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FULL-LENGTH ORIGINAL RESEARCH

Epilepsia®

Evaluation of subchronic administration of antiseizure drugs in spontaneously seizing rats

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Abstract

Objective: Approximately 30% of patients with epilepsy do not experience full seizure control on their antiseizure drug (ASD) regimen. Historically, screening for novel ASDs has relied on evaluating efficacy following a single administration of a test compound in either acute electrical or chemical seizure induction. However, the use of animal models of spontaneous seizures and repeated administration of test compounds may better differentiate novel compounds. Therefore, this approach has been instituted as part of the National Institute of Neurological Disorders and Stroke Epilepsy Therapy Screening Program screening paradigm for pharmacoresistant epilepsy.

Methods: Rats were treated with intraperitoneal kainic acid to induce status epilepticus and subsequent spontaneous recurrent seizures. After 12 weeks, rats were enrolled in drug screening studies. Using a 2-week crossover design, selected ASDs were evaluated for their ability to protect against spontaneous seizures, using a video-electroencephalographic monitoring system and automated seizure detection. Sixteen clinically available compounds were administered at maximally tolerated doses in this model. Dose intervals (1-3 treatments/d) were selected based on known half-lives for each compound.

Results: Carbamazepine (90 mg/kg/d), phenobarbital (30 mg/kg/d), and ezogabine (15 mg/kg/d) significantly reduced seizure burden at the doses evaluated. In addition, a dose-response study of topiramate (20-600 mg/kg/d) demonstrated that this compound reduced seizure burden at both therapeutic and supratherapeutic doses. However, none of the 16 ASDs conferred complete seizure freedom during the testing period at the doses tested.

Significance: Despite reductions in seizure burden, the lack of full seizure freedom for any ASD tested suggests that this screening paradigm may be useful for testing novel compounds with potential utility in pharmacoresistant epilepsy.

KEYWORDS

antiseizure drugs, chronic animal models, drug screening, epilepsy

1 | INTRODUCTION

Despite decades of research and the availability of >20 antiseizure drugs (ASDs) for the treatment of epilepsy,[1] the number of patients with refractory epilepsy has remained relatively stable.[2] Thus, the search for novel ASDs that can address this unmet need continues. Preclinical screening of ASDs has long relied on acute (eg, single stimulation) chemical or electrical seizure models to predict efficacy in the clinic.[3,4] In addition to kindling models, these acute seizure tests have served as the basis for the National Institute of Neurological Disorders and Stroke (NINDS) Epilepsy Therapy Screening Program (ETSP) for >40 years.[5] However, reliance on acute seizure and kindling models may only serve to identify ASDs with mechanisms of action similar to those of existing approved therapies.[6] Furthermore, these induced seizure models do not accurately reflect the majority of seizure types that occur in clinical populations. By contrast, animal models of epilepsy that express spontaneous, recurrent seizures more accurately reflect clinical epilepsy. Finally, a spontaneous seizure model where animals are refractory to prototype ASDs may more accurately represent clinical refractory epilepsy.

When systemically administered, kainic acid (KA) induces status epilepticus (SE) in rats, and this is followed by the development of spontaneous recurrent seizures in the majority of animals.[7,8] Previous studies have tested the ability of various ASDs to reduce seizures in this model, including topiramate (TPM),[9] carbamazepine (CBZ),[7] and carisbamate.[10] However, differing SE induction protocols and testing parameters confound direct comparisons of efficacy between compounds.[11] Furthermore, it is currently unknown to what extent seizures in this animal model of epilepsy are refractory to existing ASDs. We have developed a standardized approach where chronically seizing rats receive subchronic administration of individual ASDs to test their efficacy in preventing spontaneous recurrent seizures. Following a 7-day baseline recording period of spontaneous seizures, animals are treated with either drug or vehicle for 5 days with an intraperitoneal dosing regimen. Animals are then given a 2-day washout period, followed by a crossover to the opposing treatment arm for 5 days. Animals repeat this paradigm for up to four different ASDs. This screening approach provides spontaneous seizure outcomes, which allow for assessment of a compound's potential ability to block these seizures in an etiologically relevant model of epilepsy. This model is part of a screening and differentiation testing schema and now serves as an advanced differentiation test in the NINDS ETSP pipeline (http://www.ninds.nih.gov/Current-Research/Focus-Research/Focus-Epilepsy/ETSP).[12] This comprehensive assessment of numerous ASDs in a commonly utilized model of temporal lobe epilepsy (TLE) in a single laboratory under similar conditions has revealed that this seizure model is refractory to numerous ASDs and may therefore be an important model to identify potential compounds for the ability to prevent pharmacoresistant seizures.

