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Pharmacological profile of the novel antiepileptic drug candidate padsevonil – characterization in rodent seizure and epilepsy models

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List of nonstandard abbreviations

AED	antiepileptic drug
ADD	afterdischarge duration
BRV	brivaracetam
BZD	benzodiazepine
CBZ	carbamazepine
GAERS	Genetic Absence Epilepsy Rats from Strasbourg
HPD	hippocampal paroxysmal discharges
IP	intraperitoneal
LEV	levetiracetam
LTG	lamotrigine
MES	maximal electroshock
MTLE	mesial temporal lobe epilepsy
PHT	phenytoin
PSL	padsevonil
PTZ	pentylenetetrazol
RTG	retigabine
SC	subcutaneous
SV2	synaptic vesicle protein 2
TI	therapeutic index
TPM	topiramate
VPA	valproate

Recommended section assignment to guide listing in table of contents Neuropharmacology

ABSTRACT

The antiepileptic drug (AED) candidate, padsevonil, is the first in a novel class of drugs that bind to synaptic vesicle 2 (SV2) proteins, and to the $GABA_A$ receptor benzodiazepine site, allowing for pre- and postsynaptic activity, respectively. In acute seizure models, padsevonil provided potent, dose-dependent protection against seizures induced by administration of pilocarpine or 11-deoxycortisol, and those induced acoustically or through 6 Hz stimulation; it was less potent in the pentylenetetrazol, bicuculline, and maximal electroshock models. Padsevonil displayed dose-dependent protective effects in chronic epilepsy models, including the intrahippocampal kainate and GAERS models, which represent human mesial temporal lobe and absence epilepsy, respectively. In the amygdala kindling model, which is predictive of efficacy against focal to bilateral tonic-clonic seizures, padsevonil provided significant protection in kindled rodents; in mice specifically, it was the most potent AED compared with nine others with different mechanisms of action. Its therapeutic index was also the highest, potentially translating into a favorable efficacy and tolerability profile in humans. Importantly, in contrast to diazepam, tolerance to padsevonil's antiseizure effects was not observed in the pentylenetetrazol-induced clonic seizure threshold test. Further results in the 6 Hz model showed that padsevonil provided significantly greater protection than the combination of diazepam with either levetiracetam or brivaracetam, both selective SV2A ligands. This observation suggests that padsevonil's unique mechanism of action confers antiseizure properties beyond the combination of compounds targeting SV2A and the benzodiazepine site. Overall, padsevonil displayed robust efficacy across validated seizure and epilepsy models, including those considered to represent drug-resistant epilepsy.

SIGNIFICANCE STATEMENT

Padsevonil, a first-in-class antiepileptic drug candidate, targets synaptic vesicle 2 (SV2) proteins and the benzodiazepine site of $GABA_A$ receptors. It demonstrated robust efficacy across a broad range of rodent seizure and epilepsy models, several representing drug-resistant epilepsy. Furthermore, in one rodent model, its efficacy extended beyond the combination of drugs interacting separately with SV2 or the benzodiazepine site. Padsevonil displayed a high therapeutic index, potentially translating into a favorable safety profile in humans; tolerance to antiseizure effects was not observed.

KEY WORDS

animal models, anticonvulsants, benzodiazepines, drug tolerance/dependence, GABA receptors, kindling, safety pharmacology, seizures

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INTRODUCTION

Epilepsy is one of the most common neurological diseases worldwide, and is associated with a significant healthcare burden (Devinsky et al., 2018; Thijs et al., 2019). For most patients with epilepsy, antiepileptic drugs (AEDs) are the mainstay of therapy, which must be taken on a long-term, often lifelong basis (Trinka et al., 2012; Thijs et al., 2019). Antiepileptic drugs approved in the last decade display good safety and pharmacokinetic profiles, but improved efficacy over first-generation AEDs has not been demonstrated in clinical studies so far (Chen et al., 2018), and approximately a third of patients with epilepsy continue to experience poorly controlled seizures despite treatment, ie, drug-resistant epilepsy (DRE) (Kwan et al., 2010; Kalilani et al., 2018; Chen et al., 2018). Most AEDs were discovered by initial demonstration of their antiseizure activity in simple, classic seizure models, such as the maximal electroshock (MES) and pentylenetetrazol (PTZ) tests, which are highly predictive of clinical efficacy in epilepsy, but not DRE (Löscher et al., 2013).

Polytherapy is a frequent treatment strategy for patients with DRE, since a substantial proportion will require more than one AED to reduce their seizure burden (French and Faught, 2009; Brodie and Sills, 2011). The combination of selected AEDs should allow for synergistic or additive efficacy without any detrimental impact on safety and tolerability (French and Faught, 2009; Brodie and Sills, 2011); however, a nonclinical mechanistic rationale for clinically used AED combinations is often lacking or have not yet translated into superior efficacy.

Levetiracetam (LEV) is an AED that exerts its therapeutic activity primarily by binding to the synaptic vesicle (SV)2A protein (Lynch et al., 2004) and shows a distinctive profile in nonclinical seizure models. While ineffective in standard models used traditionally for AED discovery, such as the MES and PTZ tests, LEV provided protection against seizures in models of acquired and genetic epilepsies (Klitgaard et al., 1998), subsequently translating to broad-spectrum clinical efficacy in humans (Klitgaard and Verdru, 2007). In the audiogenic seizure and amygdala kindling models, LEV increased the potency of several AEDs and experimental agents that interfere with ligand-gated ion channels, particularly those that enhance GABA-mediated inhibition (Kaminski et al., 2009). Importantly, the increase in potency was devoid of additional

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adverse effects (ie, motor impairment) as assessed by the rotarod test; on the contrary, it was associated with an increase in the therapeutic index (Kaminski et al., 2009). Assuming a potential synergistic interaction between ligands that act via SV2 and $GABA_A$ receptors ($GABA_ARs$), a rational medicinal chemistry design program was initiated to develop a single molecular entity that could target both.

The outcome of this discovery program was the identification of padsevonil (PSL), the first rationally-designed AED candidate that acts selectively on both pre- and postsynaptic targets. Presynaptically, as a SV2 ligand, PSL displays high affinity (nM) not only for SV2A, but also for the other two protein isoforms, SV2B and SV2C. The latter markedly distinguishes the profile of PSL from that of LEV and brivaracetam (BRV), which are selective SV2A ligands and furthermore, have no established postsynaptic activity. Postsynaptically, as a positive allosteric modulator of $GABA_ARs$, PSL displays low-to-moderate (μM) binding affinity for the benzodiazepine (BZD) site in recombinant human $GABA_ARs$ and human and rat brain membrane preparations, where it shows a partial agonist profile. This profile was selected specifically to minimize CNS and respiratory adverse effects, tolerance development and abuse potential typically associated with the use of BZDs that are full agonists (Rundfeldt and Löscher, 2014). The detailed pharmacological and mechanistic profile of PSL is described in the companion paper (Wood et al., 2019). In this report, we describe the activity of PSL in a variety of rodent seizure and epilepsy models and compare its activity with that of mechanistically diverse and clinically used AEDs. We also compare the potential of PSL for development of tolerance with that of the BZD, diazepam (DZP), after chronic dosing in mice.

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MATERIALS AND METHODS

Animals

All experiments were conducted in compliance with guidelines issued by the ethics committee for animal experimentation according to Belgian law. Those conducted as part of the murine intrahippocampal kainate model of mesial temporal lobe epilepsy (MTLE) were performed at Synapcell (Grenoble, France); experiments were approved by the European Technology Platform for Global Animal Health and performed in accordance with the European Committee Council directive (2010/63/EU). All efforts were made to minimize animal suffering.

Female, genetically sound-sensitive mice (20–24 g) were derived from a DBA strain from the Laboratory of Acoustic Physiology (Paris, France) and bred in Charles River Laboratories, Italy. Male NMRI mice weighing 20–35 g were used in all other acute electrically-and chemically-induced seizure tests, as well as the rotarod and tolerance tests. Male C57BL/6J mice, weighing 25–34 g, were used for the murine model of amygdala kindling. For the rat model of amygdala kindling, male Sprague-Dawley rats weighing 300–350 g at the initiation of kindling were used. Male Wistar rats of the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) strain were used at a body weight of 280–400 g. Male Sprague-Dawley (200–240 g) rats were used for the rotarod tests. Animals were obtained from Charles River Laboratories, France, and housed in a holding room under a 12-h light-dark cycle with lights on at 06:00. Temperature was maintained at 20–24°C, relative humidity at 40–70% and the rate of air replacement was at least 15 times an hour. Animals had ad libitum access to standard dry pellet food and tap water.

