Neurotoxicology 79 (2020) 58–66

Contents lists available at ScienceDirect

Neurotoxicology

journal homepage: www.elsevier.com/locate/neuro

Evaluation of fosphenytoin, levetiracetam, and propofol as treatments for nerve agent-induced seizures in pediatric and adult rats

Emily N. Dunn[a], Liana M. Matson[a], Kari M. Haines[a], Kimberly A. Whitten[b], Robyn B. Lee-Stubbs[c], Kyle E. Berger[a], Hilary S. McCarren[a], Cherish E. Ardinger[a], Cecelia E. Jackson Piercy[a], Stephanie M. Miller-Smith[a], John H. McDonough[a,*]

[a] Neuroscience Branch, US Army Medical Research Institute of Chemical Defense, 8350 Ricketts Point Road, Aberdeen Proving Ground, MD, 21010, USA
[b] Comparative Pathology Department, US Army Medical Research Institute of Chemical Defense, 8350 Ricketts Point Road, Aberdeen Proving Ground, MD, 21010, USA
[c] Office of the Commander, US Army Medical Research Institute of Chemical Defense, 8350 Ricketts Point Road, Aberdeen Proving Ground, MD, 21010, USA

ARTICLE INFO

Keywords:
Status epilepticus
Nerve agent
Sarin
VX
Pediatric rats
Sex differences
Age differences
Anticonvulsants
Chemical countermeasures

ABSTRACT

Multiple recent instances of nerve agent (NA) exposure in civilian populations have occurred, resulting in a variety of negative effects and lethality in both adult and pediatric populations. Seizures are a prominent effect of NAs that can result in neurological damage and contribute to their lethality. Current anticonvulsant treatments for NAs are approved for adults, but no approved pediatric treatments exist. Further, the vast majority of NA-related research in animals has been conducted in adult male subjects. There is a need for research that includes female and pediatric populations in testing. In this project, adult and pediatric male and female rats were challenged with sarin or VX and then treated with fosphenytoin, levetiracetam, or propofol. In this study, fosphenytoin and levetiracetam failed to terminate seizure activity when animals were treated 5 min after seizure onset. Propofol was effective, exhibiting high efficacy and potency for terminating seizure activity quickly in pediatric and adult animals, suggesting it may be an effective anticonvulsant for NA-induced seizures in pediatric populations.

1. Introduction

Nerve agents (NA) are organophosphorus compounds that inhibit acetylcholinesterase, causing a buildup of acetylcholine that can lead to salivation, lacrimation, convulsions, status epilepticus (SE), and even death (King and Aaron, 2015). Thousands of men, women, and children have fallen victim to NAs. SE seizures can result in neurotoxicity and lethality in all age groups and are a prominent effect of NAs. Alarmingly, there are no countermeasures for NA-induced seizures in children that are currently approved. Children are especially vulnerable to the effects of NA (Hamele et al., 2014) and are more likely than adults to experience life threatening CNS effects (Rotenberg and Newmark, 2003). Experts in the field of emergency preparedness have determined that age-specific therapies are necessary to protect children against potential mass chemical exposures (American Academy of Pediatrics, 2000; Shirm et al., 2007; Henretig, 2009). Therefore, it is vital that treatment and dosing be developed for both sexes as well as for pediatric population (American Academy of Pediatrics, 2000; Shirm et al., 2007). This study evaluated the efficacy of three anticonvulsants for terminating NA-induced seizures in post-natal day (PND) 21, 28, and 70 male and female rats. In this experiment, animals were administered fosphenytoin, levetiracetam, or propofol to evaluate drug efficacy for terminating NA-induced SE following sarin or VX exposure. Both sarin and VX NAs were used because they are representative of the two main types of NAs. Sarin is slightly less volatile than water and is considered by the military as a short-term vapor hazard; VX has low volatility and is considered as a persistent percutaneous hazard. While both agents inhibit acetylcholinesterase and can elicit seizures, the onset of toxic signs and seizures is substantially more rapid with sarin than with VX when given at equitoxic doses (Shih et al., 2003).

Scholl and colleagues (Scholl et al., 2018) recently developed an animal model using electrographic (EEG) monitoring in pediatric rats. The authors observed that NAs and organophosphates (OPs) caused SE in PND21 and PND28 rats comparable to that observed in adult rats (PND70), but did not cause SE in PND14 rats. PND28 but not PND21 rats also exhibited similar neuropathology to that observed in adult rats. This information is vital for evaluating the efficacy of NA countermeasures in pediatric populations. Furthermore, this model is used in

* Corresponding author.
E-mail address: john.h.mcdonough2.civ@mail.mil (J.H. McDonough).

https://doi.org/10.1016/j.neuro.2020.03.007
Received 6 December 2019; Received in revised form 21 February 2020; Accepted 14 March 2020
Available online 24 March 2020
0161-813X/

the current study to evaluate second- and third-line anticonvulsants to treat NA-induced seizures in rat adults and pups.

Benzodiazepines are generally considered the first-line treatment for SE (Glauser et al., 2016; Brophy et al., 2012). Specifically, midazolam, lorazepam and diazepam are suggested as first-line treatments in both pre-hospital care and hospital settings for SE. Currently, midazolam is under consideration by the Department of Defense to replace diazepam as a first-line treatment for NA-induced seizures. In rats, Matson and colleagues (Matson et al., 2019) demonstrated that both diazepam and midazolam are effective at terminating NA-induced seizures in both male and female, adult and pediatric rats. Unfortunately, the effectiveness of benzodiazepines is limited with increased time of administration following seizure onset, and they are not as effective 40 min post-onset in rodents (McDonough et al., 2010). Therefore, it is critical to identify additional effective adjunctive or standalone anticonvulsants for treating NA-induced seizures. The Neurocritical Care Society suggests fosphenytoin and levetiracetam among the drugs to be considered as second-line treatments for pediatric SE (Brophy et al., 2012). Propofol is currently being utilized as a refractory status epilepticus (RSE) treatment option if first- or second-line anticonvulsants do not control seizures (Brophy et al., 2012; Loddenkemper and Goodkin, 2011). Although most of the described anticonvulsants have been tested in other seizure models, there are no data regarding their efficacy for terminating NA-induced seizures. Additionally, data are limited on the efficacy of second-line anticonvulsants for terminating NA-induced seizures in female populations.

Fosphenytoin, levetiracetam, and propofol all have differing mechanisms of action for controlling seizures. Fosphenytoin is a water soluble prodrug formulation of phenytoin, and its mechanism of action is largely due to inhibition of voltage-dependent sodium channel (Alford et al., 2015). Adverse effects can include bradyarrhythmias, hypotension, and local tissue necrosis (Abend and Loddenkemper, 2014). The mechanism of action of levetiracetam is not fully understood. The drug binds to synaptic vesicle protein 2 (SV2), which has three isoforms, 2A, 2B, and 2C. Specifically, SV2A may regulate synaptic release mechanisms, by aiding in the control of exocytosis of vesicles containing neurotransmitters (Deshpande and Delorenzo, 2014). Levetiracetam is effective in managing generalized convulsive SE (Trinka et al., 2015; Grover et al., 2016; Misra et al., 2012) and has benefits such as rapid absorption and lack of reported cardio-toxic effects when administered at appropriate doses (Ruegg et al., 2008; Wright et al., 2013). Levetiracetam is also very successful as a second-line treatment when used to suppress drug-induced seizures, with few adverse side effects (Ruegg et al., 2008; Lee et al., 2018). Disadvantages and adverse effects include irritability and sedation (Abend and Loddenkemper, 2014). Propofol increases the strength of GABA-ergic neurotransmission. It reversibly inhibits excitation at NMDA receptors. At high enough concentrations, propofol also stimulates the production and release of nitric oxide, protecting against glutamate-induced toxicity. Propofol is a widely utilized IV anesthetic. It is capable of terminating SE at sub-anesthetic doses in both animals (Holtkamp et al., 2001) and humans (Stecker et al., 1998). Propofol has a short half-life, allowing for easy titration and a rapid awakening after drug cessation (Alford et al., 2015) which is beneficial in a hospital setting. While propofol is effective for treating seizures, it poses a risk of respiratory depression (Parviainen et al., 2007; Cantrell et al., 2016). There is also a risk of developing propofol-related infusion syndrome (PRIS) with prolonged infusions in both adult and pediatric populations (Alford et al., 2015). Investigation of anticonvulsants with differing mechanisms may yield information regarding effective treatments for NA-induced seizures.