Key Points

- A novel screening approach has been established for the differentiation of potential new antiseizure therapies in a chronic model of spontaneous seizures
- This model is pharmacoresistant to a large number of currently available antiseizure drugs, and all drugs evaluated failed to produce complete seizure freedom at the doses tested
- Carbamazepine, phenobarbital, ezogabine, and topiramate significantly reduced seizure burden

2 | MATERIALS AND METHODS

2.1 | Generating a cohort of epileptic rats

Male Sprague Dawley rats (120-135 g) were obtained from Charles River. Each treatment cohort began with 48-64 rats (160-205 g at time of treatment) induced with SE using the repeated low-dose kainic acid paradigm (Tocris Bioscience, 7.5 mg/kg).[7,8] This results in the development of spontaneous recurrent seizures. Following SE, animals were housed separately in a temperature- and humidity-controlled American Association for Laboratory Animal Science–approved vivarium with a 12-hour light/dark cycle and free access to food and water. At approximately 10 weeks post-SE, surviving animals were anesthetized with isoflurane (2%-5%) and implanted with a TR50B electroencephalographic (EEG) wireless telemetry device (Kaha Telemetry Systems). A transmitter was placed in the intraperitoneal space and secured to the peritoneum with sutures. A trocar was used to separate the skin and peritoneum, and two wires were run underneath the skin to the cranium. The exposed wire tips were placed bilaterally in the epidural space. The wires were held in place using three fixation screws and dental acrylic. Animals were treated with Bicillin (MWI Animal Health) immediately following surgery and Rimadyl (MWI Animal Health, 15 mg/kg) for 2 days following surgery. Following surgery, a week-long EEG recording period coupled to video recording was evaluated for every animal. For the purposes of evaluating compounds, animals with the highest seizure rates were selected for testing (minimum = 1 seizure per week). Therefore, from the initial surgical cohort, the 24 animals with the highest seizure rates and severity were selected to be used in the experiments. The remaining animals were retained to replace any animals lost to attrition.

2.2 | Drug administration

A timeline of the experimental protocol is shown in Figure 1A. Drug treatment, as shown in Figure 1B, proceeded following the selection of the rats with the greatest seizure frequencies. For the first phase of a testing run, animals (n = 10-12) were administered either drug or vehicle. After a 5-day treatment period and a 2-day washout, animals received the opposite treatment for 5 days. This protocol was repeated up to four times in the same cohort, with an optional 1-week washout period included between tests for drugs with longer half-lives (ie, 12 hours or more). As some rats do not complete the study due to a variety of factors (eg, telemeter failure, infection, moribund behavior, or death), animals that completed at least 3 days of treatment and observation were included in the final analysis. Animals lost at any point during the treatment paradigm were replaced at the next baseline period from the cohort of unused animals.

2.3 | Data analysis

EEG and video data were continuously recorded (24 h/7 d/wk) using a custom software package[13] that synchronized the recording of EEG data from an MP150 (BIOPAC Systems) and video streams using a DVP-7020BE capture card (Advantech). EEG data were first analyzed by a custom automated seizure detection system, and any seizurelike event was marked. A human reviewer then assessed the results of the seizure detection algorithm and confirmed or rejected any detected events as seizures. Additionally, the reviewer scored the behavior on a modified Racine scale.[14] Reviewers were blinded to treatment groups.

Reviewer results were compiled using custom software that records individual injections and video-EEG seizure observations.[13] This database of seizure scores was analyzed in MATLAB (MathWorks) using custom software that provided seizure rates, seizure burdens, and seizure freedom. Seizure frequency was calculated as per-day frequency. To calculate seizure burden, the sum of all Racine seizure scores was divided by the total number of testing days. The treatment period was considered completed at the time point of 12 hours following the final injection of either ASD or vehicle. Baseline seizure burden and frequency were defined as the period of exactly 7 days prior to the first injection. Seizure freedom was defined as zero seizures occurring between the first dose of drug and 12 hours following the final injection. Although these criteria do not take pharmacokinetics into account, it remains fixed for every drug as to not bias the results. However, all seizure results are provided to the participant who provided the drug to the ETSP program, so visual evaluation of when seizures occur allows for more detailed analysis to be performed on individual drugs.

Seizure burdens and seizure frequency were compared using a Kruskal-Wallis test. Fisher exact test was used to compare the rates of seizure freedom between baseline and drug treatment as well as between drug treatment and vehicle treatments. Dose-response curves for seizure freedom were analyzed using a probit analysis.

2.4 | Drug preparation

All drugs, listed in Table 1, were prepared in 0.5% methylcellulose (Sigma), except for sodium valproate (VPA), which was prepared in saline (0.9% NaCl). CBZ, clobazam (CLB), clonazepam (CLZ), ethosuximide (ETX), ezogabine (EZG), phenobarbital