For the intrahippocampal kainate model, male C57BL/6 mice (11 weeks of age) were obtained from Janvier (France) and housed in cages on wood litter for 8 days with free access to food and water until surgery. Animal housing was maintained under artificial lighting from 8:00 to 20:00.

Drugs and chemicals

PSL ((4R)-4-(2-chloro-2,2-difluoroethyl)-1-{[2-(methoxymethyl)-6-(trifluoromethyl)imidazo[2,1-b][1,3,4]thiadiazol-5-yl]methyl}pyrrolidin-2-one, LEV (2S-(2-oxo-1-pyrrolidinyl)butanamide), and BRV (2S-2-[(4R)-2-oxo-4-propylpyrrolidin-1-yl] butanamide) were synthesized at UCB (Braine-l'Alleud, Belgium). All other reagents were of analytical grade and obtained from conventional

commercial sources. PSL was dissolved in 10 mM citrate buffer, 1.5% methylcellulose, 0.1% Tween 80, 0.1% silicone antifoam, and LEV and BRV in saline.

Experimental procedures

Murine models of acutely-induced seizures

Methods for the audiogenic, electrically- and chemically-induced seizure models have been previously described in detail (Jackson et al., 1996; Klitgaard et al., 1998; Kaminski et al., 2008; Kaminski et al., 2011; Leclercq and Kaminski, 2015).

For the audiogenic seizures model, mice were placed, one at a time, in a sound attenuated chamber, where audiogenic seizures were induced through application of an acoustic stimulus (85 dB, 10–20 kHz, 30 s). The proportion of mice protected against clonic seizures was used to determine antiseizure activity. This endpoint was chosen because a correlation between SV2A affinity and efficacy against clonic seizures has been previously demonstrated (Kaminski et al., 2008).

For electrically-induced seizures, the MES and 6 Hz models were used. In MES, 50 mA currents were delivered at a constant pulse frequency of 50 Hz and a duration of 0.2 s. The proportion of mice protected against tonic hindlimb extension after stimulation was used to determine antiseizure activity, as well as dose-response. In the 6 Hz model, 44 mA currents were delivered with 0.2 ms monopolar pulses at 6 Hz for a duration of 3 s. After stimulation, mice were observed for 30 s and the duration of immobility (stunned posture) was noted. The proportion showing immobility for <7 s was used as the endpoint for seizure protection, as previously described (Leclercq and Kaminski, 2015).

For the chemically-induced seizure models, pentylenetetrazol (PTZ; 89 mg/kg) and bicuculline (3 mg/kg) were administered subcutaneously (sc), and pilocarpine (373 mg/kg) administered intraperitoneally (ip). In the latter model, the peripheral cholinergic effect was blocked via administration of methylscopolamine (1 mg/kg, ip) 30 min before administration of pilocarpine. The proportion of mice protected against clonic seizures in all four extremities during a 60-min observation period after drug administration was used to determine antiseizure activity. 11-

deoxycortisol (1.0–1.2 mmol/kg) was infused through the lateral tail vein, and protection against generalized seizures during the 60-min observation period after infusion was used to assess antiseizure activity.

In all experiments, PSL was tested at doses ranging from 0.014 to 181.4 mg/kg. It was administered (10 ml/kg) 30 min before testing, except for audiogenic seizure testing where pre-administration time was 15 min. Testing was initiated in the audiogenic model, before having conducted thorough pharmacokinetics assessment; pre-administration time was subsequently adapted for screening in other models. Each experiment consisted of independent groups of 10–14 mice, with one group receiving vehicle (control) and the others different PSL doses. The experimenter was unaware of the nature of the compound administered.

Comparative 6 Hz study

The 6 Hz model was used to compare the protective effect of PSL with that of LEV, BRV and diazepam (DZP), as well as the combination of LEV or BRV with DZP. To allow for a direct, objective comparison, drugs were administered at doses to provide similar in vivo target occupancy. PSL was administered at a dose of 0.17 mg/kg, which is expected to provide 2% and 35% occupancy at the BZD site and SV2A, respectively, based on results of in vivo occupancy studies (Wood et al., 2019). Correspondingly, LEV and BRV were tested at 1.83 and 0.42 mg/kg, respectively, to provide 35% SV2A occupancy, and DZP at 0.017 mg/kg to provide 2% occupancy at the BZD site. All drugs were administered ip 30 min before testing except for LEV, which was administered 60 min before testing. Each experimental arm consisted of 15 or 16 mice.

Amygdala kindling

Protocols used for the both mouse and rat amygdala kindling experiments have been described previously (Löscher et al., 1986). For the rat model, experiments consisted of five groups of eight fully kindled rats, each group receiving different doses of PSL (0.14–13.9 mg/kg) administered ip (5 ml/kg) 30 min before stimulation with the same supra-maximal current (500 μA, 1 s) used for the induction of kindling. Similarly, six groups of 8–9 mice received different doses of PSL (0.014 –13.85 mg/kg) administered ip 30 min before testing with the same supra-maximal stimulation

current (250 μA, 1 s) used for the induction of kindling. Additionally, similar experiments were conducted in groups of mice receiving BRV, carbamazepine (CBZ), DZP, LEV, lamotrigine (LTG), phenytoin (PHT), topiramate (TPM), retigabine (RTG) or valproate (VPA).

The effects of drugs on three parameters were tested in fully kindled animals. First, as a measure of the drug's effect on seizure severity, the behavioral effects of the stimulation were scored according to the scale described by Racine, where 0=no reaction, 1= blinking and/or mild facial twitches and chewing, 2=head nodding and/or severe facial clonus, 3=myoclonic jerks of the forelimbs, 4=clonic seizures of the forelimbs with rearing and 5=generalized clonic seizures associated with loss of balance (Racine, 1972). Second, the proportion of animals protected against generalized seizures (scores 3–5) was used to determine the drugs' ED_{50} and antiseizure activity. Third, the electroencephalographic effect of the stimulation was determined by measuring the stimulation-induced afterdischarge duration (ADD), defined as an EEG activity with an amplitude at least twice that of the pre-stimulus recording and a frequency >1 Hz.

Murine intrahippocampal kainate mouse model of mesial temporal lobe epilepsy

Experiments were performed as previously described (Riban et al., 2002; Duveau et al., 2016). Briefly, male C57BL/6 mice (n=20) were surgically injected with kainate (1 nmol) in the right dorsal hippocampus. Bipolar electroencephalography (EEG) electrodes were implanted into the injected hippocampus, with additional monopolar surface electrodes placed over the frontoparietal cortex and cerebellum. After a 5-week period of epileptogenesis, mice (n=9) displaying hippocampal paroxysmal discharges (HPDs; ≥20/hour) without any generalized seizures were selected. Baseline EEG (20 minutes) was recorded before injection of vehicle (10 mM citrate buffer, 1.5% methylcellulose, 0.1% Tween 80, 0.1% silicone antifoam) or PSL (1, 3, 10, or 30 mg/kg; ip) and recording continued for an additional 90 minutes. Stress caused by handling and drug administration cause a transient decrease in the number of HPDs, as observed reproducibly in vehicle-treated animals. Therefore, the number and duration of HPDs were measured and analyzed for 80 min, after discarding the first 10 min post-drug administration. PSL doses were administered in a randomized crossover manner.

Spike-wave discharges in Genetic Absence Epilepsy Rat from Strasbourg (GAERS)

Four platinum electrodes were implanted bilaterally in the frontal and occipital cortices as described previously (Matagne et al., 2009). After a 2-week recovery period, rats were injected with either vehicle or PSL; EEG was recorded continuously over consecutive 20-min intervals starting 20 mins before, and up to 120 min after drug administration. The cumulative duration of spontaneous spike- and-wave discharges (SWDs) in each 20-min interval was measured by a semi-automatic program. PSL was administered at doses equal to 0.14 mg/kg, 0.43 mg/kg, 1.38 mg/kg and 4.33 mg/kg in a dose volume of 5 ml/kg body weight. Control group received vehicle injection (ip, 5 ml/kg body weight). Eight rats were used in these experiments with a cross-over design in which each animal served as its own control after injection of vehicle.

