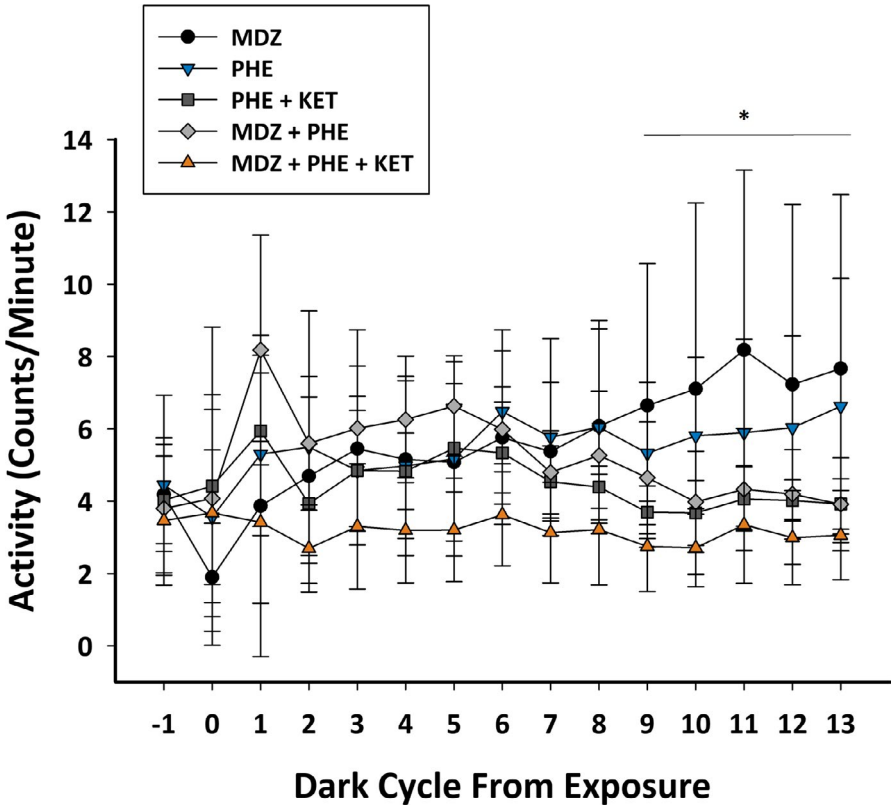

FIGURE 3 Benefit of simultaneous administration of triple antiseizure medications against soman (GD)-induced hyperactivity in rats. Midazolam (MDZ) administered 40 min after seizure onset induced by GD resulted in hyperactivity during the dark cycle (when rats are most active) in the weeks after exposure. Rats that received treatment with triple antiseizure medications of midazolam, phenobarbital, and ketamine (MDZ + PHE + KET) failed to develop hyperactivity during the dark cycle and had significantly reduced activity compared with MDZ-treated rats. No significant difference was observed in rats treated with dual therapy of phenobarbital and ketamine (PHE + KET) or midazolam and phenobarbital (MDZ + PHE) or with phenobarbital (PHE) monotherapy. Activity during the daily dark cycle (12-h period) is shown as mean ± SD. *$P < .05$ compared with MDZ monotherapy

3.4 | Phenobarbital combination therapies reduced neuropathology following soman-induced SE and delayed treatment with antiseizure medications

The triple antiseizure medication combination of phenobarbital, ketamine, and midazolam prevented or lessened damage in several brain regions, as shown with silver stain and with NeuN+ and GAD67+ immunohistochemistry 2 weeks after soman exposure. Median silver stain scores were compared in brain regions typically damaged by prolonged seizure following soman exposure. Triple therapy with phenobarbital, ketamine, and midazolam resulted in significantly less damage in fiber tracts, thalamus, amygdala, hippocampus, and piriform cortex compared with midazolam monotherapy (Figure 4). NeuN immunohistochemistry was used to measure viable neurons in the thalamus, piriform cortex, and amygdala following soman exposure. Significant loss of neurons was observed in the thalamus, amygdala, and piriform cortex in rats treated with midazolam monotherapy compared with no agent control (Figure 5). Rats receiving treatment with triple therapy following soman exposure had reduced neuronal loss compared with those that received midazolam monotherapy and were not different from no agent controls. In addition, soman-exposed rats treated with midazolam monotherapy had significantly fewer number of GAD67+ cells in the amygdala and piriform cortex compared with no agent controls (Figure 6). Treatment with triple therapy of phenobarbital, ketamine, and midazolam reduced the soman-induced loss of GAD67+ cells in the amygdala and piriform cortex. In addition, soman-exposed rats treated with phenobarbital and midazolam dual therapy had reduced loss of GAD67+ neurons in the piriform cortex compared to soman-exposed rats treated with midazolam monotherapy.