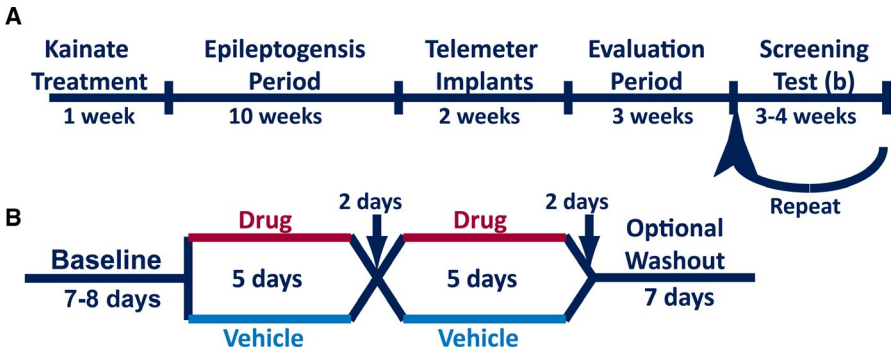

FIGURE 1 Timeline for evaluating compounds in the kainic acid (KA) status epilepticus (SE) spontaneously recurring seizure model. (A) The overall timeline of an individual cohort of animals begins with KA injections (day 1), followed by a waiting period to ensure that epilepsy has developed. Animals then receive telemeter implantation followed by a 1- to 2-wk recovery period. Once animals are verified to have sufficient spontaneous seizures, they are assigned to a treatment cohort and begin the screening paradigm. (B) The timeline of a single screening test. Following a baseline assessment period, groups of 12 rats are assigned to receive either drug or vehicle during the first 5-day treatment period. Following a 2-day washout period, each treatment group is crossed over to the opposing treatment for an additional 5 days. For compounds with a long half-life (eg, 12 hours or greater), an optional 1-week washout period is added. The screening component of the timeline in B will be repeated up to a maximum of four times with a cohort of animals

TABLE 1 Prototype drugs screened in the model sorted by mechanism of action, including sodium channel activity, GABAergic activity, and mixed mechanisms of action

Drug	Abbreviation	Mechanism of action	Dose, mg/kg	Frequency	Mixture	Half-life	Reference
Carbamazepine	CBZ	Blocks fast inactivation of NA+ Channels	30	tid	Suspension	1.2-3.5 h	16
Lacosamide	LCM	Enhances slow inactivation of NA+ channels	30	qd	Suspension	3-3.5 h	20,24
Lamotrigine	LTG	Blocks fast inactivation of NA+ Channels	30	bid	Suspension	12-30 h	16,24
Phenytoin	PHT	Blocks fast inactivation of NA+ Channels	10	bid	Suspension	1-8 h	16
Phenobarbital	PHB	$GABA_A$ receptor allosteric modulation	30	qd	Suspension	9-20 h	16
Clobazam	CLB	$GABA_A$ receptor allosteric modulation	10	qd	Suspension	1 h	16,25
Clonazepam	CLZ	$GABA_A$ receptor allosteric modulation	2	qd	Suspension	1-2 h	22,26
Tiagabine	TGB	GABA uptake inhibitor	8	bid	Solution	1 h	16
Ezogabine	EZG	K+ Channels	5	tid	Solution	2.5 h	18
Topiramate	TPM	Mixed	300	bid	Suspension	2-5 h	16
Perampanel	PER	AMPA receptor blocker	1.5	tid	Suspension	1.7 h	21
Levetiracetam	LEV	SV2A modulation	150	bid	Solution	2-3 h	16
Gabapentin	GBP	α2δ subunit of voltage-gated Ca2+ channels	300	bid	Solution	1.7 h	17
Valproate	VPA	Mixed	200	tid	Solution	1-5 h	16
Everolimus	EVR	Inhibition of mTOR pathway	6	qd	Suspension	20 h	19
Ethosuximide	ETX	T-type Ca2+ channels	150	qd	Solution	10-16 h	16

Note: Doses are listed as the single dose amount. A dose of 200 mg/kg tid would amount to 600 mg/kg/d.

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; bid, twice daily; GABA, γ-aminobutyric acid; mTOR, mammalian target of rapamycin; qd, once daily; SV2A, synaptic vesicle 2A; tid, three times daily.

(PHB), phenytoin (PHT), and tiagabine were obtained from TCI America). Lamotrigine (LTG) was obtained from AK Scientific. Lacosamide (LCM) was obtained from Axon Medchem. Everolimus (EVR) was obtained from Medchem Express. Perampanel (PER) was obtained directly from the ETSP. Drugs chosen for testing in this model were selected from the cohort of currently available clinical treatments for epilepsy. Doses were chosen based on efficacy and toxicity results from previous standard[15,16] tests in rats, including the maximal electroshock ED_{50}, the LTG-kindled rat (LTG-R) model,[16] and the minimal motor impairment toxicity test, as well as from literature references. A complete list of drugs, including their abbreviations, doses, and frequency of administration are included in Table 1, along with literature references that were used to determine an appropriate subchronic dose.[17–26] In cases where the half-life was shorter than the dosing indicates (ie, LCM, CLB, and CLZ), the secondary metabolites were considered as well.[24–26]

3 | RESULTS

3.1 | KA-SE model of spontaneous seizures

To test against spontaneous seizures, we needed to create cohorts of rats with epilepsy. Four cohorts of rats were used in this study. Following KA-SE, 41 of 48, 40 of 50, 57 of 64, and 40 of 56 animals survived; thus 82% of animals survived the initial treatment. As this model of epilepsy is a progressive disorder,[27] we did not begin testing ASDs until 12 weeks post-SE. This time point was selected to begin drug testing because previous work has demonstrated that most rats have achieved a fairly stable frequency of spontaneous seizures by this time point.[27] We observed seizures with differing levels of severity, from nonconvulsive (Racine stage 0) to Racine stage 5. The electrographic activity of these seizures is included in Figure S1.