Tolerance

To determine whether mice developed tolerance to PSL's antiseizure effects, its impact on the PTZ-induced clonic seizure threshold was tested. For comparison, the tolerance potential of diazepam, a full agonist at the BDZ site, was also evaluated. This test is widely described as a nonclinical tool for assessment of tolerance-like effects of AEDs (Rundfeldt et al., 1995). Briefly, the test consists of two steps; in the first, the PTZ threshold dose for inducing seizures and the ED_{97} of a given AED in providing protection against these PTZ-induced clonic seizures are determined. In the subsequent step, tolerance to the protective effect of the AED after repeated administration is determined.

For the first step, an iv infusion of PTZ (5 mg/ml) was administered into the tail vein of freely moving mice and the time to the three stages of seizures (twitch, clonic and tonic) was noted. Padsevonil, DZP or vehicle was administered ip (10 ml/kg) 30 minutes before PTZ infusion to determine the dose that increased the PTZ threshold dose by 97% (ED_{97}). Different treatments were randomly distributed within each group of mice (6, 8 or 10 mice per group for PSL, and 6, 10 or 11 mice per group for DZP experiments) with injections at 5 min intervals. In the second step, mice were administered with the previously selected PSL/DZP dose (ED_{97}) or vehicle, twice daily for 4 consecutive days (n=12 each group). On day 5, they were treated with PSL/DZP or vehicle 30 min before assessment of their respective seizure threshold, following iv infusion of PTZ. There were four experimental groups, as described in Table 1.

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Rotarod

The impact of PSL on motor activity was evaluated using the rotarod test in both mice and rats using previously described protocols (Klitgaard et al., 1998). Animals were trained and only those able to remain on the rod for at least 60 s in three consecutive trials were used in the tests. In mice, PSL was administered ip (10 ml/kg) 30 min before testing; one group (control) received vehicle and the others PSL doses 4.3–77.9 mg/kg (n=10 each group). In rats, PSL was administered ip (5 ml/kg) 30 min before testing; one group (control) received the vehicle and the others PSL doses 4.3–43.3 mg/kg (n=8 each group). The median tolerated dose, at which toxicity, or impairment of motor coordination occurs in 50% of animals (TD_{50}) was calculated and used for determining the therapeutic index (TI) of PSL. The TI is defined as ratio between doses producing motor impairment (TD_{50}) and doses providing protection against seizures (ED_{50}). To compare the TI of PSL with that of other AEDs in amygdala kindling model, the TD_{50} of the following drugs was also determined in naïve mice: BRV, CBZ, DZP, LEV, LTG, PHT, TPM, RTG and VPA.

Data analysis

Unless otherwise noted, ED_{50} and its associated 95% confidence intervals (CI) were calculated using a non-linear fitting of the dose-response curve with GraphPad Prism version 4 (GraphPad Software, San Diego, CA, USA). In the 6 Hz comparative study, Fisher's exact test was used for statistical comparisons of the number of animals protected with PSL and with the combinations of LEV or BRV with DZP using GraphPad Prism (as above).

Amygdala kindling

Significant differences between compound and vehicle in the median behavioral seizure score, in protection against generalized seizures, and in the ADD were evaluated with Wilcoxon signed rank test, Fisher's exact test and Mann-Whitney U-test, respectively. All statistical analyses were performed with GraphPad Prism (as above).

Intrahippocampal kainate model

Statistical analyses were performed with GraphPad Prism version 7 using two-way analysis of variance (ANOVA) for repeated measures, with the factors time and compound dose (with

repeated measures applying only on the time factor), followed by Bonferroni's multiple comparison test.

Spike-wave discharges in GAERS

For each treatment, the mean cumulative duration of SWDs (± SEM) was calculated for each 20-min interval. Results for each 20-min interval were compared with those of vehicle treatment using a two-way analysis of variance (ANOVA) with repeated measures, followed by a post hoc Bonferroni multiple comparison test ($p<0.05$), using GraphPad Prism. Due to the high variability of the responses observed in each 20-min interval for different rats, data were further analyzed using the cumulative duration of SWDs covering the total post-drug observation period (120 min). This allowed application of non-linear regression curve fitting of the results and estimation of the protective ED_{50}.

Tolerance

The effective dose increasing the PTZ threshold by 97% (ED_{97}) was calculated using a non-linear fitting of individual values of the dose-response curve (SAS/STAT^R Software version 9.1). A one-way ANOVA followed by a Tukey multiple comparison test were performed with individual calculated doses of PTZ inducing clonic seizures in the four groups of mice; statistically significant differences between (chronic vehicle + test compound ED_{97} dose) and (chronic test compound ED_{97} dose + test compound ED_{97} dose) were used for assessing development of tolerance.

RESULTS

Murine models of acutely-induced seizures

Administration of PSL provided potent, dose-dependent protection against seizures induced by 6 Hz stimulation, an acoustic stimulus and a bolus dose of pilocarpine (ED_{50} 0.16, 0.17 and 0.19 mg/kg, respectively). The potency of PSL in these three models was greater than that of LEV and BRV (ineffective in the pilocarpine model) (Table 2). PSL also provided dose-dependent protection against clonic seizures induced by a bolus dose of PTZ. Its potency in this model was higher than that of BRV, while LEV was ineffective. In the 11-deoxycortisol model, PSL provided dose-dependent and almost complete protection against seizures; at the highest dose tested (43.3 mg/kg), 90% of animals were protected. Brivaracetam was ineffective in this model, while LEV provided only limited protection at the highest doses tested. PSL showed low potency against seizures induced by a bolus of bicuculline, while LEV and BRV were ineffective in this model. The lowest potency was seen in the MES model (ED_{50} 92.8 mg/kg). The lack of activity or low potency in this model was also observed with LEV and BRV.

Comparative 6 Hz study

The protective effect of PSL in the 6 Hz model was compared with that of LEV, BRV and DZP alone, and with the combinations of LEV or BRV with DZP at doses expected to provide similar occupancy at SV2A (35%) or the BZD site (2%). PSL protected a greater proportion of mice than LEV, BRV and DZP alone or in combination (Figure 1). The difference in the protection offered by PSL and that of the LEV/DZP and BRV/DZP combinations was statistically significant ($p=0.021$ and $p=0.0008$, respectively, Fisher's exact test). The difference in the protection provided by BRV and the BRV/DZP combination or the LEV and LEV/DZP combination was not significant ($p=0.4$ and $p=0.145$, respectively).

Amygdala kindling

The protective effect of PSL against seizures was evaluated in fully kindled animals using three parameters. In rats, PSL provided dose-dependent and complete protection against focal to bilateral seizures (secondary generalized seizures). The reduction in the proportion of rats displaying generalized seizures at doses of 2.4, 4.3 and 13.9 mg/kg was statistically significant,

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with 100% of animals protected at the highest dose (Figure 2, right panel). The ED_{50} was estimated to be 2.43 (2.41–2.46) mg/kg. Significant, dose-dependent reductions in the median seizure severity score and ADD were also observed with PSL, starting from a dose of 2.4 mg/kg.

In mice, just as in rats, PSL significantly reduced the proportion of animals with focal to bilateral seizures and the median seizure severity score starting from a dose of 1.4 mg/kg (Figure 2, left panel). Based on the proportion of mice protected from focal to bilateral seizures (secondary generalized seizures), the ED_{50} was estimated to be 1.2 (0.43–3.40) mg/kg. PSL also reduced the ADD, but only at the highest dose tested (13.9 mg/kg); at lower doses an increase was observed, with the increase (40%) at the 1.38 mg/kg dose being statistically significant.

The TI of PSL in kindled mice was 9.8, which was relatively high compared with that of BRV and VPA, 2.8 and 1.2, respectively (Table 3). Other AEDs tested in this model displayed only partial protection against generalized seizures; therefore, it was not possible to calculate their TI.

Intrahippocampal kainate model

PSL administration (1, 3, 10, or 30 mg/kg) resulted in dose-dependent and statistically significant reductions in the number of HPDs compared with vehicle or baseline, between 30 and 70 minutes after administration. PSL 10 and 30 mg/kg doses were associated with significant reductions in the number of HPDs from 10 to 30 minutes after administration (Figure 3, top panel). Dose-dependent effects of PSL were also observed when the cumulated duration of HPDs was calculated, with all PSL doses associated with significant reductions compared with vehicle 50–70 minutes after administration. Maximal effects were observed with 10 and 30 mg/kg doses after 10–30 minutes (Figure 3, bottom panel).