4 | DISCUSSION

In the current study, we report on the beneficial effects of combining the antiseizure medication phenobarbital

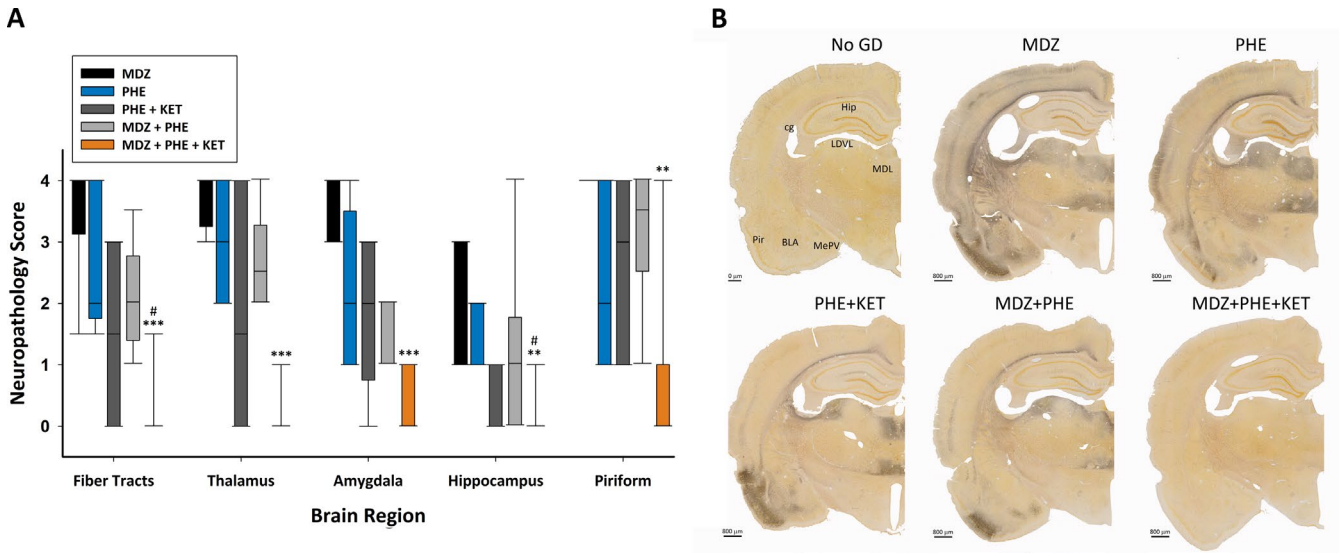

FIGURE 4 Benefit of combination triple therapy with the antiseizure medications phenobarbital, ketamine, and midazolam against soman (GD)-induced neurodegeneration in rats. Brain samples were collected 2 weeks after soman exposure and processed with silver stain to visualize neurodegeneration. A, Midazolam (MDZ) administered 40 min after GD-induced seizure failed to prevent severe neurodegeneration in fiber tracts, thalamus, amygdala, hippocampus, and piriform cortex. Rats that received treatment with triple antiseizure medications of midazolam, phenobarbital, and ketamine (MDZ + PHE + KET) had less neurodegeneration in fiber tracts, thalamus, amygdala, hippocampus, and piriform cortex compared with those receiving MDZ monotherapy. *$P < .05$; **$P < .01$; and ***$P < .001$. In addition, rats administered MDZ + PHE + KET had less neurodegeneration in fiber tracts and hippocampus compared with phenobarbital (PHE) monotherapy rats (#$P < .05$). No difference was observed between MDZ-treated rats and rats treated with phenobarbital and ketamine (PHE + KET) or midazolam and phenobarbital (MDZ + PHE) dual therapy. B, Representative images illustrate soman-induced neurodegeneration in fiber tracts including the cingulum (cg), in the thalamus including the mediodorsal lateral thalamus (MDL) and the lateral dorsal ventrolateral thalamus (LDVL), in the amygdala, including the basolateral and lateral amygdala (BLA/LA) and the medial posterior ventral amygdala (MePV), in the hippocampus (Hip), and in the piriform cortex (Pir) of rats treated with monotherapy or dual therapy, but not with triple therapy