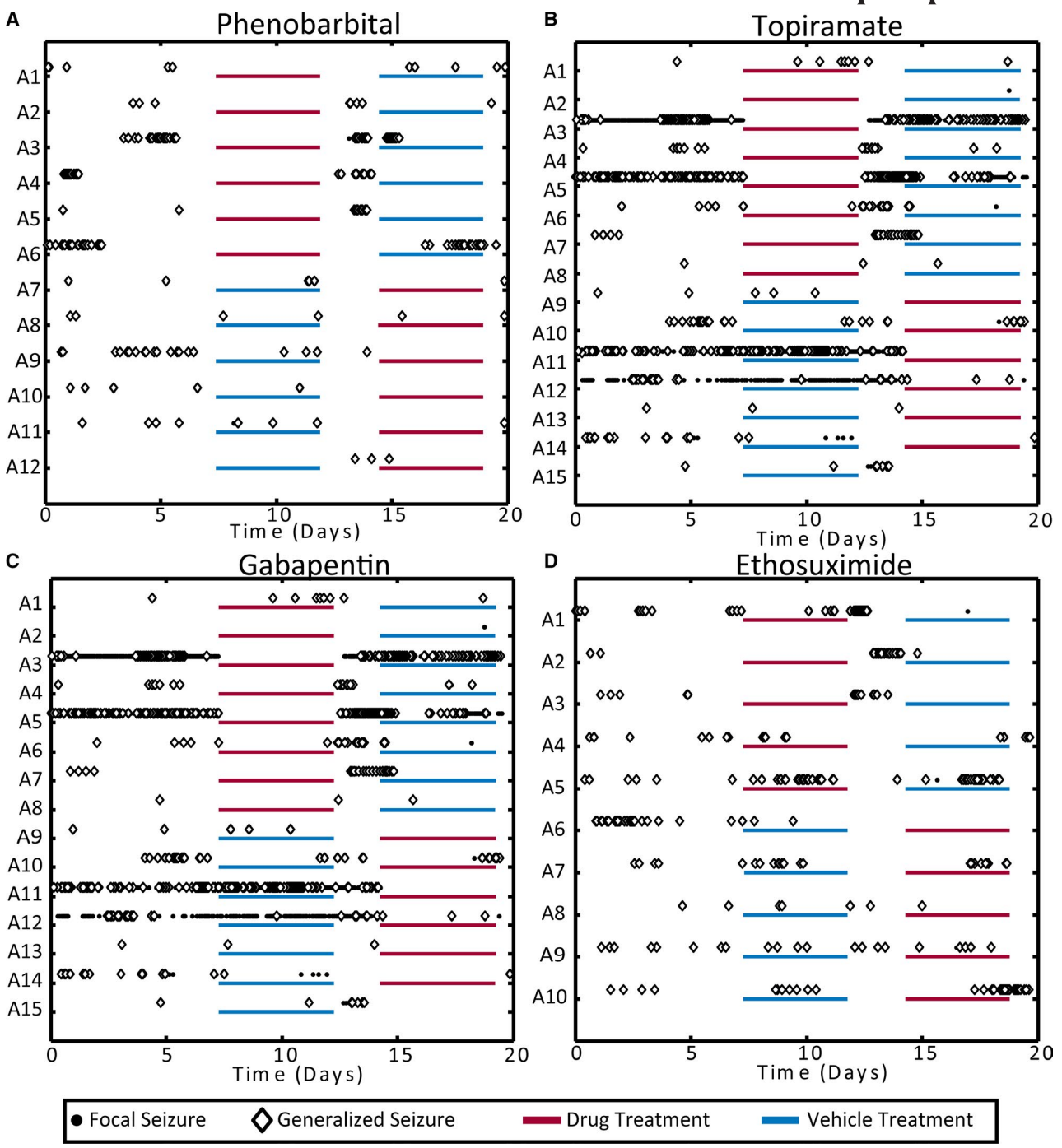

FIGURE 2 Raw data from three different drugs tested at various doses in this model. These data demonstrate the high interanimal variability in seizure incidence. Circles represent stage 0-2 seizures (focal), and diamonds represent stage 3-5 seizures (generalized). Red lines indicate drug treatment periods, and blue lines indicate vehicle treatment periods. (A) Phenobarbital (30 mg/kg once daily) showed the highest efficacy of all drugs tested. (B) Topiramate (300 mg/kg twice daily) showed a high degree of efficacy in preventing seizures in the absence of notable adverse events. (C) Gabapentin (300 mg/kg twice daily) provided moderate efficacy in preventing seizures. (D) Ethosuximide (150 mg/kg twice daily) was not effective in preventing seizures

3.2 | Evaluation of prototype ASDs

Visual examination of the results of the study allows for easy interpretation of the data. The seizure history of rats treated with four different compounds is shown in Figure 2: PHB (30 mg/kg once daily [qd]), TPM (300 mg/kg twice daily [bid]), gabapentin (GBP; 300 mg/kg bid), and ETX (150 mg/kg bid). These representative ASDs illustrate the range of effects noted in this study. Effects of drugs not shown here are included in Figures S2-S12. At the doses tested, these compounds were either highly efficacious (eg, reduced seizure burden to at least 20% of baseline; PHB, TPM), moderately

efficacious (20%-80% of baseline seizure burden; GBP), or without efficacy (>80% of baseline seizure burden; ETX; see also Table 2).