Spike-wave discharges in GAERS

PSL (0.14–4.33 mg/kg) produced a dose-related suppression in spontaneous SWDs, which was statistically significant from the 0.43 mg/kg dose – the suppression was almost complete at a dose of 4.33 mg/kg (Figure 4). The effect was apparent in the first 20-min test interval and persisted throughout the recording period (up to 120 min). Treatment with PSL also resulted in a

dose-dependent reduction in the cumulative duration of spontaneous SWDs recorded over the 120 min post-drug period (ED_{50} 0.87 mg/kg).

Tolerance

Having established the PTZ threshold dose for inducing clonic seizures, PSL and DZP were tested. Both drugs increased the seizure threshold in a dose-dependent manner; the ED_{97} of PSL was 15.9 mg/kg and that of DZP 2.1 mg/kg.

Animals that were treated twice daily for 4 days with vehicle, PSL or DZP at the calculated ED_{97} dose, were injected again on day 5 with the same dose before assessment of the seizure threshold following iv PTZ infusion. Treatment with PSL (15.9 mg/kg) caused a significant increase in the PTZ threshold dose with a similar magnitude in both groups (mice chronically treated with vehicle or drug). The difference in the mean doses of PTZ that induced seizures in mice treated chronically with vehicle and those treated chronically with PSL was not statistically significant (Figure 5).

Diazepam (2.1 mg/kg) also caused a significant increase in the PTZ threshold dose for clonic seizures in both groups, but with a much lower magnitude in mice chronically treated with DZP, reflecting development of tolerance to its antiseizure effects. The mean dose of PTZ inducing clonic seizures in mice treated chronically with the vehicle was comparable to the mean dose calculated in mice treated chronically with DZP (Figure 5).

Rotarod

Administration of PSL resulted in a dose-dependent impairment in the performance of both mice and rats in the rotarod test; TD_{50} values were 11.8 (9.2–15.2) mg/kg and 24.4 (15.0–39.7) mg/kg, respectively. Using these, and ED_{50} values determined in various models, the TI of PSL was calculated. PSL had a TI of 28 in the GAERS and 10 in the rat amygdala kindling models. In mice, the TI was calculated to be 69 in the audiogenic, 62 in the pilocarpine-induced, 74 in the 6 Hz-induced and 2.5 in the PTZ-induced seizure tests. As noted above, the TI in the murine amygdala kindling model was 9.8.

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DISCUSSION

PSL is the first in a novel class of drugs that bind to SV2 proteins and the BZD site on $GABA_A$Rs. As shown in studies reported here, this pre- and postsynaptic activity results in a distinct pharmacological profile across a wide range of seizure and epilepsy models representing focal and generalized epilepsy in humans.

The MES and PTZ tests, considered gold standards for early detection of antiseizure activity, are used for screening candidate compounds (Bialer and White, 2010; Klitgaard, 2005). LEV is inactive in both models, while BRV, a more potent and selective SV2A ligand than LEV shows weak activity in both models (Matagne et al., 2008). Similarly, PSL showed activity in both models, but its potency, while greater than that of BRV, was also relatively weak. PSL's effect was greater in the PTZ than in the MES test, which is likely to be mediated partially via the BZD site, since BZDs show high potency in this model (Löscher et al., 2011). PSL also showed relatively low potency in the bicuculline test, where typical BZDs are active, but not abecarnil, a partial agonist at the BZD site (Turski et al.,1990); consequently, low activity was expected, since both LEV or BRV are inactive in this test, and PSL shows a partial agonist profile.

PSL provided potent, dose-dependent protection against seizures induced in sound-sensitive mice, a genetic model of generalized epilepsy. BRV is active in this model, while LEV shows lower potency, correlating with their SV2A binding affinity (Matagne et al., 2008; Kaminski et al., 2008). PSL also provided strong protection against pilocarpine-induced clonic seizures, where in contrast to the audiogenic model, BRV is ineffective, while LEV shows relatively high potency. Among acute models, PSL displayed the highest potency in the 6 Hz model (ED_{50} 0.16 mg/kg), used as a test for protection against drug-resistant focal seizures, since many older (eg, CBZ, phenobarbital, PHT) and newer AEDs (eg, felbamate, LTG, tiagabine, TPM) fail to fully protect animals (Barton et al., 2001). This model was also used to compare the efficacy of PSL against LEV, BRV, DZP and LEV/DZP and BRV/DZP combinations. Importantly, for this comparison, doses calculated to provide similar SV2A and BZD site occupancy were used for the SV2 ligands and DZP, 35% and 2%, respectively. Given that LEV and BRV require 80% SV2A for antiseizure activity in nonclinical models (Gillard et al., 2011), low level occupancy was selected

in these experiments to further differentiate PSL activity. Protection offered by PSL, even at 35% SV2A occupancy was almost 70% and significantly greater than that provided by either LEV or BRV in combination with DZP. These observations suggest that PSL's antiseizure properties are due to a differentiated mode of action that provides greater protection than co-administration of an SV2A ligand and a BZD. Furthermore, the interaction of PSL with SV2B and SV2C may also contribute to enhanced antiseizure effects.

The 11-deoxycortisol model is also considered to represent drug-resistant seizures (Kaminski et al., 2011). LEV offers only partial protection at the highest doses, while phenytoin, carbamazepine and valproate are ineffective; BRV has also proven to be ineffective. Padsevonil, however, demonstrated robust efficacy, providing dose-dependent protection with an ED_{50} of 10 mg/kg. 11-deoxycortisol induces paroxysmal epileptiform network activity and seizures by significantly reducing GABAergic neurotransmission, which may explain why many AEDs, but not PSL, fail to suppress seizures (Kaminski et al., 2011).

The intrahippocampal kainate model displays many features of human MTLE (Riban et al., 2002, Pernot et al., 2011). Unilateral injection of kainate in the dorsal hippocampus results in neuronal loss, mossy fiber sprouting, and dispersion of granule cells, followed by spontaneous and recurrent HPDs observed on EEG (Suzuki et al., 1995; Mitsuya et al., 2009). Focal seizures remain frequent and stable during the animal's life, and importantly, resistant to most AEDs (Riban et al., 2002, Duveau et al., 2016), as in human MTLE (Engel et al., 1997). PSL displayed dose-dependent protective effects, with almost complete and long-lasting inhibition of HPDs at the highest dose (30 mg/kg).

The GAERS model is considered predictive of human absence epilepsy (Danober et al., 1998; van Luijtelaar et al., 2002). LEV has a weak effect in this model, while BRV suppresses spontaneous SWDs with complete inhibition at the highest dose (67.9 mg/kg), which again correlates with their affinity for SV2A (Matagne et al., 2008; Kaminski et al., 2008). PSL showed higher potency than BRV and markedly suppressed spontaneous SWDs with almost complete inhibition at the highest dose (4.33 mg/kg), providing further evidence for PSL's broad spectrum of activity against both focal and generalized seizures.

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AED activity in the amygdala kindling model is predictive of efficacy against focal to bilateral tonic-clonic seizures in the clinical setting (Löscher and Schmidt, 1988). Electrographic and behavioral symptoms of seizures are initially localized at the site of stimulation, but rapidly evolve to bilateral activity, with seizures increasing in length and severity upon repeated stimulation (Löscher et al., 2011, White et al., 2003). In the rat model, PSL significantly reduced the proportion of animals displaying seizures, with 100% of animals protected at the highest dose. PSL also reduced the seizure severity score and the ADD, indicating effects on both local seizure discharge and seizure spread, or evolution to bilateral seizures. PSL was substantially more potent than LEV and BRV; while BRV significantly reduces the ADD at only high doses, LEV has no effect (Klitgaard et al., 2016). PSL's effects in the mouse kindling model mirrored those in the rat model, with one exception; it reduced the ADD only at the highest dose. The reduction in ADD at the 13.9 mg/kg dose and the increase at the 1.38 mg/kg dose were both statistically significant, somewhat similar to the effects of low BRV doses (Matagne et al., 2008). In the mouse model, PSL was the most potent compared with nine other mechanistically different AEDs. It was only possible to determine the ED_{50} of BRV and VPA since the others failed to provide full protection at high doses. Results were also used to compare the TI of AEDs, a measure of the margin between antiseizure and adverse effects, expressed by the ratio between doses producing adverse effects and seizure protection (TD_{50}/ED_{50}); the greater the TI, the greater the separation between toxic and therapeutic doses. In mice, PSL TD_{50} was 12 mg/kg and the ED_{50} 1.2 mg/kg, resulting in a TI of 10. In comparison, the TI of BRV and VPA were 3 and 1, respectively. Since the protective ED_{50} of the remaining AEDs could not be determined, due to limited efficacy, their TI could not be calculated. Overall, these findings indicate that PSL has full efficacy in the kindling model, displaying a high TI, potentially translating into higher efficacy and improved tolerability in humans.