with ketamine and midazolam to counter the effects of soman-induced SE in rats. The therapeutic limitations of benzodiazepines highlight the need for improved antiseizure medications to treat pharmacoresistant seizure. Our current findings provide further support that targeting cholinergic-induced maladaptive glutamatergic (increased NMDAR and AMPAR) and GABAergic (reduced $GABA_AR$) components that contribute to pharmacoresistance following OPNA exposure is an effective approach to reducing cholinergic-induced seizure and neuropathology. Our laboratory and others previously reported on the beneficial effect of administering the NMDAR antagonist ketamine in combination with a benzodiazepine in reducing cholinergic-induced seizure severity, epileptogenesis, functional impairment, and brain pathology in rodent models.[8-10,12,13,30-32] Previous studies also showed the importance of simultaneous administration of antiseizure medications, as opposed to sequential drug administration, in reducing cholinergic-induced seizure severity.[33] The inclusion of a third antiseizure medication, valproate, in combination with ketamine and midazolam further reduces seizure severity, epileptogenesis, and neuronal loss following soman exposure.[3,10,11,21] Our current findings report on the benefits of phenobarbital when used as an adjunct to midazolam and that inclusion of ketamine provides additional protection against soman-induced SE, similar to our previous observations with combination therapy of valproate with ketamine and midazolam. Phenobarbital monotherapy or dual therapy of phenobarbital (and midazolam or ketamine) improved outcome, but the greatest benefit of treatment was in the group that received the triple therapy of phenobarbital, ketamine, and midazolam.

Although early administration of benzodiazepines is effective at reducing cholinergic-induced seizure,[34] the therapeutic window for terminating seizure is limited to 40 minutes or less after seizure onset,[27,35-37] and treatment success with benzodiazepines is inversely correlated with seizure duration.[35] In the weeks after exposure, many rats and mice develop SRS.[24,26,38,39] Since the time spent in seizure correlates with neuronal cell damage,[5] prompt control of seizure activity is critical. Phenobarbital reduced time in the initial seizure when administered as a monotherapy, as a dual therapy (with ketamine or midazolam) or as a triple therapy (with ketamine and midazolam) 40 minutes after soman-induced seizure compared