Anticonvulsant treatments for NA-induced SE should display high efficacy, high potency, fast seizure termination latency, long-term treatment success, and bioavailability, and should prevent brain damage caused by the seizure activity. In this experiment, the second-line anticonvulsants fosphenytoin, levetiracetam, and propofol, were evaluated for effectiveness to treat NA-induced seizures using an established pediatric rat model (Scholl et al., 2018). The calculated median effective anticonvulsant dose values (ED50; the dose stopping seizures in 50 % of the animals), seizure termination latency, and neuropathology data were included in the evaluation of propofol. Due to the inefficacy of fosphenytoin and levetiracetam, ED50 values could not be determined for these drugs; however, lethality and neuropathology data are reported for both drugs.

2. Methods

The below methods are the same as were used in Matson et al. (2019), with the exception of specific information for anticonvulsant formulation and dosing.

2.1. Animals

Pregnant Sprague-Dawley rats (13–15 days gestation) were received from Charles River (Raleigh, NC); pups were delivered in the animal facility approximately 1 week following arrival of the pregnant female. Litters were culled to 8 or 10 pups to maintain consistency for weights. Animals were kept on a 12 h light-dark cycle and had access to food and water *ad libitum*. All surgical procedures and experiments were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011) and the Animal Welfare Act of 1966 (P.L. 89-544) as amended.

2.2. EEG implantation

Rats were implanted with stainless steel electroencephalographic (EEG) 3-channel electrodes and headpieces (Plastics One, Roanoke, VA). All animals received 1 mg/kg meloxicam subcutaneously (SC) 15 min prior to surgery or 0.03 mg/kg buprenorphine (SC) immediately following surgery for analgesia. At PNDs 19 and 25 or 26 for pups and PND63 or 64 for adults, the animals were anesthetized with isoflurane (5% induction; 0.5–3.0 % maintenance, with oxygen) and placed into a stereotaxic frame. The skull was exposed with a midline incision. Small burr holes were drilled in the skull using a hand drill equipped with a stop set at $\sim 0.5-1.5$ mm to prevent penetration into the brain with the exact depth based on the age of the animal. Sterilized stainless steel screw electrodes served as cortical EEG leads, and were placed in the holes. The leads were connected to a miniature connector plug via wires already soldered to the heads of the screws. PND21 and PND28 animals were implanted with smaller EEG electrodes. Two small holes were drilled and screws were placed in the holes. These simply served as anchor screws, as wires connected to the headpiece were looped at the end and touched the skull in comparable areas to those of the PND70 rats. The screws and plug were held in place using either acrylic dental cement or glass ionomer dental cement for both adults and rat pups. The skin incision around the head mount was sutured. Immediately after surgery all animals received warmed Ringer's solution. Animals of all age groups were placed in a heated recovery chamber, and were returned to single-housed home cages once they were alert and ambulating.

2.3. Test compounds

Stocks of sarin ((RS)-propan-2-yl methylphosphonofluoridate) and VX (O-ethyl S-diisopropylaminomethyl methylphosphonothiolate), both from the USAMRICD and > 97 % pure by phosphorus NMR analysis, were aliquoted in saline and stored at −80 °C. NAs were thawed on experiment days and remained on ice during the experiment. Pralidoxime Cl (2PAM; USP) was purchased from ScienceLabs (Houston, TX), atropine methyl nitrate (AMN; > 98 % purity) was purchased from Sigma-Aldrich (St. Louis, MO), and atropine sulfate

($ATSO_4$; USP) was purchased from Sparhawk Labs (Lenexa, KA).

Propofol and fosphenytoin were tested in 1/8 $\log_{10}$ steps while levetriacetam was tested in 1/4 $\log_{10}$ steps using the up and down testing method which is described in further detail below (Dixon and Massey, 1983). Initial doses of 18 mg/kg (pediatric animals) and 75 mg/kg (adults) of propofol (10 mg/mL) were used in this study. Propofol was purchased from Activas Pharma, Inc. (Parsippany, NJ). Starting doses of levetiracetam (500 mg/mL) were 560 mg/kg (PND28 s) or 320 mg/kg (adults). Levetiracetam, mixed with sterile H_2O, was purchased from Tocris (Bristol BS11 OQL, UK) or Sigma-Aldrich (St. Louis, MO). The initial doses utilized for fosphenytoin (75 mg/mL) were 48 mg/kg (32 PE) or 203 mg/kg (135 PE) (start doses of fosphenytoin increased once the experiment began and treatments were not successful at the lower dose). It should be noted when using fosphenytoin, the amount of drug used is always expressed in phenytoin sodium equivalents, or PE. Thus, 1.5 mg of fosphenytoin sodium is equivalent to 1 mg of phenytoin sodium after administration (McDonough et al., 2004). As such, for the rest of the paper, all doses of fosphenytoin will be expressed in PE. Fosphenytoin was purchased from Parke-Davis (Morris Plains, NJ).

2.4. Procedure

Although dependent on litter characteristics, attempts were made to test an equal number of males and females as well as age groups for each test day. On PND21, PND28 or PND70 the animals were pretreated with a 25 mg/kg dose of 2-PAM Cl and then challenged SC 20 min later with the NAs sarin or VX. These rat PND ages represent human equivalent ages of 3–6 years old, 8–10 years old and 15–18 years old, respectively, depending upon the method used to calculate equivalence (Sengupta, 2013). Doses and concentrations of VX and sarin varied depending on the age group, but were determined in previous experiments to be ideal for eliciting seizures (Scholl et al., 2018; Matson et al., 2019).