PSL was designed to exert its therapeutic activity via two distinct mechanisms: as a SV2 ligand, and as a partial agonist at the BZD site of the $GABA_AR$. Partial agonism was selected based on evidence suggesting that the likelihood of developing tolerance to therapeutic effects is lower compared with full agonists (Miller et al., 1990; Serra et al., 1994; Rundfeldt et al., 2014). Clinical evidence supports these observations. Clobazam, a BZD and partial agonist, has been used

successfully for the treatment of patients with Lennox-Gastaut syndrome (Faulkner, 2015; Gauthier and Mattson, 2015). Results of a long-term trial demonstrated sustained seizure control at stable dosages over a 3-year period (Conry et al., 2014; Gidal et al., 2016). Another partial agonist, abecarnil, has shown efficacy in the treatment of patients with photosensitive epilepsy without development of tolerance (Kasteleijn-Nolst Trenité et al., 2016). To evaluate PSL's tolerance potential, the PTZ-induced clonic seizure threshold test was used, where the ability of AEDs to increase the seizure threshold is assessed after acute, and twice daily PTZ infusion for 4 days at the ED_{97} dose (Rundfeldt et al., 1995). Under both regimens, PSL increased the threshold for PTZ-induced seizures to the same extent, indicating that tolerance was not developed; in contrast, DZP showed significant loss in its ability to increase the threshold.

The precise role of SV2A in synaptic transmission, and how ligand binding translates into antiseizure activity remain to be fully elucidated, yet the strength of ligands' antiseizure activity correlates with their binding affinity – BRV's greater affinity for SV2A over that of LEV translated into superior antiseizure activity in animal models (Matagne et al., 2008; Kaminski et al., 2008). In turn, PSL's affinity for SV2A has been shown to be greater than that of BRV (Wood et al., 2019). PSL's additional actions on SV2B and SV2C, and the $GABA_AR$ BZD site, have resulted in a nonclinical profile that differs substantially from that of other AEDs. Additional evidence from the present studies suggest that the pre- and postsynaptic mechanism of action confers enhanced antiseizure properties beyond the combination of compounds targeting SV2A and the BZD site. PSL's highly differentiated antiseizure profile suggests a robust therapeutic benefit, an observation supported by results of a Phase IIb proof-of-concept trial (Muglia et al., 2017).

CONFLICTS OF INTEREST

All authors are current or former employees of UCB Pharma.

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AUTHORSHIP CONTRIBUTIONS

Participated in research design: K Leclercq, A Matagne L Provins, H Klitgaard, RM Kaminski

Conducted experiments: K Leclercq, RM Kaminski

Contributed new reagents or analytic tools: L Provins

Performed data analysis: K Leclercq, RM Kaminski

Wrote or contributed to the writing of the manuscript: K Leclercq, H Klitgaard, RM Kaminski

REFERENCES

Barton ME, Klein BD, Wolf HH, White HS (2001). Pharmacological characterization of the 6 Hz psychomotor seizure model of partial epilepsy. Epilepsy Res 47:217–227.

Bialer M, White HS (2010). Key factors in the discovery and development of new antiepileptic drugs. Nat Rev Drug Discov 9:68–82.

Brodie MJ, Sills GJ (2011). Combining antiepileptic drugs--rational polytherapy? Seizure 20:369–375.

Chen Z, Brodie MJ, Liew D, Kwan P (2018). Treatment outcomes in patients with newly diagnosed epilepsy treated with established and new antiepileptic drugs: a 30-year longitudinal cohort study. JAMA Neurol 75:279–286.

Conry JA, Ng Y-T, Kernitsky L, Mitchell WG, Veidemanis R, Drummond R, Isojarvi J, Lee D, Paolicchi JM; OV-1004 Study Investigators (2014). Stable dosages of clobazam for Lennox-Gastaut syndrome are associated with sustained drop-seizure and total-seizure improvements over 3 years. Epilepsia 55:558–567.

Crevecoeur J, Kaminski RM, Rogister B, Foerch P, Vandenplas C, Neveux M, Mazzuferi M, Kroonen J, Poulet C, Martin D, Sadzot B, Rikir E, Klitgaard H, Moonen G, Deprez M (2014). Expression pattern of synaptic vesicle protein 2 (SV2) isoforms in patients with temporal lobe epilepsy and hippocampal sclerosis. Neuropathol Appl Neurobiol 40:191–204.

Danober L, Deransart C, Depaulis A, Vergnes M, Marescaux C (1998). Pathophysiological mechanisms of genetic absence epilepsy in the rat. Prog Neurobiol 55:27–57.

Detrait E, Leclercq K, Matagne A, Klitgaard H (2008). Protective activity of brivaracetam in the 6 Hz model of partial epilepsy: comparison with levetiracetam and older antiepileptic drugs. Epilepsia 49 (Suppl 7):1.252 Abstract.

Devinsky O, Vezzani A, O'Brien TJ, Jette N, Scheffer IE, de Curtis M, Perucca P (2018). Epilepsy. Nat Rev Dis Primers 4:18024.

Duveau V, Pouyatos B, Bressand K, Bouyssières C, Chabrol T, Roche Y, Depaulis A, Roucard C (2016). Differential effects of antiepileptic drugs on focal seizures in the intrahippocampal kainate mouse model of Mesial Temporal Lobe Epilepsy. CNS Neurosci Ther 22:497–506.

Engel JJ, Williamson PD, Wieser HG (1997). Mesial temporal lobe epilepsy, in A comprehensive textbook (Engel J. and Pedley TA, editors). Raven press, Philadelphia.

Faulkner MA (2015). Comprehensive overview: efficacy, tolerability, and cost-effectiveness of clobazam in Lennox–Gastaut syndrome. Ther Clin Risk Manag 11:905–914.

French JA, Faught E (2009). Rational polytherapy. Epilepsia 50 Suppl 8:63–68.

Gauthier AC, Mattson RH (2015). Clobazam: A Safe, Efficacious, and Newly Rediscovered Therapeutic for Epilepsy. CNS Neurosci Ther 21:543–8.

Gidal BE, Wechsler RT, Sankar R, Montouris GD, White HS, Cloyd JC, Kane MC, Peng G, Tworek DM, Shen V, Isojarvi J (2016). Deconstructing tolerance with clobazam: Post hoc analyses from an open-label extension study. Neurology 87:1806–1812.

Gillard M, Fuks B, Leclercq K, Matagne A (2011). Binding characteristics of brivaracetam, a selective, high affinity SV2A ligand in rat, mouse and human brain: relationship to anti-convulsant properties. Eur J Pharmacol 664:36–44.

Jackson HC, Hansen HC, Kristiansen M, Suzdak PD, Klitgaard H, Judge ME, Swedberg MD (1996). Anticonvulsant profile of the imidazoquinazolines NNC 14-0185 and NNC 14-0189 in rats and mice. Eur J Pharmacol 308:21–30.

Kalilani L, Sun X, Pelgrims B, Noack-Rink M, Villanueva V (2018). The epidemiology of drug-resistant epilepsy: A systematic review and meta-analysis. Epilepsia 59:2179-2193.

Kaminski RM, Matagne A, Leclercq K, Gillard M, Michel P, Kenda B, Talaga P, Klitgaard H (2008). SV2A protein is a broad-spectrum anticonvulsant target: functional correlation between protein binding and seizure protection in models of both partial and generalized epilepsy. Neuropharmacology 54:715–20.

Kaminski R, Matagne A, Patsalos PN, Klitgaard H (2009). Benefit of combination therapy in epilepsy: A review of the preclinical evidence with levetiracetam. Epilepsia 50:387–97.

Kaminski R, Fu Z, Venkatesan K, Mazzuferi M, Leclercq K, Seutin V, Vicini (2011). 11-Deoxycortisol impedes GABAergic neurotransmission and induces drug-resistant status epilepticus in mice. Neuropharmacol 60:1098–1108.