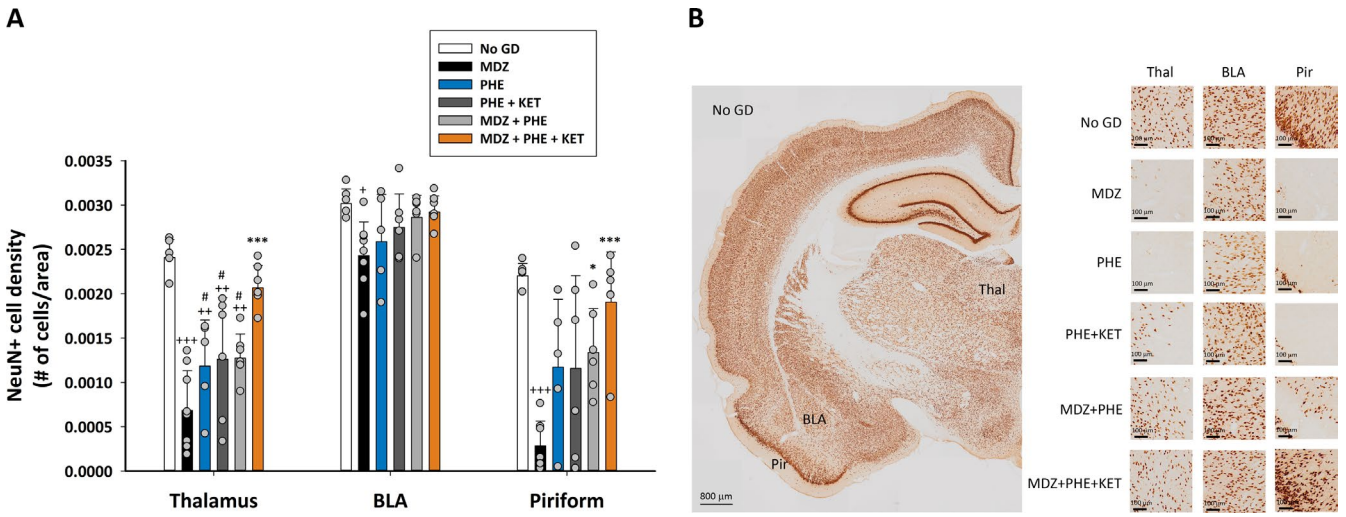

FIGURE 5 Benefit of combination triple therapy with the antiseizure medications phenobarbital, ketamine, and midazolam against soman (GD)-induced neuronal loss in rats. Brain samples collected 2 weeks after GD exposure were processed with NeuN immunohistochemistry to label viable neurons. A, Rats treated with monotherapy of midazolam (MDZ) or phenobarbital (PHE) or with dual therapy of phenobarbital and ketamine (PHE + KET) or midazolam and phenobarbital (MDZ + PHE) 40 min after GD-induced seizure had significant loss of neurons in the medial thalamus compared with no agent controls (No GD). In contrast, rats that were treated with the triple antiseizure medications midazolam, phenobarbital, and ketamine (MDZ + PHE + KET) were not different from No GD and had significantly more neurons in the medial thalamus compared to rats treated with MDZ monotherapy. Rats treated with MDZ also had significantly fewer neurons in the piriform cortex compared with No GD rats, while those treated with PHE or MDZ + PHE were not different from No GD. In addition, rats treated with MDZ + PHE or those treated with MDZ + PHE + KET had more neurons in the piriform cortex compared to rats treated with MDZ. In the basolateral amygdala (BLA), MDZ-treated rats had fewer neurons compared with control. $+P < .05$; $++P < .01$; and $+++P < .001$ compared with No GD; $*P < .05$; $**P < .01$; and $***P < .001$ compared with MDZ monotherapy; $\#P < .05$ compared with MDZ + PHE + KET triple therapy. B, Hemicoronal image of No GD animal (left) and representative images of treatment groups (right) illustrate GD-induced loss of neurons in the thalamus (Thal), BLA, and piriform cortex (Pir) of rats treated with MDZ monotherapy, with neuronal protection provided in GD-exposed animals treated with MDZ + PHE + KET triple therapy

with midazolam monotherapy. In agreement with the antiseizure effects of phenobarbital against soman exposure, phenobarbital at this dose (30 mg/kg) was also effective against kainate-induced seizure and brain damage.[40] In the current study, behavioral seizure as measured on the Racine scale was primarily reduced in animals that received triple therapy; these animals had significantly less seizure than those treated with monotherapy (midazolam or phenobarbital) or dual therapy (phenobarbital plus midazolam). Treatment efficacy of triple therapy to reduce behavioral seizure occurred within 30 minutes of treatment and persisted for the duration of toxic sign monitoring. In addition, treatment with triple therapy (phenobarbital with midazolam and ketamine), but not phenobarbital monotherapy or dual therapy, reduced the number of SRS compared with midazolam monotherapy. No animals that received triple therapy following soman exposure developed SRS compared with 75% of animals that received midazolam monotherapy. In mice exposed to a seizure-inducing dose of soman, a low dose of phenobarbital monotherapy (20 mg/kg) resulted in a delay in onset to SRS but did not reduce neuropathology.[41] Higher doses of phenobarbital as evaluated against DFP in rats[17,18] or combination of lower doses of phenobarbital with other antiseizure medications as used in the current study may be needed to improve neuroprotection. These findings demonstrate the efficacy of combining subanesthetic doses of antiseizure medications in reducing cholinergic-induced SE and epileptogenesis.

Triple therapy with phenobarbital, ketamine, and midazolam also provided significant protection from neuropathological damage following soman-induced seizure. Animals that received the triple therapy had less neurodegeneration illustrated with silver stain in fiber tracts, thalamus, amygdala, hippocampus, and piriform cortex compared with those that received midazolam monotherapy. Rats that received phenobarbital monotherapy or dual therapy of phenobarbital with ketamine or midazolam were not significantly different from rats that received midazolam monotherapy. Using antibodies against a neuron-specific marker (NeuN) and a GABAergic marker (GAD67), we visualized the overall populations of mature neurons and inhibitory interneurons to determine whether treatment with combination antiseizure medications would reduce soman-induced neuronal loss compared with midazolam