Animals administered sarin received an admixture of atropine sulfate ($ATSO_4$; 0.5 mg/kg) and methyl atropine nitrate (AMN; 2 mg/kg) directly after agent exposure; PND21 and PND28 animals given VX received the AMN and $ATSO_4$ at first signs of toxicity; PND70 animals exposed to VX received the AMN immediately after exposure and the $ATSO_4$ at first signs of toxicity. For PND21 and PND28 rats all injections (including NA) were SC; PND70 rats also received NA SC but received 2PAM, $ATSO_4$, and AMN IM due to the larger muscle mass in the adult animals. These treatments significantly reduced the early lethal NA effects without preventing the development of SE. The animals were then monitored for the development of EEG seizure activity using a tethered system. Fig. 1 provides a time-line and summarizes procedural details for all age groups. Five min after the onset of sustained seizure activity the animals were treated intraperitoneally (IP) with a dose of the test compound, which was determined using the Dixon and Massey (1983) up-down experimental method. In the up-down method, the initial testing dose was chosen based on pilot work, and then a succession of doses in 1/8 (propofol, fosphenytoin) or 1/4 (levetiracetam) $\log_{(10)}$ units above and below this starting dose were established as fixed steps between doses. If the initial dose tested in the first animal stopped the seizure, the next lower dose was tested in the second animal; if the initial test dose did not stop the seizure, the next higher dose was tested in the second animal. Testing proceeded in this fashion until 4 reversals occurred. This procedure was followed for each unique age group, sex, NA and drug treatment combination. In the cases of fosphenytoin and levetiracetam, the failure of all the doses tested to control seizures elicited by either NA led to a decision to suspend testing. This is described in more detail in the results. The EEG was then monitored for 4 h after anticonvulsant treatment. If an animal displayed sustained seizure activity at the 4-hr time point, they were candidates for early removal and were euthanized as described below. To be rated as a treatment success all spiking and/or rhythmic waves had to stop within 1 h of drug treatment, and the EEG had to remain free of epileptiform activity for a minimum of 1 h. If the seizure activity was controlled by the treatment, the animals were administered 2 − 5 ml saline or lactated ringer's solution and placed back in their holding room. The animals were then returned to the EEG recording chambers 24 h later for another 30 min EEG recording session. All EEG recordings were done with CED 1902 amplifiers to display and record the EEG signals on a computer with Spike2 software (Cambridge Electronic Design, Ltd., Cambridge, UK).

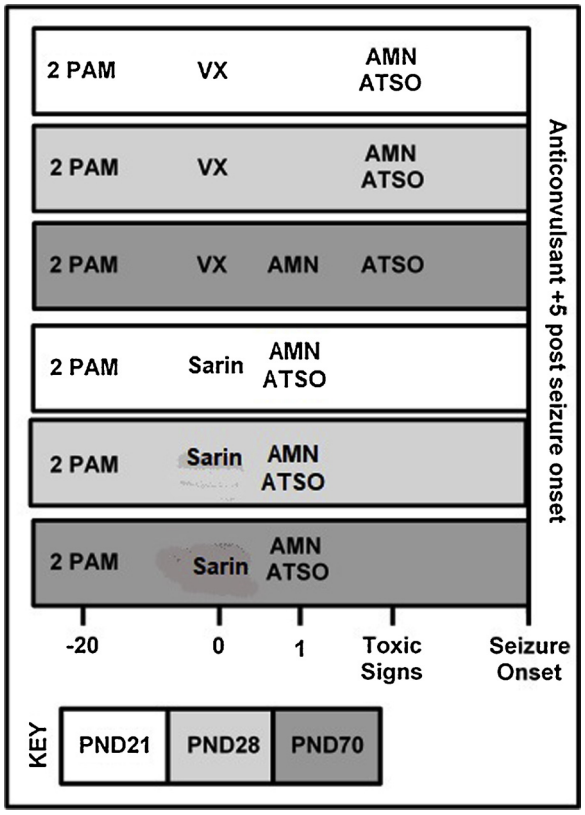

Fig. 1. Description of the test procedure. All animals received 2-PAM prior to nerve agent exposure. All PND21 and PND28 VX exposed animals received AMN and ATSO at the onset of toxic signs; PND70 VX exposed animals received AMN 1 min after VX and ATSO at the onset of toxic signs. All sarin exposed animals received atropine methyl nitrate (AMN) and atropine sulfate (ATSO) 1 min after exposure. Test anticonvulsants were given 5 min after seizure onset.

2.5. Tissue collection

Either at four or 24 h after treatment, rats were anesthetized with 75–100 mg/kg sodium pentobarbital, IP (Vortech Pharmaceuticals, Dearborn, MI) and perfused with 0.9 % phosphate buffered saline (PBS) followed by 4% paraformaldehyde (FD Neurotechnologies, Columbia, MD). Brains were removed and post-fixed in 4% paraformaldehyde. Three paraffin embedded sections were consecutively cut at five microns from Bregma (one at 1 mm, one at -2.64 to -2.76 mm, and one at 5.04–5.16 mm) using a Leica RM225 microtome. Brain tissue was then stained with hematoxylin and eosin (H&E) and scored for damage by a board-certified veterinary pathologist who was unaware of exposure conditions or treatment outcome. The six brain areas that were evaluated and scored for neuronal damage were the lateral cortex, dorsal cortex, piriform cortex, amygdala, thalamus, and hippocampus using a previously defined scale of 0–4 (0, no damage; 1, minimal damage (1–10 %); 2, mild damage (11–25 %); 3, moderate damage (26–45 %); 4, severe damage (> 45 %) (McDonough et al., 1995; Matson et al., 2019). The scores for the six individual areas were summed to yield a total neuropathology score for each animal. Total neuropathology

scores could range from 0 to a maximum score of 24 for an individual animal.

2.6. Data analyses

The formula and tables in Dixon and Massey (1983) were utilized to determine anticonvulsant ED50 values for propofol in the pediatric groups. An ad hoc analysis was conducted on the $\log_{10}$ of the ED50 values to compare the values among age, agent, and sex. To reduce variability in the data, the $\log_{10}$ values of the ED50 s were used in the analysis using JMP 13.1.0 (SAS Institute Inc.) statistical software. A three-factor ANOVA was used with all two-factor interactions. Due to an initial misclassification of animals (i.e., some PND70 animals were initially classified as treatment failures and were later reevaluated and classified as treatment successes), there was an insufficient number of reversals in the adult propofol groups, and the ED50 values for the propofol-treated PND70 groups could therefore not be calculated using the Dixon and Massey (1983) up-down procedure. However, there was sufficient data from all PND70 animals to estimate the ED50 for propofol using a regression analysis with a probit transformation using the IBM SPSS Statistics program (Version 26). Since fosphenytoin and levetiracetam failed to terminate seizures in this experiment, no ED50 value information could be gathered for these two anticonvulsants.

Several doses of propofol were used to treat SE in PND21, 28 and 70 male and female rats. Doses were not used as a factor in estimating the mean seizure termination latencies for the propofol-treated animals. A parametric survival analysis was performed using several different distributions (Weibull, Lognormal, Exponential, Frechet and Loglogistic). The Frechet distribution was determined to be the best fit of the data and used to compare agent, age, sex and all two-factor interactions. Animals that did not experience a seizure termination were considered a treatment failure, given a maximum time of 60 min for the analysis and were treated as censored data in the survival analysis. Further comparisons of age within each agent group with respect to seizure termination time were made using a Product-Limit Survival fit. JMP 13.1.0 (SAS Institute Inc., Cary, NC) statistical software was used for all of these analyses.

Neuropathology scores for propofol-treated animals were categorized and compared based upon whether seizures terminated (treatment success) or did not terminate (treatment failure) following administration of the anticonvulsant. Since the total of the neuropathology scores was categorical in nature and thus not normally distributed, a generalized linear model was used to assess age (PND21, 28, 70), agent (sarin, VX) and seizure termination status (treatment success, treatment failure) differences. Neuropathology scores of animals treated with fosphenytoin or levetiracetam were also examined, but due to the limited number of animals per group, no statistical analyses were conducted on these groups of animals.