Results from evaluation of each drug tested in this model are included in Table 2, sorted by mechanism of action. CBZ was the only sodium channel blocker that reduced seizure burden and increased seizure freedom, whereas LTG, LCM, and PHT had no effect on either seizure burden or seizure freedom. Of all the compounds tested in this study, only LTG resulted in adverse effects over the course of treatment, with animals exhibiting ataxia and lethargy starting on the 3rd day of treatment, requiring one animal's withdrawal from the study due to these effects. Compounds targeting γ-aminobutyric acid type A receptors (eg, PHB, CLB, and CLZ) were all effective in this model and significantly reduced seizure burden and increased seizure freedom. The potassium channel opener EZG also significantly reduced seizure burden and increased seizure freedom. ASDs targeting calcium channels had mixed effects, with GBP (α2δ-binding on voltage-gated calcium channels) reducing seizure burden and increasing seizure freedom, whereas ETX (blockade of T-type calcium channels) was ineffective. TPM, a compound with multiple mechanisms of action, reduced seizure burden and increased seizure freedom, whereas VPA (mixed mechanism of action) was without effect on seizure burden or seizure freedom. PER (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor antagonist) and levetiracetam (LEV; SV2A-acting) moderately reduced seizure burden. Of note, none of the ASDs at the doses used in this study resulted in complete seizure freedom, and several ASDs had no significant effect on seizure burden (EVR, LCM, PHT, LTG, VPA, and ETX).

3.3 | Dose-response evaluation of TPM and PHB

To determine whether this screening approach was sensitive enough to determine the ED_{50} of tested ASDs, dose-response studies were performed using TPM and PHB. The initial screening dose of TPM was selected based on a maximum tolerated dose (300 mg/kg bid).[15] Although this dose was effective in reducing seizures, previously published data suggest it also corresponds to plasma levels well above the therapeutic range.[17,28] Therefore, additional lower doses of TPM (10, 19, 37.5, 75, 150 mg/kg) were evaluated (see Table 3). In general, TPM reduced seizure burden by at least 50%, with

TABLE 2 Seizure burden and seizure freedom results from highest doses of prototype compounds tested

	Seizure freedom			Seizure burden			Seizure frequency		
Drug	**Baseline**	**Drug**	**Vehicle**	**Baseline**	**Drug**	**Vehicle**	**Baseline**	**Drug**	**Vehicle**
CBZ	2/11	7/11**,***	0/11	4.5 ± 2.1	0.6 ± 0.3**,***	9.3 ± 3.8	1.1 ± 0.5	0.2 ± 0.1*,****	2.1 ± 0.9
LCM	3/10	1/10	3/9	1.8 ± 0.7	1.5 ± 0.3	1.8 ± 0.7	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.2
LTG	0/12	1/12	2/11	13.9 ± 3.6	16.2 ± 5.9	8.2 ± 3.7	5.1 ± 1.9	7.1 ± 1.9	2.7 ± 0.9
PHT	1/11	1/11	2/11	6.3 ± 2.0	8.0 ± 4.1	8.5 ± 3.8	1.4 ± 0.4	1.8 ± 1.0	1.8 ± 0.8
PHB	1/12	10/12*,****	4/12	8.0 ± 3.1	0.2 ± 0.1*,***	7.0 ± 3.5	1.4 ± 0.5	<0.1*,***	1.5 ± 0.7
CLB	0/12	6/12*	2/12	14.5 ± 3.1	2.2 ± 1.1*,****	12.2 ± 5.9	2.9 ± 0.6	0.5 ± 0.2**,***	2.6 ± 1.3
CLZ	0/10	5/10**	1/10	6.3 ± 1.3	3.8 ± 2.5**	16.3 ± 11.8	1.7 ± 0.4	0.9 ± 0.6****	4.0 ± 2.9
TGB	0/12	2/12	1/12	15.0 ± 6.8	9.4 ± 4.5	8.3 ± 3.0	6.0 ± 3.4	2.4 ± 1.1	3.9 ± 1.7
EZG	0/11	8/10*,***	0/10	8.5 ± 2.0	0.2 ± 0.2*,***	13.2 ± 3.3	1.9 ± 0.4	0.1 ± 0.0*,***	2.9 ± 0.8
TPM	1/15	10/14*,***	0/15	15.7 ± 6.0	1.1 ± 0.5*,***	15.4 ± 6.6	6.2 ± 3.1	0.2 ± 0.1*,***	6.6 ± 2.8
PER	0/10	4/10**	2/10	6.7 ± 2.2	1.9 ± 0.9**	5.0 ± 1.1	3.1 ± 1.8	0.9 ± 0.6****	1.5 ± 0.5
LEV	3/11	4/11	1/11	7.2 ± 3.0	2.2 ± 1.3****	9.4 ± 2.7	2.5 ± 0.8	0.8 ± 0.4**	2.6 ± 0.8
GBP	1/15	7/15**,****	2/15	11.8 ± 6.2	4.4 ± 2.4**,****	9.8 ± 3.0	6.7 ± 3.6	3.1 ± 1.9**,***	3.3 ± 1.3
VPA	0/10	4/10**	2/9	10.4 ± 4.1	3.3 ± 1.0	7.7 ± 3.6	1.9 ± 0.8	0.7 ± 0.2	3.9 ± 2.3
EVR	0/11	2/11	2/11	4.9 ± 1.2	3.4 ± 1.5	3.4 ± 1.1	1.2 ± 0.3	1.0 ± 0.4	0.8 ± 0.2
ETX	0/10	3/10	1/10	5.6 ± 1.5	14.2 ± 6.0	6.5 ± 3.4	1.2 ± 0.3	3.0 ± 1.2	1.4 ± 0.7

Note: Efficacy was compared to both the baseline and vehicle treatment periods. Drugs are ordered by mechanism of action. For probability, Wilcoxon rank sum test was used for seizure burden and seizure frequency, and Fisher exact test for seizure freedom.