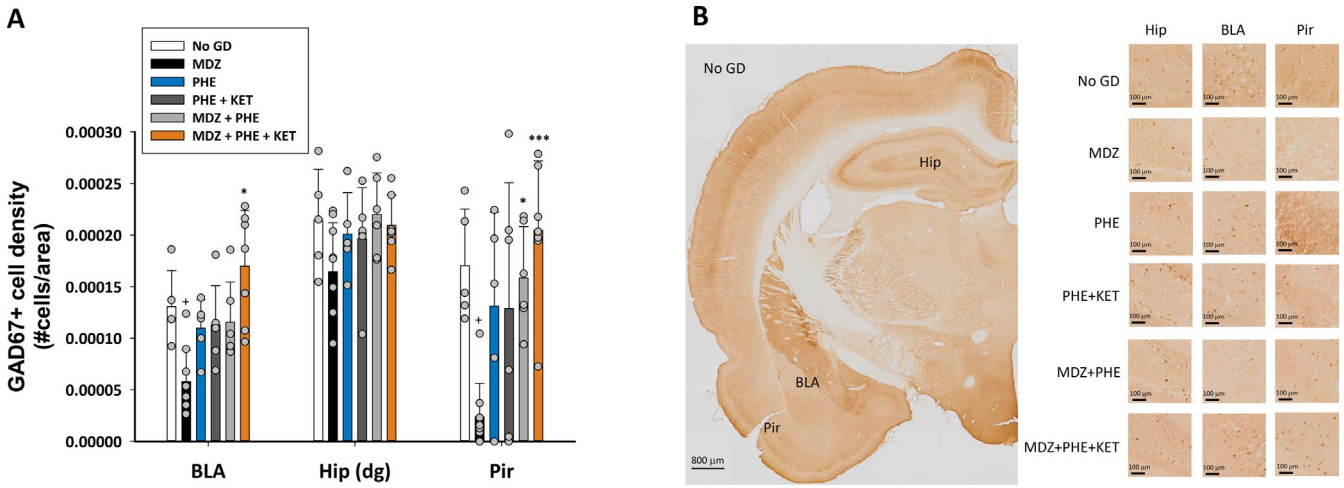

FIGURE 6 Benefit of combination triple therapy with the antiseizure medications phenobarbital, ketamine, and midazolam against soman (GD)-induced loss of GABA interneurons in rats. Brain samples were collected at 2 weeks after GD exposure and processed with GAD67 immunohistochemistry to label GABA interneurons. A, Rats treated with monotherapy of midazolam (MDZ) had significant reduction in GAD67+ cells in the amygdala and piriform cortex compared with no agent controls (No GD). In contrast, rats that were treated with the triple antiseizure medications of midazolam, phenobarbital, and ketamine (MDZ + PHE + KET) were not different from No GD and had significantly greater GAD67+ cells in the amygdala and piriform cortex compared to rats treated with MDZ monotherapy. In the piriform cortex, rats treated with phenobarbital and midazolam dual therapy (MDZ + PHE) or MDZ + PHE + KET triple therapy had protection of GAD67+ cells compared with MDZ monotherapy. +$P < .05$ compared with No GD; *$P < .05$ and ***$P < .001$ compared with MDZ; B, Representative images of GAD67+ cells in the hippocampus (Hip), basolateral amygdala (BLA), and piriform cortex (Pir) of rats treated with MDZ monotherapy, phenobarbital (PHE) monotherapy, phenobarbital and ketamine (PHE + KET) dual therapy, MDZ + PHE dual therapy, or MDZ + PHE + KET triple therapy

monotherapy. Animals treated with delayed midazolam had extensive neuronal loss as evidenced by reduced NeuN+ cells in the thalamus, amygdala, and piriform cortex. Treatment with triple therapy of phenobarbital, ketamine, and midazolam protected neurons in the thalamus and piriform cortex, while treatment with phenobarbital in combination with midazolam reduced loss of neurons in the piriform cortex. In agreement with our findings of reduced neuronal loss 2 weeks after soman exposure in rats treated with phenobarbital (30 mg/kg, IP) as an adjunct to midazolam (3 mg/kg, IP), higher doses of phenobarbital (56 or 100 mg/kg, IP) in combination with midazolam (1.8 mg/kg, IM) reduced cell death (FluoroJade B) measured 24 hours after soman exposure.[16] In addition, rats treated with phenobarbital (50 mg/kg) 30 minutes prior to pilocarpine exposure had reduced brain damage shown with cresyl violet.[42] The current findings show added protection of neurons when ketamine is used as combination with phenobarbital and midazolam.

GABA interneurons play a crucial role in excitatory and seizure activity.[43-45] An antibody against GAD67, commonly used as a selective marker of neurons that synthesize the neurotransmitter GABA,[46,47] was used to quantify loss of inhibitory neurons after soman exposure. Our laboratory previously reported that delayed benzodiazepine treatment of soman exposure in rats led to loss of GAD67+ cells in the amygdala, vital for seizure regulation, and in the piriform cortex, implicated in seizure propagation.[29] The loss of GABA interneurons in these regions following soman exposure may contribute to the development of epileptogenesis. We currently report that midazolam-treated rats had significant loss of GAD67+ cells in the amygdala and piriform cortex and that triple therapy with phenobarbital, ketamine, and midazolam attenuated loss. In the piriform cortex, phenobarbital dual therapy with midazolam also reduced the loss of GAD67+ cells following soman exposure. Protection from loss of GABA interneurons by phenobarbital in combination with midazolam and ketamine may reduce the potential to develop epileptogenesis.

Following exposure to a seizure-inducing dose of soman, animals develop a biphasic change in motor activity, with reduced motor activity and startle response in the first 24 hours followed by increased activity and startle and perseverative behaviors in the weeks after exposure,[26,48,49] with increased hyperactivity predominant during the dark cycle when rodents are most active. Soman-exposed animals also are hyper-reactive, to include biting during handling and exaggerated startle to air-puff.[50] In the current study, triple therapy with phenobarbital, ketamine, and midazolam prevented the development of hyperactivity following soman exposure, while rats treated with midazolam monotherapy had increased activity by postexposure day 9.