3. Results

3.1. Fosphenytoin and levetiracetam

Neither fosphenytoin nor levetiracetam was successful in controlling sarin- or VX-induced seizures in any of the age groups. Animals treated with these drugs typically seized continuously through the day post-treatment (Fig. 2). Across both sexes and all age groups, only 1 of 24 (4%) fosphenytoin-treated animals qualified as a treatment success (Table 1), while the rest were considered failures. Four of 24 (17 %) died post-treatment, all of which were treated at the highest fosphenytoin dose administered (203 mg/kg; 135 PE) in the experiment. When the PND70 male and female age group animals reached the 135 PE dose level of fosphenytoin and there was no evidence of seizure control, two additional male and female animals were tested with each agent and the 135 PE fosphenytoin dose. All were treatment failures. Since there was no evidence of fosphenytoin (76 PE) exerting any anticonvulsant effect in the PND21 or PND28 animals of either sex exposed to either NA, and given the failure of the higher dose in the PND70 s, a decision was made to suspend testing with these younger animals at higher doses. Similarly, across both sexes and all age groups, no animals treated with levetiracetam were considered to be a treatment success (Table 1). Four of 18 (22 %) levetiracetam-treated rats died post-treatment, all four of which were treated with one of the two highest doses (560 mg/kg or 1000 mg/kg) administered in the experiment. Because there was no evidence of treatment success in the PND70 animals at any dose of levetiracetam, only limited testing was performed in younger animals at the 560 mg/kg (PND28, N = 3) or 1000 mg/kg (PND21, N = 1; PND28, N = 3) dose, again with no evidence of an anticonvulsant effect in any animal.

3.2. Propofol

Propofol was highly effective in controlling NA seizures in all age groups. EEG examples of successful seizure control with propofol in all age groups are displayed in Fig. 3. When a dose was effective in terminating the seizure the animal was initially sedated and this could last several hours, but most animals that were successfully treated had regained the ability to right themselves by the end of the initial recording session. Comparison of the anticonvulsant ED50 s showed no significant two-factor interactions. However, a significant difference in ED50 s was observed between age groups. The PND28 animals had a significantly higher mean ED50 than the PND21 animals, $p < 0.05$ (PND28 ED50 value: 26.72 mg/kg; PND21 ED50 value: 11.11 mg/kg). No other significant effects of either NA or sex were observed. Due to limited data for PND70 animals, it was not possible to estimate ED50 s for each sex by agent group. However, since analysis of PND21 and PND28 propofol anticonvulsant ED50 s with sarin and VX did not show a significant sex or agent difference, it was assumed that the same would be true for PND70 animals. Therefore, the data was combined across sex and agent in order to estimate one anticonvulsant ED50 for all PND70 propofol animals using a probit analysis. Table 2 displays all the data used in this analysis. The estimated anticonvulsant ED50 of propofol for PND70 animals was 57.82 mg/kg, however, no confidence interval was able to be estimated. This ED50 dose of propofol in PND70 animals is more than two-fold higher than the ED50 of the PND28 animals and more than five-fold higher than the ED50 of the PND21 animals.

No significant effects of sex, age, or agent were observed for seizure termination latency. Since agent and sexes were not significantly different, a further comparison and estimation of mean seizure termination latency for ages within each agent group was made. No significant differences were observed in seizure termination latency among PND21, PND28, or PND70 rats for either sarin or VX (Fig. 4). The percent of animals with seizure termination and the seizure termination latencies are given in Table 3.

3.3. Neuropathology

A generalized linear model on total neuropathology score for NA (VX, sarin), age (PND21, 28, 70), and treatment status (treatment success, treatment failure) indicated that there were main effects of age ($p = .022$) and treatment outcome ($p < .001$), but not NA ($p = .149$) in propofol-treated animals. Rats that were considered treatment successes had significantly lower total neuropathology scores than animals that were considered treatment failures ($p < .001$). PND28 animals had similar total scores to PND70 animals. Animals that had their seizures successfully controlled by propofol displayed no neural damage regardless of agent or age group; Fig. 5 illustrates total neuropathology scores across age and treatment outcome for propofol-treated animals. Fig. 6a demonstrates the amygdala in PND21, 28, and 70 animals successfully treated with propofol. All animals treated with either fosphenytoin or levetriacetam exhibited total neuropathology scores of 4 or higher. Total neuropathology scores in treatment failures ranged

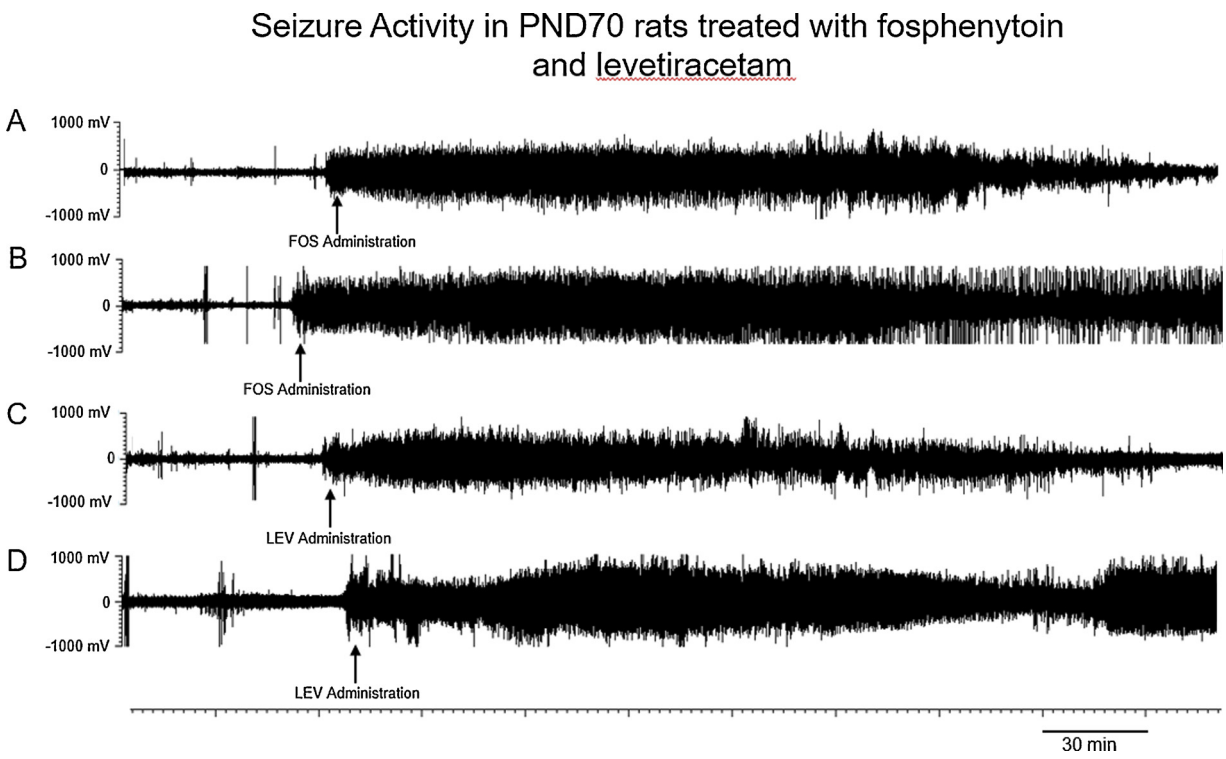

Fig. 2. EEG recordings of PND70 animals administered high doses of fosphenytoin (FOS) and levetiracetam (LEV). A VX-exposed male (A) and a sarin-exposed female (B) animal treated 5 min after seizure onset with fosphenytoin (135 PE). A VX-exposed female (C) and a sarin-exposed male (D) animal treated 5 min after seizure onset with levetiracetam (1000 mg/kg). These animals seized continuously throughout the day after they were treated with each drug and were all classified as treatment failures.