Kasteleijn-Nolst Trenité DG, Groenwold R, Schmidt B, Löscher W (2016). Single dose efficacy evaluation of two partial benzodiazepine receptor agonists in photosensitive epilepsy patients: A placebo-controlled pilot study. Epilepsy Res 122:30–6.

Klitgaard H, Matagne A, Gobert J, Wülfert E (1998). Evidence for a unique profile of levetiracetam in rodent models of seizures and epilepsy. Eur J Pharmacol 353:191–206.

Klitgaard H (2005). Antiepileptic drug discovery: lessons from the past and future challenges. Acta Neurol Scand 112 (Suppl 181):68–72.

Klitgaard H, Verdru P (2007). Levetiracetam: the first SV2A ligand for the treatment of epilepsy. Expert Opin Drug Discov 2:1537–1545.

Klitgaard H, Matagne A, Nicolas J-M, Gillard M, Lamberty Y, De Ryck M, Kaminski RM, Leclercq K, Niespodziany I, Wolff C, Wood M, Hannestad J, Kervyn S, Kenda B (2016). Brivaracetam: Rationale for discovery and preclinical profile of a selective SV2A ligand for epilepsy treatment. Epilepsia 57:538–548.

Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Allen Hauser W, Mathern G, Moshé SL, Perucca E, Wiebe S, French J (2010). Definition of drug resistant epilepsy: consensus proposal by the ad hoc Task Force of the ILAE Commission on Therapeutic Strategies. Epilepsia 51:1069–1077.

Leclercq K, Kaminski RM (2015). Genetic background of mice strongly influences treatment resistance in the 6 Hz seizure model. Epilepsia 56:310–318.

Löscher W, Jäckel R, Czuczwar SJ (1986). Is amygdala kindling in rats a model for drug resistant partial epilepsy? Exp Neurol 93:211–226.

Löscher W, Rundfeldt C, Hönack D, Ebert U (1996). Long-term studies on anticonvulsant tolerance and withdrawal characteristics of benzodiazepine receptor ligands in different seizure models in mice. I. Comparison of diazepam, clonazepam, clobazam and abecarnil. J Pharmacol Exp Ther 279:561–572.

Löscher W, Schmidt D (1988). Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. Epilepsy Res 2:145–181.

Löscher W (2011). Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. Seizure 20: 359–368.

Löscher W, Klitgaard H, Twyman RE, Schmidt D (2013). New avenues for anti-epileptic drug discovery and development. Nat Rev Drug Discov 12:757–776.

Löscher W, Gillard M, Kaminski R, Klitgaard H (2016). Synaptic Vesicle Glycoprotein 2A Ligands in the Treatment of Epilepsy and Beyond. CNS Drugs 30:1055–1077.

Lynch BA, Lambeng N, Nocka K, Kensel-Hammes P, Bajjalieh SM, Matagne A, Fuks B (2004). The synaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam. Proc Natl Acad Sci USA 101:9861–9866.

Matagne A, Margineanu DG, Kenda B, Michel P, Klitgaard H (2008). Anti-convulsive and anti-epileptic properties of brivaracetam (ucb 34714), a high-affinity ligand for the synaptic vesicle protein, SV2A. Br J Pharmacol 154:1662–1671.

Matagne A, Margineanu DG, Potschka H, Löscher W, Michel P, Kenda B, Klitgaard H (2009). Profile of the new pyrrolidone derivative seletracetam (ucb 44212) in animal models of epilepsy. Eur J Pharmacol 614:30–37.

Miller LG, Galpern WR, Greenblatt DJ, Lumpkin M, Shader RI (1990). Chronic benzodiazepine administration. VI. A partial agonist produces behavioral effects without tolerance or receptor alterations. J Pharmacol Exp Ther 254:33–38.

Mitsuya K, Nitta N, Suzuki F (2009). Persistent zinc depletion in the mossy fiber terminals in intrahippocampal kainate mouse model of mesial temporal lobe epilepsy. Epilepsia 50:1979–90.

Muglia P, Toledo M, Steinhoff BJ, Majoie M, Webster E, Otoul C, De Bruyn S, Van Paesschen W, Boon P, Werhahn KJ (2017). Efficacy and tolerability of adjunctive padsevonil in adults with drug-resistant focal onset seizures: a randomized, double-blind, placebo-controlled, proof-of-concept trial. American Epilepsy Society (AES) Annual Meeting Abstract Database. AESnet.org

Pernot F, Heinrich C, Barbier L, Peinnequin A, Carpentier P, Dhote F, Baille V, Beaup C, Depaulis A, Dorandeu F (2011). Inflammatory changes during epileptogenesis and spontaneous seizures in a mouse model of mesiotemporal lobe epilepsy. Epilepsia 52:2315–2325.

Racine RJ (1972). Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol 32:281–294.

Riban V, Bouilleret V, Pham-Lê BT, Marescaux C, Fritschy JM, Depaulis A (2002). Evolution of hippocampal epileptic activity during the development of hippocampal sclerosis in a mouse model of temporal lobe epilepsy. Neuroscience 112:101–111.

Rundfeldt C, Wlaź P, Hönack D, Löscher W (1995). Anticonvulsant tolerance and withdrawal characteristics of benzodiazepine receptor ligands in different seizure models in mice. Comparison of diazepam, bretazenil and abecarnil. J Pharmacol Exp Ther 275:693–702.

Rundfeldt C, Löscher W (2014). The pharmacology of imepitoin: the first partial benzodiazepine receptor agonist developed for the treatment of epilepsy. CNS Drugs 28:29–43.

Serra M, Ghiani CA, Motzo C, Porceddu ML, Biggio G (1994). Long-term treatment with abecarnil fails to induce tolerance in mice. Eur J Pharmacol 259:1–6.

Suzuki F, Junier MP, Guilhem D, Sorensen JC, Onteniente B (1995). Morphogenetic effect of kaïnate on adult hippocampal neurons associated with a prolonged expression of brain-derived neurotrophic factor. Neuroscience 64:665–674.

Thijs RD, Surges R, O'Brien TJ, Sander JW (2019). Epilepsy in adults. Lancet 393:689–701.

Trinka E (2012) Ideal characteristics of an antiepileptic drug: how do these impact treatment decisions for individual patients? Acta Neurol Scand Suppl 194:10–18.

Turski L, Stephens DN, Jensen LH, Petersen EN, Meldrum BS, Patel S, Hansen JB, Löscher W, Schneider HH, Schmiechen R (1990). Anticonvulsant action of the beta-carboline abecarnil: studies in rodents and baboon, Papio papio. J Pharmacol Exp Ther 253:344–352.

van Luijtelaar EL, Drinkenburg WH, van Rijn CM, Coenen AM (2002). Rat models of genetic absence epilepsy: what do EEG spike-wave discharges tell us about drug effects? Methods Find Exp Clin Pharmacol 24 Suppl D:65–70.

White HS (2003). Preclinical development of antiepileptic drugs: past, present, and future directions. Epilepsia 44 Suppl 7:2–8.

Wolff C, Mullier B, Ghisdal P, Provins L, Kaminski RM (2017). Functional characterization of padsevonil on GABA-A receptors. American Epilepsy Society (AES) Annual Meeting Abstract Database. AESnet.org

Wood M, Daniels V, Provins L, Wolff C, Kaminski RM, Gillard M (2019). Pharmacological profile of the antiepileptic drug candidate padsevonil – interactions with synaptic vesicle 2 proteins and GABAA receptors. J Pharmacol Exp Ther

FOOTNOTES

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FIGURE LEGENDS

Figure 1

Protective effect of padsevonil (0.17 mg/kg) in the 6 Hz model compared with that of levetiracetam (1.83 mg/kg), brivaracetam (0.42 mg/kg), and diazepam (0.017 mg/kg), as well as the combination of diazepam with levetiracetam or brivaracetam. The drugs were administered at doses associated with similar in vivo SV2A (35%) and benzodiazepine site (2%) occupancies (comparisons were made using Fisher's exact test).

Figure 2

Effect of padsevonil on seizure parameters recorded after supra-threshold stimulation in fully kindled rats (right panel) and mice (left panel). Control recordings were performed 48 h before testing with padsevonil. Values are mean ± standard error of mean for afterdischarge duration. Comparisons between drug and control in protection against generalized seizures, seizure severity score and afterdischarge duration were evaluated with Wilcoxon signed rank test, Fisher's exact test and Mann-Whitney U-test, respectively, with *indicating statistically significant differences ($p<0.05$).