Abbreviations: CBZ, carbamazepine; CLB, clobazam; CLZ, clonazepam; ETX, ethosuximide; EVR, everolimus; EZG, ezogabine; GBP, gabapentin; LCM, lacosamide; LTG, lamotrigine; LEV, levetiracetam; PER, perampanel; PHB, phenobarbital; PHT, phenytoin; TGB, tiagabine; TPM, topiramate; VPA, valproate.

*$P < .01$, significantly different from baseline.

**$P < .05$, significantly different from baseline.

***$P < .01$, significantly different from vehicle.

****$P < .05$, significantly different from vehicle.

TABLE 3 Seizure burden and seizure freedom results from dose-response studies

	Seizure freedom			Seizure burden			Seizure frequency		
Dose	**Baseline**	**Drug**	**Vehicle**	**Baseline**	**Drug**	**Vehicle**	**Baseline**	**Drug**	**Vehicle**
TPM									
10	1/10	3/10	1/10	7.7 ± 4.7	1.9 ± 0.6****	6.5 ± 2.9	1.8 ± 1.1	0.4 ± 0.1	1.4 ± 0.6
19	0/9	3/9	0/9	8.0 ± 2.4	3.4 ± 2.3**,****	7.9 ± 2.2	1.5 ± 0.5	0.8 ± 0.5**	1.6 ± 0.5
37.5	2/10	6/9	4/10	5.1 ± 3.5	0.6 ± 0.4**	2.6 ± 1.3	1.0 ± 0.7	0.1 ± 0.1****	0.6 ± 0.3
75	1/9	8/9*,***	2/9	18.6 ± 10.5	0.2 ± 0.2*,***	4.6 ± 1.4	3.6 ± 1.9	0.0 ± 0.0*,***	1.0 ± 0.3
150	0/10	7/10*,***	1/10	9.6 ± 3.3	0.5 ± 0.3*,***	5.4 ± 1.2	1.9 ± 0.6	0.1 ± 0.1*,***	1.1 ± 0.3
300	1/15	10/14*,***	0/15	15.7 ± 6.0	1.1 ± 0.5*,***	15.4 ± 6.6	6.2 ± 3.1	0.2 ± 0.1*,***	6.6 ± 2.8
PHB									
7.5	1/10	4/10	1/10	11.6 ± 4.0	3.0 ± 1.2	10.0 ± 3.8	2.4 ± 0.8	0.6 ± 0.2	2.2 ± 0.9
15	1/10	5/10****	0/10	6.7 ± 2.0	2.8 ± 1.9**,****	10.5 ± 2.2	1.6 ± 0.5	1.4 ± 1.2*,****	2.3 ± 0.5
30	1/12	10/12*,****	4/12	8.0 ± 3.1	0.2 ± 0.1*,***	7.0 ± 3.5	1.4 ± 0.5	<0.1*,***	1.5 ± 0.7

Note: Drug efficacy was compared to both the baseline and vehicle treatment results. For probability, Wilcoxon rank sum test was used for seizure burden and seizure frequency, and Fisher exact test for seizure freedom.

Abbreviations: PHB, phenobarbital; TPM, topiramate.

*$P < .01$, significantly different from baseline.

**$P < .05$, significantly different from baseline.

***$P < .01$, significantly different from vehicle.

****$P < .05$, significantly different from vehicle.

a maximal reduction in seizure burden observed at doses of 75 and 100 mg/kg (see Table 3). In addition, TPM treatment increased seizure freedom in a dose-dependent manner; 10-19 mg/kg resulted in 33% seizure freedom, 37.5 mg/kg resulted in 67% seizure freedom, and 75-300 mg/kg resulted in 70%-88% seizure freedom (see Table 3). A probit analysis of seizure freedom calculated an ED_{50} of 31.1 mg/kg, with a 95% confidence interval of 1.0-69.8 mg/kg.

The initial screening dose of PHB was selected based on a maximum tolerated dose (30 mg/kg qd) in naive rats.[15] Although this dose was well tolerated, it was determined to be the maximum tolerable dose in this assay. Therefore, we also evaluated the effects on seizure burden and seizure freedom using 7.5 mg/kg (qd) and 15 mg/kg (qd). Treatment of rats at 15 mg/kg resulted in a significant reduction of seizure burden, whereas 7.5 mg/kg was without significant effect (Table 3). Furthermore, probit analysis of seizure freedom following PHB treatment calculated an ED_{50} of 12.0 mg/kg, with a 95% confidence interval of 0.13-21.7 mg/kg.