Table 1
Fosphenytoin and levetiracetam treatment success and mortality rates. The table shows the number of treatment successes and mortality rates among both fosphenytoin (FOS) and levetiracetam (LEV) treated rats. The numbers represent both male and female rats and were collapsed across age groups and NAs.

Drug	Dose	Treatment Successes	Mortality Post- Treatment
FOS	32 PE	0/3	0/3
FOS	43 PE	0/2	0/2
FOS	57 PE	0/2	0/2
FOS	76 PE	1/4	0/4
FOS	101 PE	0/1	0/1
FOS	135 PE	0/12	4/12
LEV	320 mg/kg	0/3	0/3
LEV	560 mg/kg	0/7	2/7
LEV	1000 mg/kg	0/8	2/8

from 3 to 24 in propofol-treated animals, 4–24 in fosphenytoin-treated animals, and 5–23 in levetiracetam-treated animals. Note that animals treated with either fosphenytoin or levetiracetam were treatment failures (with the exception of one, which still exhibited neuropathology), and therefore, no treatment successes are shown in the figure for either drug (Fig. 6b).

4. Discussion

Testing new compounds in both adult and pediatric populations is important for the protection of civilians of all ages in the event of a mass chemical attack. In this experiment, three approved anticonvulsants were evaluated for their ability to control NA-induced seizures. Fosphenytoin and levetiracetam were unable to mitigate seizures in this model, while propofol was able to effectively terminate seizures following NA exposure in all age groups.

Neither fosphenytoin nor levetiracetam was able to terminate seizure activity when given 5 min after seizure onset in either sarin- or VX-exposed animals in any age group, suggesting that neither drug is effective for treating NA-induced seizures. The single treatment success in the fosphenytoin-treated group had a seizure that terminated 49 min. following treatment and only stayed off for 2 h and 14 min. Even though the rat was considered to be a treatment success, it was in SE at the four-hour observation time. Furthermore, this subject's total neuropathology score was 4 out of 24, indicating that it had modest brain damage. Neither fosphenytoin nor levetiracetam terminated seizures in either adult or pediatric animals in this model, yet both drugs are utilized as second-line SE treatments in clinical settings (Kapur et al., 2019; Lyttle et al., 2019). As second-line treatments, they are given when substantial doses of benzodiazepines have already been administered. This could make a difference in their efficacy in a hospital setting; however, in this model, both drugs proved to be ineffective when given IP and utilized with only $ATSO_4$ and AMN. Standard immediate treatment of NA casualties is typically done using IM autoinjector drug administration, while treatment in a hospital setting would most likely use intravenously (IV) drug administration. The IP route of anticonvulsant administration was chosen in this model to provide as rapid drug absorption as possible and because IM administration of such large volumes of some of the test drugs (e.g., 100 mg/kg propofol; 1000 mg/kg levetiracetam) would be totally impractical especially in the immature animals given their smaller muscle mass. While both fosphenytoin and levetiracetam may have been more successful if administered IV at clinical doses and with associated supportive care, the highest doses used in this study appear to have met or exceeded those used in clinical practice even though they were given IP. The recommended loading dose for fosphenytoin in humans is 15 – 20 mg/kg (Walker et al., 1995; Heafield, 2000; Kapur et al., 2019), and the highest dose administered in this study was 203 mg/kg PE. This equates to approximately 32 mg/kg PE in human equivalent dosing (HED) using guidelines for extrapolating animal doses to humans (Food and Drug Administration, 2005). Clinical studies conducted with IV

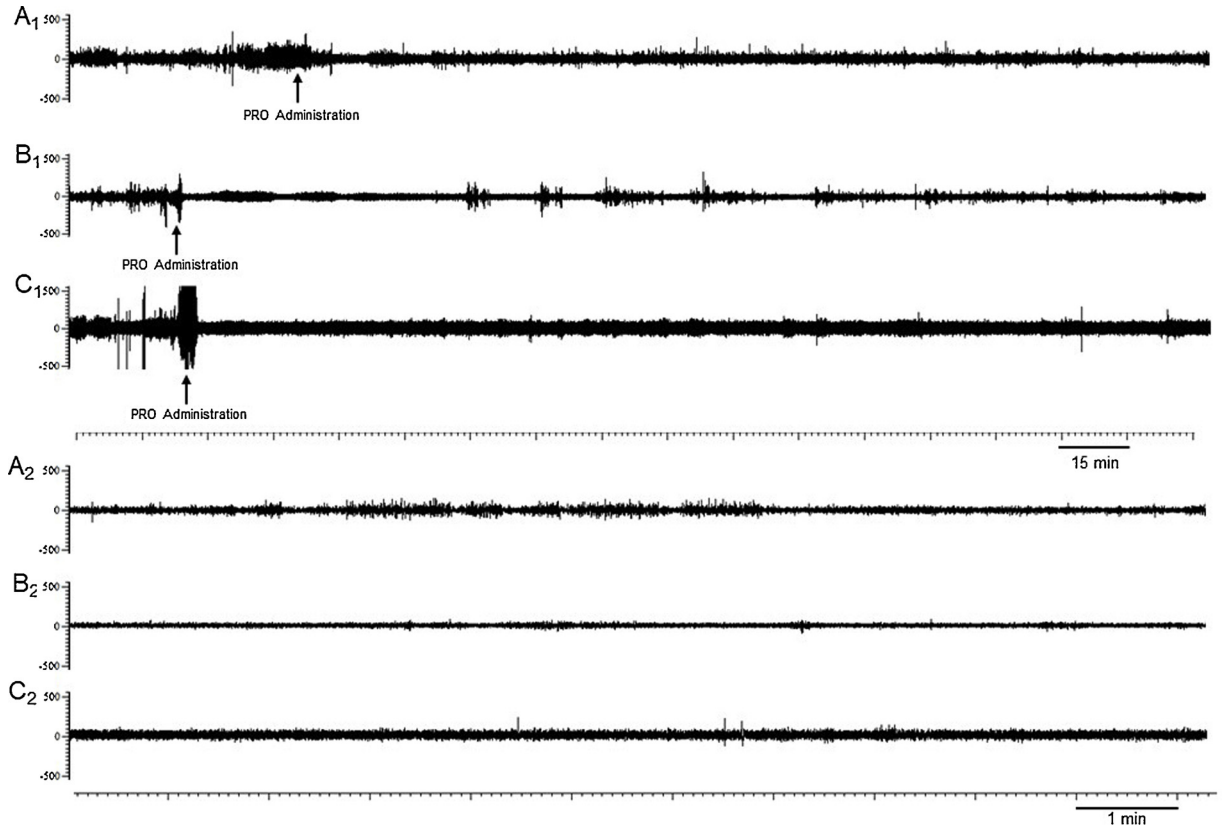

Fig. 3. EEG recordings of a PND21 (A; female, sarin exposed, 13.5 mg/kg propofol), a PND28 (B; female, sarin exposed, 42.0 mg/kg propofol) and a PND70 (C; male, VX exposed, 56 mg/kg propofol) animal that had their seizures successfully treated with propofol. The top three traces (A_1, B_1, C_1) display ~4 h of EEGs of the exposure day; animals were treated 5 min after seizure onset (arrows). The bottom three traces (A_2, B_2, C_2) display the EEG records of the same three animals 24 h after exposure and treatment. Note the rapid and continuous control of NA seizure activity in all three age groups and contrast that to the EEGs displayed in Fig. 2.

Table 2
Doses of propofol administered to PND70 animals. The number of animals that were considered treatment successes and failures at each dose are displayed in the table.