In sum, our current findings suggest that the antiseizure medication phenobarbital is more beneficial than midazolam monotherapy at treating soman-induced SE when treatment is delayed and that the addition of ketamine and midazolam provided increased benefit over that of monotherapy or dual therapy in preventing epileptogenesis. In addition, although dual therapy of phenobarbital with midazolam provided some neuroprotection in the piriform cortex, triple therapy with phenobarbital, ketamine, and midazolam protected additional brain regions from excitatory and inhibitory neuronal loss.

ACKNOWLEDGMENTS

This research was supported by a grant from the National Institute of Neurological Disorders and Stroke [U01 NS074926] by Merit Review Award # I01 BX000273-07 from the United States Department of Veterans Affairs (Biomedical Laboratory Research and Development Service) to Dr Claude Wasterlain. The authors acknowledge Ms Erica Kundrick, Mr Sean O'Brien, Ms Celinia Ondeck, Mr Michael Pham, Ms Katie Walker, Dr Mark Moffett, and Dr Matthew Rice for assistance with animal handling, agent exposures, data collection, and/or data compilation.

CONFLICT OF INTEREST

Jerome Niquet and Claude Wasterlain have a patent on polytherapy of cholinergic seizures (UC Case No. 2012-172-2). Other authors have no conflict of interest to disclose.

ETHICAL APPROVAL

We confirm that we have read the Journal's position on issues involved in the ethical publication and affirm that this report is consistent with those guidelines.

ORCID

Lucille A. Lumley https://orcid.org/0000-0003-4162-8817

REFERENCES

1. Newmark J. Therapy for acute nerve agent poisoning: an update. Neurol Clin Pract. 2019;9:337–42.
2. Niquet J, Baldwin R, Suchomelova L, Lumley L, Naylor D, Eavey R, et al. Benzodiazepine-refractory status epilepticus: pathophysiology and principles of treatment. Ann N Y Acad Sci. 2016;1378:166–73.
3. Niquet J, Lumley L, Baldwin R, Rossetti F, Suchomelova L, Naylor D, et al. Rational polytherapy in the treatment of cholinergic seizures. Neurobiol Dis. 2020;133:104537.
4. Shih TM, Koviak TA, Capacio BR. Anticonvulsants for poisoning by the organophosphorus compound soman: pharmacological mechanisms. Neurosci Biobehav Rev. 1991;15:349–62.
5. Shih TM, Duniho SM, McDonough JH. Control of nerve agent-induced seizures is critical for neuroprotection and survival. Toxicol Appl Pharmacol. 2003;188:69–80.
6. Naylor DE, Liu H, Wasterlain CG. Trafficking of GABA(A) receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. J Neurosci. 2005;25:7724–33.
7. Naylor DE, Liu H, Niquet J, Wasterlain CG. Rapid surface accumulation of NMDA receptors increases glutamatergic excitation during status epilepticus. Neurobiol Dis. 2013;54:225–38.
8. Martin BS, Kapur J. A combination of ketamine and diazepam synergistically controls refractory status epilepticus induced by cholinergic stimulation. Epilepsia. 2008;49:248–55.
9. Niquet J, Baldwin R, Norman K, Suchomelova L, Lumley L, Wasterlain CG. Midazolam-ketamine dual therapy stops cholinergic status epilepticus and reduces Morris water maze deficits. Epilepsia. 2016;57:1406–15.
10. Lumley L, Niquet J, Marrero-Rosado B, Schultz M, Rossetti F, de Araujo Furtado M, et al. Treatment of acetylcholinesterase inhibitor-induced seizures with polytherapy targeting GABA and glutamate receptors. Neuropharmacology. 2021;185:108444.
11. Lumley LA, Rossetti F, de Araujo Furtado M, Marrero-Rosado B, Schultz CR, Schultz MK, et al. Dataset of EEG power integral, spontaneous recurrent seizure and behavioral responses following combination drug therapy in soman-exposed rats. Data Brief. 2019;27:104629.
12. Marrero-Rosado BM, de Araujo Furtado M, Kundrick ER, Walker KA, Stone MF, Schultz CR, et al. Ketamine as adjunct to midazolam treatment following soman-induced status epilepticus reduces seizure severity, epileptogenesis, and brain pathology in plasma carboxylesterase knockout mice. Epilepsy Behav. 2020;111:107229.
13. Marrero-Rosado BM, Stone MF, de Araujo FM, Schultz CR, Cadieux CL, Lumley LA. Novel genetically modified mouse model to assess soman-induced toxicity and medical countermeasure efficacy: human acetylcholinesterase knock-in serum carboxylesterase knockout mice. Int J Mol Sci. 2021;22:1893.
14. Twyman RE, Rogers CJ, Macdonald RL. Differential regulation of gamma-aminobutyric acid receptor channels by diazepam and phenobarbital. Ann Neurol. 1989;25:213–20.
15. Nardou R, Yamamoto S, Bhar A, Burnashev N, Ben-Ari Y, Khalilov I. Phenobarbital but not Diazepam Reduces AMPA/kainate receptor mediated currents and exerts opposite actions on initial seizures in the neonatal rat hippocampus. Front Cell Neurosci. 2011;5:16.
16. Jackson C, Ardinger C, Winter KM, McDonough JH, McCarren HS. Validating a model of benzodiazepine refractory nerve agent-induced status epilepticus by evaluating the anticonvulsant and neuroprotective effects of scopolamine, memantine, and phenobarbital. J Pharmacol Toxicol Methods. 2019;97:1–12.
17. Reddy DS, Perumal D, Golub V, Habib A, Kuruba R, Wu X. Phenobarbital as alternate anticonvulsant for organophosphate-induced benzodiazepine-refractory status epilepticus and neuronal injury. Epilepsia Open. 2020;5:198–212.
18. Spampanato J, Bealer SL, Smolik M, Dudek FE. Delayed adjunctive treatment of organophosphate-induced status epilepticus in rats with phenobarbital, memantine, or dexmedetomidine. J Pharmacol Exp Ther. 2020;375:59–68.
19. Mehta AK, Ticku MK. Comparison of anticonvulsant effect of pentobarbital and phenobarbital against seizures induced