4 | DISCUSSION

In the present study, we evaluated 16 prototype ASDs, evaluated at therapeutically relevant doses, in the KA-SE model of recurrent seizures, and although some of these ASDs reduced seizure burden and conferred seizure freedom in some rats in this model, none of the compounds tested was able to produce full seizure freedom. This suggests that this model mirrors human pharmacoresistant epilepsy in that none of the currently approved ASDs can produce full seizure freedom, and thus seizures are refractory to some commonly used ASDs. This indicates that this model may be useful in identifying new potential drug compounds that produce seizure freedom where no other currently available therapy has succeeded.

Of the 16 compounds tested in this study, only PHB and EZG were highly effective in this model, as they significantly reduced seizure burden and produced seizure freedom in at least 75% of the animals tested at doses devoid of adverse effects. Comparable doses of PHB have been previously shown to suppress seizures to varying extents in post-SE spontaneously seizing rats.[29] Although the efficacy of PHB against spontaneous seizures in animal models suggests clinical utility of this drug in pharmacoresistant epilepsy, PHB has notable dose-limiting side effects, namely sedation.[30] By contrast, EZG has not been evaluated in chronic spontaneous seizure models to a great extent, although a dose of 40 mg/kg was effective against amygdala kindled seizures[31] and EZG can also block seizures in a rapid kindling model and the LTG kindled rat model.[32] Efficacy of EZG described in this report is in agreement with the broad spectrum of activity in numerous animal models of seizures of this compound[33] and suggests the potential usefulness in pharmacoresistant epilepsy of compounds targeting the Kv7 family of potassium channels. Although EZG was met with limited clinical use and has been removed from the market[34] due to adverse events, efficacy in this model suggests that potassium channel openers remain a viable target for therapy development.

Compounds with moderate efficacy in this model include those that significantly reduced seizure burden and produced seizure freedom in 25%-50% of animals tested. These include CBZ, CLB, CLZ, PER, LEV, GBP, and TPM. Previously, comparable doses of CBZ have been either effective[29] or ineffective[35] in reducing spontaneous seizures in post-SE models. By contrast, CLB, CLZ, PER, and GBP have not been previously evaluated for their effects against post-SE spontaneous seizures, although a comparable dose of PER was effective in reducing electrographic seizures in a mouse model of TLE.[36] LEV has previously been shown to reduce spontaneous seizures following pilocarpine-induced[37] or electrically induced[38] SE, but this effect can decline quickly after treatment is initiated.[38] TPM has previously been shown to dose-dependently inhibit spontaneous seizures.[9] In agreement with these findings, we observed a dose-dependent reduction of spontaneous seizures with TPM. Furthermore, we observed ~50% seizure reduction at doses of 10-37.5 mg/kg. Additional doses of TPM would be needed to verify a loss of this partial activity at doses < 10 mg/kg. Nevertheless, this model of spontaneous seizures is sensitive to many clinically available ASDs, suggesting that novel compounds found to be efficacious in this model would be potentially useful compounds to advance to clinical trials.

Compounds that were unable to reduce seizure burden or confer seizure freedom in this model include LCM, LTG, PHT, VPA, and ETX. LCM has not previously been evaluated for its effects against spontaneous seizures, and therefore our findings suggest limited use of this compound against pharmacoresistant epilepsy. LTG has previously been shown to be effective against post-SE spontaneous seizures in an amygdala stimulation model, and this efficacy was observed at a lower dose than evaluated in the present study.[35] Furthermore, in animals previously shown to be resistant to PHB in an electrically induced post-SE epilepsy model, LTG was effective in reducing seizures.[39] Therefore, LTG has had a history of mixed performance in refractory models of epilepsy, and its efficacy may be model-specific. Likewise, PHT has been previously shown to be effective in post-SE spontaneous seizure models, although doses were greater than the dose used in this study.[29,40] Furthermore, the dose of PHT used in this study was the maximum recommended dose (ie, maximally tolerated dose), and therefore higher doses were not attempted. The discrepancy between the present findings and those reported previously for PHT may therefore warrant further investigation. Conversely, a comparable dose of VPA was previously shown to be effective in post-SE spontaneously seizing animals,[29] and our findings with ETX are in agreement with previous studies,[29,35] demonstrating minimal efficacy against spontaneous seizures. Lack of efficacy of these compounds (LCM, LTG, PHT, VPA, and ETX) reaffirms that this model demonstrates notable pharmacoresistance. Furthermore, as each compound was evaluated at an estimated maximum tolerated dose, any seizure reduction that would have been observed at higher doses would have been difficult to interpret.

Mechanism of action did not appear to be a predictor of efficacy for ASDs tested in this model. Drugs highly efficacious at reducing seizure burden and increasing the number of seizure-free animals, namely CBZ, PHB, TPM, and EZG, came from distinct classes of ASDs. However, no drug resulted in complete seizure freedom for all animals, which may be the ideal benchmark for a new potential therapy. The model is also sensitive to drugs with moderate efficacy, including CLB, GBP, and LEV. There was no significant efficacy observed for EVR, LCM, LTG, PHT, CLZ, ETX, and VPA. Given that several compounds with clinical utility for focal seizures were unable to prevent seizures in the KA-SE model of TLE, we conclude that this animal model of epilepsy is a refractory model and thus fits well in the new testing scheme of the ETSP.[41]