Sex	Agent	Dose	Total No. of Animals	No. of Treatment Successes	No. of Treatment Failures
Male	Sarin	75 mg/kg	2	0	2
		100 mg/kg	2	2	0
Female	Sarin	56 mg/kg	1	1	0
		75 mg/kg	2	2	0
		100 mg/kg	1	1	0
		133 mg/kg	1	0	1
		180 mg/kg	2	2	0
		240 mg/kg	1	1	0
		320 mg/kg	1	1	0
Male	VX	42 mg/kg	2	0	2
		56 mg/kg	3	2	1
		75 mg/kg	2	2	0
Female	VX	56 mg/kg	1	0	1
		75 mg/kg	2	2	0
		100 mg/kg	3	1	2
		133 mg/kg	1	1	0

levetiracetam in humans indicated that a 40–60 mg/kg loading dose was safe and effective in terminating seizures in children and adults, respectively (Wheless et al., 2009; Kapur et al., 2019; Lyttle et al., 2019), and the highest dose administered in this study was 1000 mg/kg. The HED of this is approximately 161 mg/kg using the above animal-to-human extrapolation. Thus, the highest doses of both fosphenytoin and levetiracetam administered in this study were well above the recommended loading doses in humans, and still seizures persisted and caused neuropathological damage. To date, no studies have evaluated levetiracetam or fosphenytoin in conjunction with supportive care or administered IV as possible anticonvulsants for NA-induced SE termination and neuroprotection. This would be interesting to assess, as results may change with route of administration and other advantages of care in a hospital setting.

Previous studies have shown that fosphenytoin and levetiracetam can be neuroprotective, at least in cases involving pilocarpine administration. In a study utilizing IV levetiracetam to treat pilocarpine-induced seizures, levetiracetam significantly reduced neuropathology, but did not prevent it completely (Zheng et al., 2010). Similarly, in a study utilizing phenytoin-treated pilocarpine-exposed animals, phenytoin attenuated but did not prevent neuropathology (Cunha et al., 2009). The two drugs helped to moderate the brain damage that occurred during pilocarpine-induced seizures, indicating that they may have some neuroprotective ability. In the present study, all fosphenytoin- and levetiracetam-treated animals presented with neuropathology following all-day, continuous seizing. Likewise, propofol-treated animals that were treatment failures also had neuropathology, while those animals that had the seizures controlled were free of any neuropathology. These findings reiterates the point that failing to stop NA-induced seizures, or any prolonged SE for that matter, results in neuropathology and loss of normal functioning and that rapid seizure termination is the essential goal of any anticonvulsant treatment (McDonough and Shih, 1997; Pessah et al., 2016).

It is shown here that propofol was an effective treatment for pediatric and adult rats. The substantial differences in ED50 doses across the ages as shown in the present study is surely a factor to consider for treatment development, because different options would be needed in a mass chemical exposure situation. Traditionally, propofol has been considered a third-line treatment for SE and is administered IV. Because

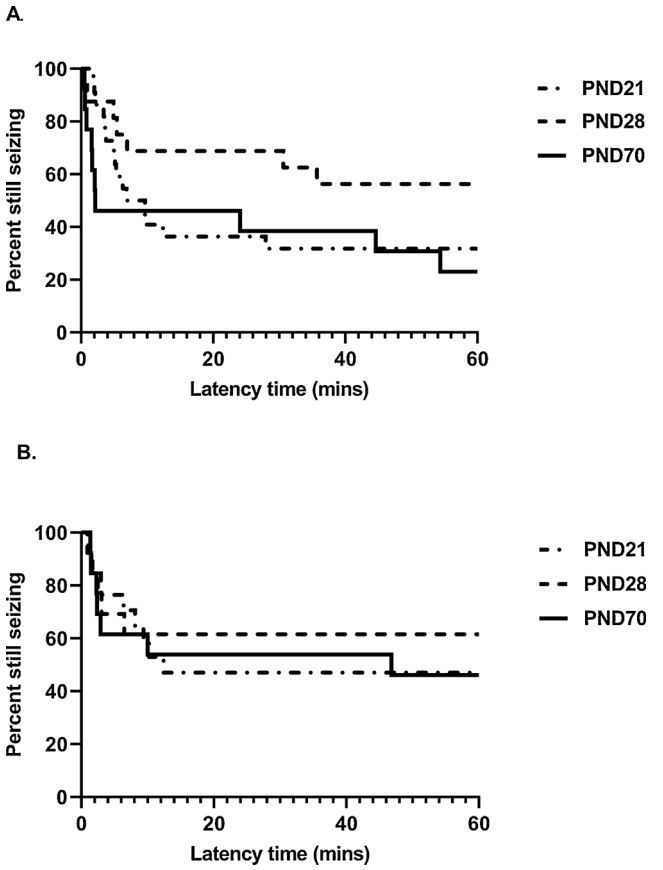

Fig. 4. Seizure termination latencies displayed as survival curves for (A) sarin- and (B) VX-exposed animals treated with propofol. There were no significant age differences observed in seizure termination latency in both sarin- and VX-exposed rats. Sarin exposed animals: PND21 (N = 22: male = 10, female = 12); PND28 (N = 16: male = 5; female = 11); PND70 (N = 13: male = 4; female = 9). VX exposed animals: PND21 (N = 16; male = 6; female = 10); PND28 (N = 13; male = 6; female = 7); PND70 (N = 14, male = 7; female = 7).

Table 3
Percent of seizures terminated and Mean (Std Error) seizure termination times for animals treated with propofol. The table shows the percent of animals whose seizures terminated (treatment successes) and mean seizure termination latency in each group. In all groups, mean seizure termination latency was no longer than 26 min.

Agent	Age	% Seizure Terminated	Mean (Std Error) [mins]
Sarin	PND21	68.2% (15 /22)	13.67 (2.44)
	PND28	43.8% (7/16)	25.37 (3.98)
	PND70	76.9% (10/13)	22.74 (7.11)
VX	PND21	50.0% (8/16)	9.46 (1.08)
	PND28	38.4% (5/13)	5.14 (0.64)
	PND70	57.1% (8/14)	24.93 (6.28)

propofol is rapidly metabolized, continuous infusion over at least several hours would likely be required to achieve lasting seizure control. Additionally, doses of propofol that effectively control seizures in a clinical setting often depress respiration to the point of necessitating ventilator support. Thus, it will likely not be the best candidate for use in pre-hospital care for NA-induced seizures, but could be advanced as a standalone or first-line treatment for in-hospital care of NA-induced seizures.