by maximal electroshock and picrotoxin in rats. Pharmacol Biochem Behav. 1986;25:1059–65.
20. Prasad A, Williamson JM, Bertram EH. Phenobarbital and MK-801, but not phenytoin, improve the long-term outcome of status epilepticus. Ann Neurol. 2002;51:175–81.
21. Niquet J, Lumley L, Baldwin R, Rossetti F, Schultz M, de Araujo Furtado M, et al. Early polytherapy for benzodiazepine-refractory status epilepticus. Epilepsy Behav. 2019;101:106367.
22. Nissinen J, Lukasiuk K, Pitkänen A. Is mossy fiber sprouting present at the time of the first spontaneous seizures in rat experimental temporal lobe epilepsy? Hippocampus. 2001;11:299–310.
23. Racine RJ. Modification of seizure activity by electrical stimulation: II. Motor seizure. Electroencephalogr Clin Neurophysiol. 1972;32:281–94.
24. de Araujo FM, Zheng A, Sedigh-Sarvestani M, Lumley L, Lichtenstein S, Yourick D. Analyzing large data sets acquired through telemetry from rats exposed to organophosphorous compounds: an EEG study. J Neurosci Methods. 2009;184:176–83.
25. Moffett MC, Schultz MK, Schwartz JE, Stone MF, Lumley LA. Impaired auditory and contextual fear conditioning in soman-exposed rats. Pharmacol Biochem Behav. 2011;98:120–9.
26. Schultz MK, Wright L, de Araujo Furtado M, Stone MF, Moffett MC, Kelley NR, et al. Caramiphen edisylate as adjunct to standard therapy attenuates soman-induced seizures and cognitive deficits in rats. Neurotoxicol Teratol. 2014;44:89–104.
27. Schultz MK, Wright L, Stone MF, Schwartz JE, Kelley NR, Moffett MC, et al. The anticholinergic and antiglutamatergic drug caramiphen reduces seizure duration in soman-exposed rats: synergism with the benzodiazepine diazepam. Toxicol Appl Pharmacol. 2012;259:376–86.
28. Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem. 1981;29:577–80.
29. Marrero-Rosado B, Rossetti F, Rice MW, Moffett MC, Lee RB, Stone MF, et al. Age-related susceptibility to epileptogenesis and neuronal loss in male fischer rats exposed to soman and treated with medical countermeasures. Toxicol Sci. 2018;164:142–52.
30. Ballough G, Newmark J, Levine E, Filbert M. Neuroprotection as a treatment for nerve agent survivors. In: Tuorinsky SD, Medical aspects of chemical warfare. Washington, D.C.: Department of the Army, Office of The Surgeon General and Borden Institute, 2008. p. 221–42.
31. Dorandeu F, Barbier L, Dhote F, Testylier G, Carpentier P. Ketamine combinations for the field treatment of soman-induced self-sustaining status epilepticus. Review of current data and perspectives. Chem Biol Interact. 2013;203:154–9.
32. Lewine JD, Weber W, Gigliotti A, McDonald JD, Doyle-Eisele M, Bangera N, et al. Addition of ketamine to standard-of-care countermeasures for acute organophosphate poisoning improves neurobiological outcomes. Neurotoxicology. 2018;69:37–46.
33. Niquet J, Baldwin R, Norman K, Suchomelova L, Lumley L, Wasterlain CG. Simultaneous triple therapy for the treatment of status epilepticus. Neurobiol Dis. 2017;104:41–9.
34. Lipp JA. Effect of diazepam upon soman-induced seizure activity and convulsions. Electroencephalogr Clin Neurophysiol. 1972;32:557–60.
35. McDonough JH Jr, Dochterman LW, Smith CD, Shih TM. Protection against nerve agent-induced neuropathology, but not cardiac pathology, is associated with the anticonvulsant action of drug treatment. Neurotoxicology. 1995;16:123–32.