Figure 3

Padsevonil (PSL) activity in the murine intrahippocampal kainate model – effect on the mean number (top panel) and the mean cumulated duration of hippocampal paroxysmal discharges (HPDs; bottom panel). Values are mean ± standard error of the mean (n=9); comparisons are *$p<0.05$, **$p<0.01$, ***$p<0.001$, **** $p<0.0001$ vs baseline (Bonferroni's multiple comparison test).

Figure 4

Padsevonil (PSL) activity in the GAERS model – effect on the duration of spontaneous spike-and-wave discharges. Values are mean ± standard error of the mean (n=8 per group), with * indicating statistically significant difference with respective time point in vehicle-treated group ($p<0.05$ Bonferroni's multiple comparison test).

Figure 5

Effect of chronic (4 days) treatment with padsevonil (PSL; 15.9 mg/kg) or diazepam (DZP; 2.1 mg/kg) on the pentylenetetrazol (PTZ)-induced seizure threshold. On the fifth day, PSL increased the threshold to the same extent in animals that had been treated chronically with vehicle or PSL. In contrast, there was a significant decrease in the ability of DZP to increase the threshold in animals that had been treated chronically with DZP, indicating development of tolerance. Development of tolerance assessed based on statistically significant differences between (chronic vehicle + test compound ED_{97} dose) and (chronic test compound ED_{97} dose + test compound ED_{97} dose) using a one-way ANOVA followed by a Tukey multiple comparison test.

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Table 1

Experimental groups in the pentylenetetrazol-induced clonic seizure threshold test.

	Days 1 – 4	Day 5
Group 1	Vehicle twice daily	Vehicle administered 30 mins before iv PTZ
Group 2	Vehicle twice daily	PSL/DZP at ED_{97} administered 30 mins before iv PTZ
Group 3	PSL/DZP twice daily at ED_{97}	Vehicle administered 30 mins before iv PTZ
Group 4	PSL/DZP twice daily at ED_{97}	PSL/DZP at ED_{97} administered 30 mins before iv PTZ

DZP=diazepam; iv=intravenous; PSL= padsevonil; PTZ=pentylenetetrazol

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Table 2

Potency of padsevonil and selective SV2A ligands in murine models of acutely-induced seizures.

Model	Padsevonil	Brivaracetam	Levetiracetam
	ED_{50} (mg/kg)		
6 Hz	0.16 (0.1–0.2)	4.4[a]	19.2[a]
Audiogenic	0.17 (0.1–0.2)	2.4[a]	30[a]
Pilocarpine	0.19 (0.1–0)	NE[a]	7.1[b]
Pentylenetetrazol	4.8 (1.7–10.8)	30[a]	NE[a]
11-deoxycortisol	9.9 (4.6–21.0)	NE[c]	540*[c]
Bicuculline	27.3 (17.2–43.1)	NE[b]	NE[b]
Maximal electroshock	92.8 (74.3–115.9)	113[a]	NE[a]

NE=not effective
All drugs were administered intraperitoneally 15 mins before testing in the audiogenic seizures model, 30 mins in others
*Minimally active dose, defined as the lowest dose providing statistically significant protection against seizures
[a] Klitgaard H, et al (2016). Epilepsia 57:538–48
[b] Klitgaard H, et al (1998). Eur J Pharmacol 353:191–206
[c] Kaminski RM, et al (2011). Neuropharmacol 60:1098–1108.

Table 3

Comparison of the therapeutic index of padsevonil and nine other antiepileptic drugs with different mechanisms of action determined in the mouse amygdala kindling model and rotarod test. Other than padsevonil, brivaracetam and valproate, the TD_{50}, and therefore, the therapeutic index of the other antiepileptic drugs could not be calculated.

	Amygdala kindling ED_{50} (mg/kg)	MAD (mg/kg) | protection against seizures[a] (%)	MTD (mg/kg) | protection against seizures[a] (%)	Rotarod test TD_{50} (mg/kg)	Therapeutic index
Padsevonil	1.2 (0.4–3.4)	1.4 | 60	13.9 | 100	12 (9–15)	9.8
Brivaracetam	68 (39–118)	134 | 90	212 | 91	195 (133–245)	2.8
Levetiracetam	–	540 | 60	540 | 60	1389 (962–2041)	–
Valproate	239 (169–338)	250 | 56	400 | 89	298 (201–418)	1.2
Phenytoin	–	>70 | 0	70 | 0	129 (76–194)	–
Carbamazepine	–	56 | 89	56 | 89	36 (27–48)	–
Lamotrigine	–	>56 | 33	56 | 33	20 (13–27)	–
Diazepam	–	3 | 89	3 | 89	3 (2–4)	–
Topiramate	–	>300 | 25	300 | 25	249 (150–357)	–
Retigabine	–	15 | 100	15 | 100	12 (8–18)	–

[a]Protection against focal to bilateral tonic-clonic seizures

MAD=minimally active dose, defined as the lowest dose providing statistically significant protection against focal to bilateral tonic-clonic seizures

MTD=maximal tested dose, defined as the highest dose tested in the amygdala kindling model