There were also no significant differences between sex, age, and agent in seizure termination latency. When propofol terminated seizure activity, it functioned relatively quickly, with some group average

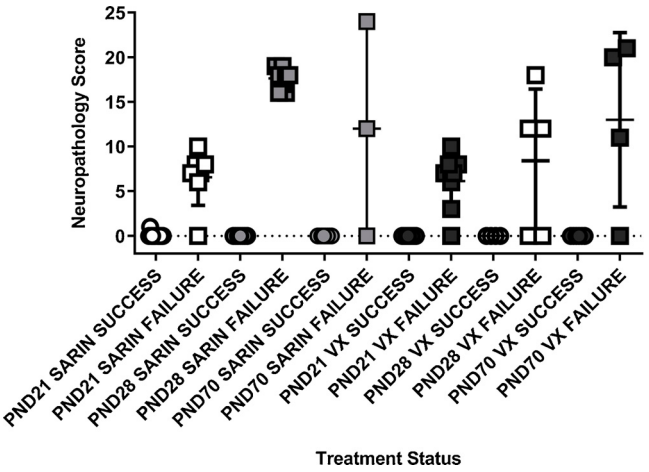

Fig. 5. Total neuropathology scores in propofol-treated rats. The figure shows total neuropathology scores of individual PND21, PND28, and PND70 rats treated with propofol after nerve agent exposure and categorized as treatment successes (seizure terminated; circles) or treatment failures (seizure not terminated; squares). The group mean and +/- standard deviation are displayed by the lines. Animals in all age groups categorized as treatment success had total neuropathology scores of 0, with the exception of one male PND21 animal challenged with sarin and treated with 10 mg/kg propofol that had a total neuropathology score of 1. Of the animals considered treatment failures, PND21 rats had significantly lower mean total neuropathology scores than either the PND28 or PND70 rats. All animals that were considered treatment failures had higher neuropathology scores than did treatment successes in all three age groups. All animals categorized as treatment successes were euthanized and perfused 24 h after propofol treatment; all animals categorized as treatment failures were euthanized and perfused 4 h after propofol treatment.

latencies as low as 5 min post-treatment (Table 3). Although the potency of propofol is substantially less than the benzodiazepines diazepam or midazolam, the speed of seizure control by propofol across agents and age groups is comparable to what was seen with when these drugs were tested in this model (Matson et al., 2019). Propofol also exhibited the ability to prevent neuropathology when the seizures were controlled within an hour of treatment, again a finding virtually identical to the results with diazepam and midazolam (Matson et al., 2019). Together, propofol's high efficacy, fast seizure termination latency, and ability to prevent neuropathology make the drug a promising treatment possibility for NA-induced seizures.

Transparency document

The Transparency document associated with this article can be found in the online version.

CRediT authorship contribution statement

Emily N. Dunn: Investigation, Writing - original draft. **Liana M. Matson:** Investigation, Writing - review & editing. **Kari M. Haines:** Investigation. **Kimberly A. Whitten:** Formal analysis. **Robyn B. Lee-Stubbs:** Formal analysis. **Kyle E. Berger:** Investigation. **Hilary S. McCarren:** Writing - review & editing. **Cherish E. Ardinger:** Investigation. **Cecelia E. Jackson Piercy:** Investigation. **Stephanie M. Miller-Smith:** Methodology. **John H. McDonough:** Conceptualization, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare no conflict of interest.

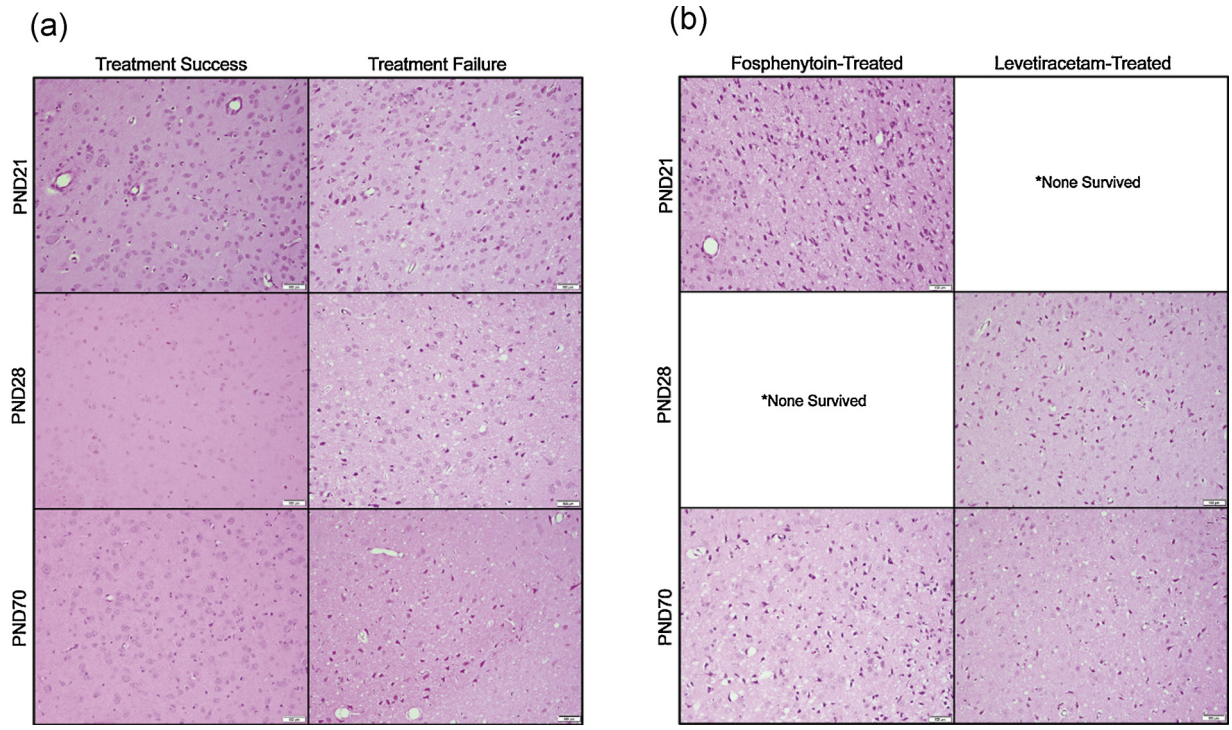

Fig. 6. a Upper left: a VX-exposed PND21 female rat treated successfully with18.0 mg/kg propofol; survival 24 h; undamaged neurons in the amygdala. Upper right: a VX-exposed PND21 male rat; treated unsuccessfully with 13.5 mg/kg propofol; survival 4 h; extensive degeneration and necrosis of neurons in the amygdala with neuropil vacuolation. Middle left: a VX-exposed PND28 male rat treated successfully with 24.0 mg/kg propofol; survival 24 h; undamaged neurons in the amygdala. Middle right: a sarin-exposed PND28 female rat treated unsuccessfully with 24.0 mg/kg propofol; survival 4 h; extensive degeneration and necrosis of neurons in the amygdala with neuropil vacuolation. Lower left: a VX-exposed exposed PND70 male rat treated successfully with 75.0 mg/kg propofol; survival 24 h; undamaged neurons in the amygdala. Lower right: a sarin-exposed PND70 female rat treated unsuccessfully with 100 mg/kg propofol; survival 4 h; extensive degeneration and necrosis of neurons in the amygdala with neuropil vacuolation. Fig. 6b All fosphenytoin- and levetiracetam-treated rats were treatment failures and those that survived were euthanized 4 h after treatment. No fosphenytoin-treated PND28 rats or levetiracetam-treated PND21 rats survived. All images were stained with H&E and taken at 10 × . Upper left: sarin-exposed PND21 male rat; treated unsuccessfully with 76 PE of fosphenytoin; extensive degeneration and necrosis of neurons in the amygdala with neuropil vacuolation. Middle right: VX-exposed PND28 female rat; treated unsuccessfully with 1000 mg/kg levetiracetam; extensive degeneration and necrosis of neurons in the amygdala with neuropil vacuolation. Lower left: VX-exposed PND70 female rat; treated unsuccessfully with 135 PE fosphenytoin; extensive degeneration and necrosis of neurons in the amygdala with neuropil vacuolation. Lower right: VX-exposed PND70 male rat; treated unsuccessfully with 1000 mg/kg levetiracetam; extensive degeneration and necrosis of neurons in the amygdala with neuropil vacuolation.