36. McDonough JH Jr, Shih TM. Pharmacological modulation of soman-induced seizures. Neurosci Biobehav Rev. 1993;17:203–15.
37. Shih T, McDonough JH Jr, Koplovitz I. Anticonvulsants for soman-induced seizure activity. J Biomed Sci. 1999;6:86–96.
38. Kundrick E, Marrero-Rosado B, Stone M, Schultz C, Walker K, Lee-Stubbs RB, et al. Delayed midazolam dose effects against soman in male and female plasma carboxylesterase knockout mice. Ann N Y Acad Sci. 2020;1479:94–107.
39. Marrero-Rosado B, Araujo Furtado M, Schultz CR, Stone M, Kundrick E, Walker K, et al. Soman-induced status epilepticus, epileptogenesis, and neuropathology in carboxylesterase knockout mice treated with midazolam. Epilepsia. 2018;59:2206–18.
40. Diaz-Ruiz A, Mendez-Armenta M, Galván-Arzate S, Manjarrez J, Nava-Ruiz C, Santander I, et al. Antioxidant, anticonvulsive and neuroprotective effects of dapsone and phenobarbital against kainic acid-induced damage in rats. Neurochem Res. 2013;38:1819–27.
41. McCarren HS, Eisen MR, Nguyen DL, Dubée PB, Ardinger CE, Dunn EN, et al. Characterization and treatment of spontaneous recurrent seizures following nerve agent-induced status epilepticus in mice. Epilepsy Res. 2020;162:106320.
42. Turski WA, Cavalheiro EA, Coimbra C, da Penha BM, Ikonomidou-Turski C, Turski L. Only certain antiepileptic drugs prevent seizures induced by pilocarpine. Brain Res. 1987;434:281–305.
43. Botterill JJ, Nogovitsyn N, Caruncho HJ, Kalynchuk LE. Selective plasticity of hippocampal GABAergic interneuron populations following kindling of different brain regions. J Comp Neurol. 2017;525:389–406.
44. During MJ, Spencer DD. Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. Lancet. 1993;341:1607–10.
45. Shetty AK, Upadhya D. GABA-ergic cell therapy for epilepsy: advances, limitations and challenges. Neurosci Biobehav Rev. 2016;62:35–47.
46. Almeida-Suhett CP, Prager EM, Pidoplichko V, Figueiredo TH, Marini AM, Li Z, et al. GABAergic interneuronal loss and reduced inhibitory synaptic transmission in the hippocampal CA1 region after mild traumatic brain injury. Exp Neurol. 2015;273:11–23.
47. Di Maio R, Cannon JR, Greenamyre JT. Post-status epilepticus treatment with the cannabinoid agonist WIN 55,212–2 prevents chronic epileptic hippocampal damage in rats. Neurobiol Dis. 2015;73:356–65.
48. Haggerty GC, Kurtz PJ, Armstrong RD. Duration and intensity of behavioral change after sublethal exposure to soman in rats. Neurobehav Toxicol Teratol. 1986;8:695–702.
49. Langston JL, Wright LK, Connis N, Lumley LA. Characterizing the behavioral effects of nerve agent-induced seizure activity

in rats: increased startle reactivity and perseverative behavior. Pharmacol Biochem Behav. 2012;100:382–91.
50. Shih TM, Lenz DE, Maxwell DM. Effects of repeated injection of sublethal doses of soman on behavior and on brain acetylcholine and choline concentrations in the rat. Psychopharmacology. 1990;101:489–96.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Lumley LA, Marrero-Rosado B, Rossetti F, et al. Combination of antiseizure medications phenobarbital, ketamine, and midazolam reduces soman-induced epileptogenesis and brain pathology in rats. Epilepsia Open. 2021;6:757–769. https://doi.org/10.1002/epi4.12552