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Figure 1

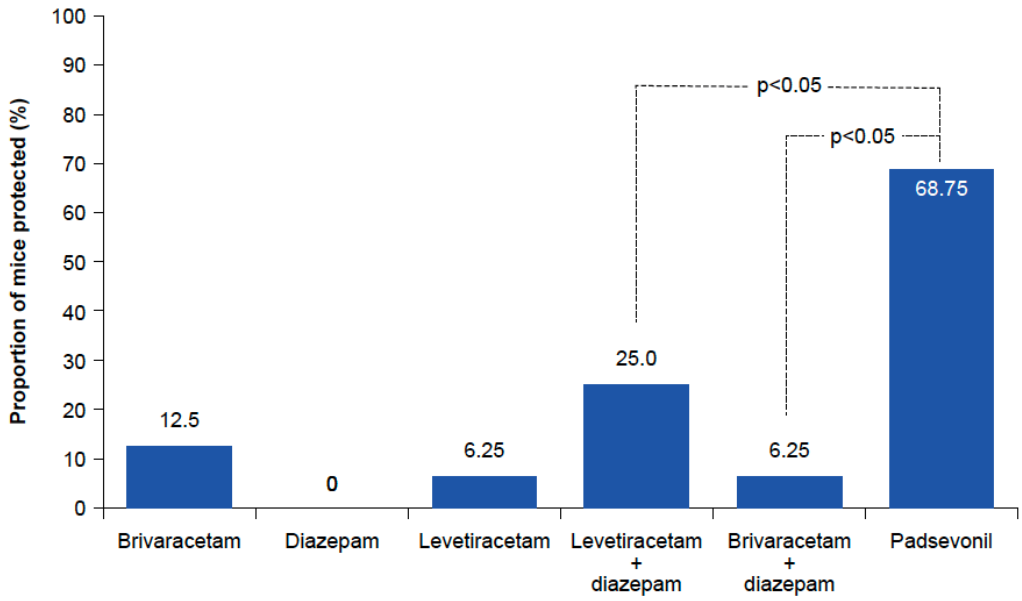

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Figure 2

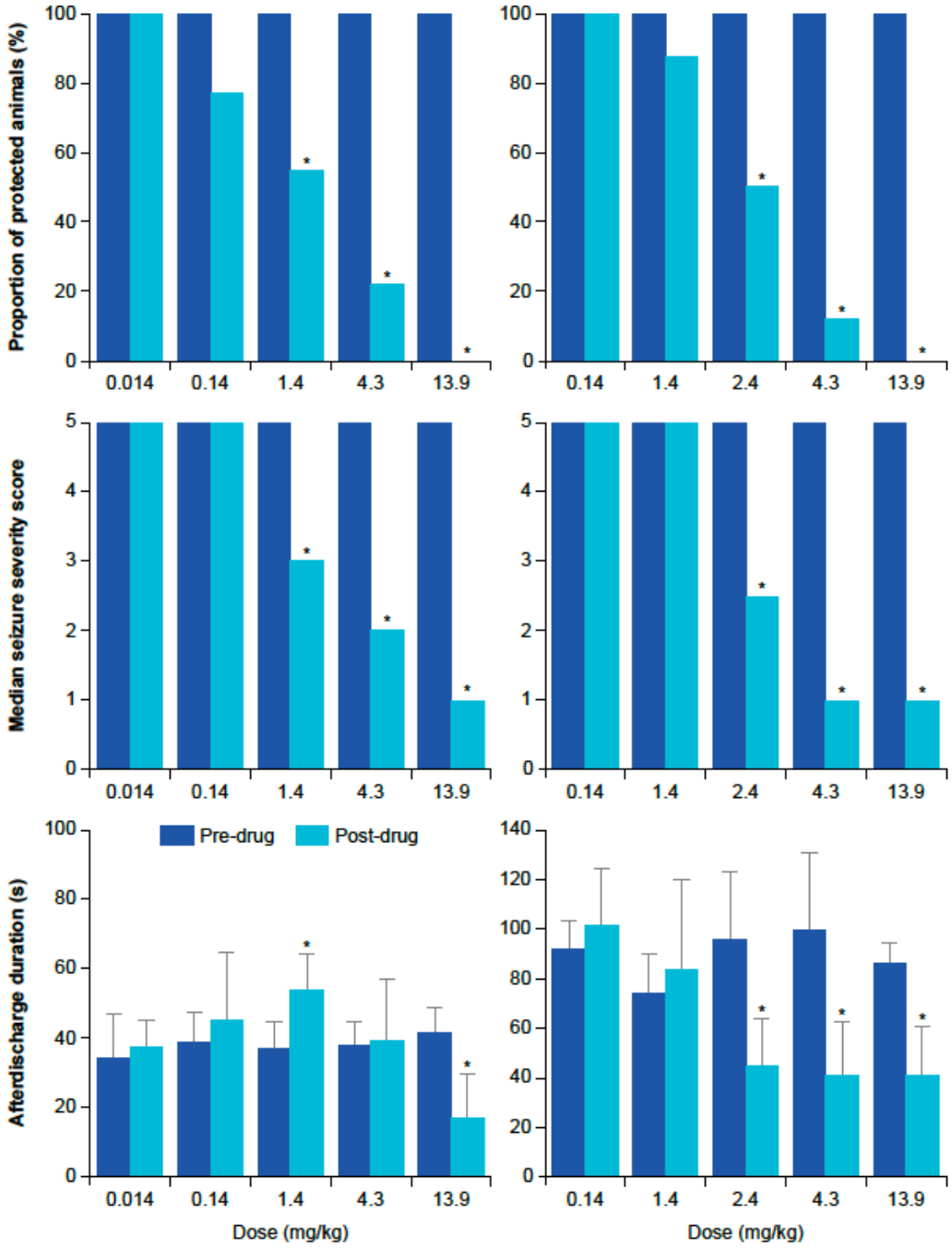

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Figure 3

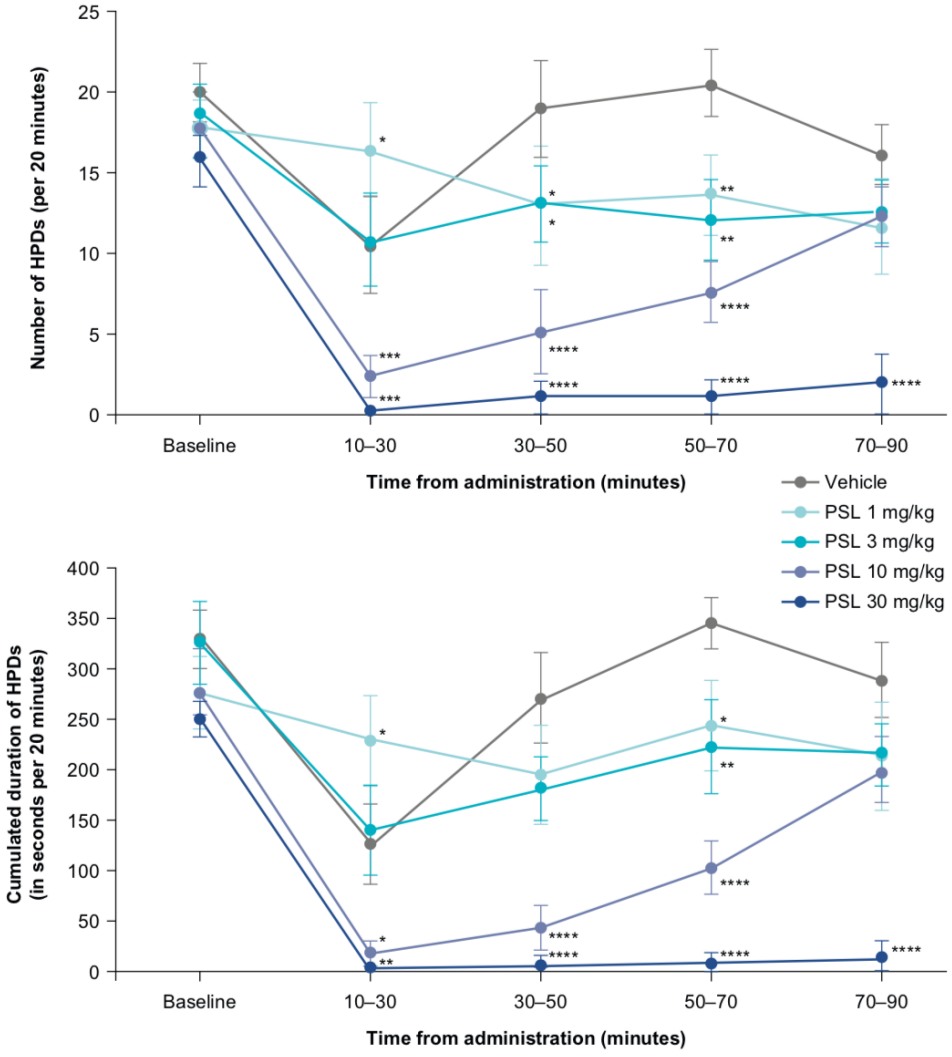

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Figure 4

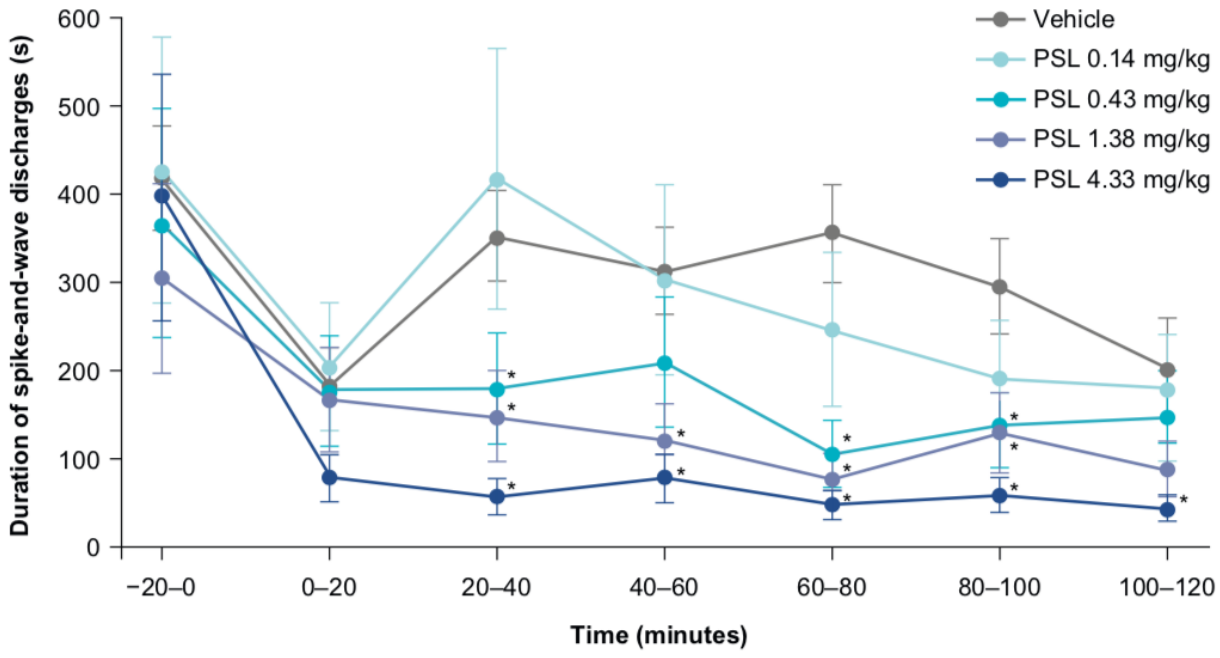

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Figure 5