Acknowledgements

Support was provided by an Inter-Agency Agreement between NIH/NINDS (Y1-O6-9613-01) and USAMRICD (A120-B.P2009-2). This research was supported in part by appointments to the Postgraduate Research Participation Program at the U.S. Army Medical Research Institute of Chemical Defense administered by the Oak Ridge Institute for Science and Education through an Inter-Agency Agreement between the U.S. Department of Energy and U.S. Army Medical Research and Development Command. The views expressed in this report are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. 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, and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

References

Abend, N.S., Loddenkemper, T., 2014. Management of pediatric status epilepticus. Curr. Treat. Options Neurol. 16 (7), 301.
Alford, E.L., Wheless, J.W., Phelps, S.J., 2015. Treatment of generalized convulsive status epilepticus in pediatric patients. J. Pediatr. Pharmacol. Ther. 20 (4), 260–289.
American Academy of Pediatrics, 2000. Committee on Environmental Health and Committee on Infectious Diseases. Chemical-biological terrorism and its impact on children: a subject review. Pediatrics 105 (3 Pt 1), 662–670.
Brophy, G.M., et al., 2012. Guidelines for the evaluation and management of status epilepticus. Neurocrit. Care 17 (1), 3–23.
Cantrell, F.L., Wardi, G., O'Connell, C., 2016. Propofol use for toxin-related seizures. Pharmacotherapy 36 (6), 702–704.
Cunha, A.O., et al., 2009. Neuroprotective effects of diazepam, carbamazepine, phenytoin and ketamine after pilocarpine-induced status epilepticus. Basic Clin. Pharmacol. Toxicol. 104 (6), 470–477.
Deshpande, L.S., Delorenzo, R.J., 2014. Mechanisms of levetiracetam in the control of status epilepticus and epilepsy. Front. Neurol. 5, 11.
Dixon, W.J., Massey, F.J., 1983. Sensitivity experiments. Introduction to Statistical Analysis. McGraw-Hill, New York, pp. 426–441.
Food and Drug Administration, 2005. Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. US Department of Health and Human Services.
Glauser, T., et al., 2016. Evidence-based guideline: treatment of convulsive status epilepticus in children and adults: report of the Guideline Committee of the American Epilepsy Society. Epilepsy Curr. 16 (1), 48–61.
Grover, E.H., Nazzal, Y., Hirsch, L.J., 2016. Treatment of convulsive status epilepticus. Curr. Treat. Options Neurol. 18 (3), 11.
Hamele, M., Poss, W.B., Sweney, J., 2014. Disaster preparedness, pediatric considerations in primary blast injury, chemical, and biological terrorism. World J. Crit. Care Med. 3 (1), 15–23.
Heafield, M.T., 2000. Managing status epilepticus. New drug offers real advantages. BMJ 320 (7240), 953–954.
Henretig, F., 2009. Preparation for terrorist threats: biologic and chemical agents. Clin. Pediatr. Emerg. Med. 10 (3), 130–135.
Holtkamp, M., Tong, X., Walker, M.C., 2001. Propofol in subanesthetic doses terminates status epilepticus in a rodent model. Ann. Neurol. 49 (2), 260–263.
Kapur, J., et al., 2019. Randomized trial of three anticonvulsant medications for Status Epilepticus. New England J. Med. Surg. Collat. Branches Sci. 381, 2103–2113.
King, A.M., Aaron, C.K., 2015. Organophosphate and carbamate poisoning. Emerg. Med. Clin. North Am. 33 (1), 133–151.
Lee, T., et al., 2018. Levetiracetam in toxic seizures. Clin. Toxicol. (Phila) 56 (3),

175–181.
Loddenkemper, T., Goodkin, H.P., 2011. Treatment of pediatric status epilepticus. Curr. Treat. Options Neurol. 13 (6), 560–573.
Lyttle, M.D., et al., 2019. Levetiracetam versus phenytoin for second-line treatment of paediatric convulsive status epilepticus (EcLiPSE): a multicenter, open-label, randomized trial. Lancet 393 (10186), 2125–2134.
Matson, L., et al., 2019. Evaluation of first-line anticonvulsants to treat nerve agent-induced seizures and prevent neuropathology in adult and pediatric rats. Neurotoxicology 74, 203–208.
McDonough, J.H., Shih, T.-M., 1997. Neuropharmacological mechanisms of nerve agent-induced seizures and neuropathology. Neurosci. Biobehav. Rev. 21, 559–579.
McDonough, J.H., et al., 1995. Protection against nerve agent-induced neuropathology, but not cardiac pathology, is associated with the anticonvulsant action of drug treatment. Neurotoxicology 16 (1), 123–132.
McDonough, J.H., et al., 2004. Effects of fosphenytoin on nerve agent-induced status epilepticus. Drug Chem. Toxicol. 27 (1), 27–39.
McDonough, J.H., McMonagle, J.D., Shih, T.M., 2010. Time-dependent reduction in the anticonvulsant effectiveness of diazepam against soman-induced seizures in guinea pigs. Drug Chem. Toxicol. 33 (3), 2279–2283.
Misra, U.K., Kalita, J., Maurya, P.K., 2012. Levetiracetam versus lorazepam in status epilepticus: a randomized, open labeled pilot study. J. Neurol. 259 (4), 645–648.
Parviainen, I., Kalviainen, R., Ruokonen, E., 2007. Propofol and barbiturates for the anesthesia of refractory convulsive status epilepticus: pros and cons. Neurol. Res. 29 (7), 667–671.
Pessah, I.N., et al., 2016. Models to identify treatments for the acute and persistent effects of seizure-inducing chemical threat agents. Ann. N.Y. Acad. Sci. 1378 (1), 124–136.
Rotenberg, J.S., Newmark, J., 2003. Nerve agent attacks on children: diagnosis and management. Pediatrics 112 (3 Pt 1), 648–658.
Ruegg, S., et al., 2008. Intravenous levetiracetam: treatment experience with the first 50 critically ill patients. Epilepsy Behav. 12 (3), 477–480.
Scholl, E.A., et al., 2018. Age-dependent behaviors, seizure severity and neuronal damage in response to nerve agents or the organophosphate DFP in immature and adult rats. Neurotoxicology 66, 10–21.
Sengupta, P., 2013. The laboratory rat: relating its age with human's. Int. J. Prev. Med. 4 (6), 624–630.
Shih, T.-M., Duniho, S.M., McDonough, J.H., 2003. Control of nerve agent-induced seizures is critical for neuroprotection and survival. J.Appl. Toxicol. 188, 69–80.
Shirm, S., et al., 2007. Prehospital preparedness for pediatric mass-casualty events. Pediatrics 120 (4), e756–761.
Stecker, M.M., et al., 1998. Treatment of refractory status epilepticus with propofol: clinical and pharmacokinetic findings. Epilepsia 39 (1), 18–26.
Trinka, E., et al., 2015. Pharmacotherapy for status epilepticus. Drugs 75 (13), 1499–1521.
Walker, M.C., Smith, S.J., Shorvon, S.D., 1995. The intensive care treatment of convulsive status epilepticus in the UK. Results of a national survey and recommendations. Anesthesia 50 (2), 130–135.
Wheless, J.W., et al., 2009. Rapid infusion of a loading dose of intravenous levetiracetam with minimal dilution: a safety study. J. Child Neurol. 24 (8), 946–951.
Wright, C., et al., 2013. Clinical pharmacology and pharmacokinetics of levetiracetam. Front. Neurol. 4, 192.
Zheng, Y., et al., 2010. Intravenous levetiracetam in the rat pilocarpine-induced status epilepticus model: behavioral, physiological and histological studies. Neuropharmacology 58 (4-5), 793–798.