There were unexpected tolerability issues experienced by all of the animals treated with LTG. The dose tested (30 mg/kg bid) was selected based on the literature[35] and acute models with this compound.[16] Treatment with LTG resulted in ataxia and lethargy, but these were not observed until several doses had been administered. This is suggestive of an accumulation effect of the drug or a metabolite, but verification of this observation was beyond the scope of this study. Due to the technical considerations and cost associated with this model, observations with LTG suggest the utility of tolerability assessments to confirm dose and dose interval prior to testing. Therefore, prior to using any investigational compound within the testing scheme of the ETSP, subchronic dosing for 3 days is evaluated in rats with KA-SE induced epilepsy prior to testing in a full study. Furthermore, the animals will be subjected to an extensive behavioral assay, the Irwin test,[42] to assess any potential tolerability issues. Participants will be strongly encouraged to provide pharmacokinetic data prior to being screened in this test, as these will enhance the interpretability of the experiments.

4.1 | Considerations of the experimental approach

One issue inherent to testing against any model of chronic seizures is the variability of seizure frequency and severity. As demonstrated in Tables 2 and 3, the present studies confirm that seizure frequency following KA-SE is highly variable. Although seizure variability is consistent with observations in human epilepsy,[43,44] evaluation of ASDs in this model is challenging and requires sufficiently powered treatment groups. Initial and post hoc analyses suggest that these studies are sufficiently powered to detect differences of at least 80% between drug and vehicle treatments with the sample sizes used. Therefore, differences in seizure burden

of <80% (moderate efficacy) should be interpreted carefully. This approach to evaluating novel ASDs also suggests that compounds must produce substantial differences in seizure burden to be considered as potential therapies for pharmacoresistant epilepsy.

In this approach, animals with the highest seizure frequencies in each cohort were selected for inclusion in each study. This bias toward a higher seizure burden was intentional, as the goal of this model is to identify compounds effective against pharmacoresistant epilepsy. The higher frequency of seizures increases the chance for seizures to be captured during the subchronic window in which drugs are administered. In some cases, where animals were lost to attrition during the study, replacement animals were used to maintain a sufficient group size. The replacement animals were those not initially enrolled in the study and therefore had a lower relative seizure rate during the evaluation period. However, there is no specific distinction drawn between "high seizing" and "low seizing" animals. It is a continuous spectrum and varies from cohort to cohort.

A concern of using a 2-day washout between treatment arms of the study is that it may be too short to avoid any carryover effects of drugs with a long half-life. This is a legitimate concern, and future studies, depending on known pharmacokinetics of a compound, may allow for more time between treatment arms of an individual study. Furthermore, only four drugs in this study, PHB, LTG, EVR, and ETX, demonstrated a potentially longer elimination half-life than the 2-day washout period (ie, five half-lives being longer than the 60 hours between a drug treatment and a vehicle treatment). Three of those treatments had no effect on seizure incidence or severity (LTG, EVR, and ETX) and therefore did not have an effect of carrying over into the vehicle arm of the study. For PHB, seizures were observed during and immediately following the crossover period (Figure 1A), suggesting that no carryover effect was present following the crossover to the vehicle treatment arm of the study. Furthermore, if the same cohort of animals was used for multiple drug studies, a minimum of 9 days occurred from the last day of drug treatment in the first study to the first day of the next drug treatment arm. Further, a new 7-day baseline period is collected between drug studies. Thus, in the present study, there was likely very little carryover effect of a compound to either the next treatment arm of an individual study or the next study.

Each cohort of animals used in these studies received up to four different ASDs during the course of the experiments. Previous exposure to ASDs may impact subsequent responses to additional ASDs in a single cohort of animals. Such disease-modifying changes have previously been demonstrated, and serve as the basis of the LTG-resistant amygdala kindling model.[31,45] Although ideally each compound would be tested in a new cohort, this would be cost-prohibitive as part of a screening paradigm. One strategy to address this would be to perform dose-response studies in naive cohorts of animals once a "hit" on an investigational compound is identified. However, given that patients with refractory epilepsy often receive a battery of different ASDs in the quest for seizure freedom, the approach we have undertaken to identify novel ASDs for refractory epilepsy is therefore translationally relevant.

5 | CONCLUSION

The subchronic dosing approach in the KA-SE model of spontaneous seizures described herein was implemented to establish a paradigm that balances moderate throughput with maintaining clinical translatability by challenging ASDs against spontaneous seizures in rodents with epilepsy. In addition, by using a large battery of prototype ASDs, we have determined that the seizures observed in the KA-SE model are refractory to numerous ASDs. Thus, this model is now being used as part of advanced testing in the screening for novel compounds for the treatment of refractory epilepsy in the NINDS-funded ETSP. The hope is that this novel approach could help to identify the next new ASD that will address the needs of the significant number of patients with uncontrolled, refractory epilepsy.

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CONFLICT OF INTEREST

K.E.T., T.G.N., J.H., S.F.E., and P.J.W. declare no conflicts of interest. C.S.M. serves as a consultant for Sea Pharmaceuticals. K.S.W. serves on the scientific advisory board for FutureNeuro,The Wholistic Research and Education Foundation, and the Cannabidiol Product Board for the Utah Department of Health. K.S.W. is a paid consultant for Xenon Pharmaceuticals. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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