OV329, a novel highly potent γ-aminobutyric acid aminotransferase inactivator, induces pronounced anticonvulsant effects in the pentylenetetrazole seizure threshold test and in amygdala-kindled rats

Malte Feja1,2 | Sebastian Meller1 | Lillian S. Deking1 | Edith Kaczmarek1 | Matthew J. During3 | Richard B. Silverman4,5 | Manuela Gernert1,2

Summary

Objective: An attractive target to interfere with epileptic brain hyperexcitability is the enhancement of γ-aminobutyric acidergic (GABAergic) inhibition by inactivation of the GABA-metabolizing enzyme GABA aminotransferase (GABA-AT). GABA-AT inactivators were designed to control seizures by raising brain GABA levels. OV329, a novel drug candidate for the treatment of epilepsy and addiction, has been shown in vitro to be substantially more potent as a GABA-AT inactivator than vigabatrin, an antiseizure drug approved as an add-on therapy for adult patients with refractory complex partial seizures and monotherapy for pediatric patients with infantile spasms. Thus, we hypothesized that OV329 should produce pronounced anticonvulsant effects in two different rat seizure models.

Methods: We therefore examined the effects of OV329 (5, 20, and 40 mg/kg ip) on the seizure threshold of female Wistar Unilever rats, using the timed intravenous pentylenetetrazole (ivPTZ) seizure threshold model as a seizure test particularly sensitive to GABA-potentiating manipulations, and amygdala-kindled rats as a model of difficult-to-treat temporal lobe epilepsy.

Results: GABA-AT inactivation by OV329 clearly increased the threshold of both ivPTZ-induced and amygdala-kindled seizures. OV329 further showed a 30-fold greater anticonvulsant potency on ivPTZ-induced myoclonic jerks and clonic seizures compared to vigabatrin investigated previously. Notably, all rats were responsive to OV329 in both seizure models.

Significance: These results reveal an anticonvulsant profile of OV329 that appears to be superior in both potency and efficacy to vigabatrin and highlight OV329 as a highly promising candidate for the treatment of seizures and pharmacoresistant epilepsies.

Keywords
amygdala kindling, epilepsy, GABA-AT, metrazol, PTZ, vigabatrin
1 | INTRODUCTION

Epilepsy is one of the most common neurological diseases globally, affecting more than 70 million people worldwide. In light of the finding that ~30% of epilepsy patients have persistent seizures despite use of different currently approved drugs and combinations thereof, there is a critical need for the development of new antiseizure drugs (ASDs), particularly for drug-resistant seizures. Among various approaches to interfering with epileptic brain hyperexcitability, an attractive target is the enhancement of γ-aminobutyric acidergic (GABAergic) inhibition by inactivation of GABA amino transferase (GABA-AT), the enzyme responsible for GABA catabolism. Therefore, GABA-AT inactivators were designed to control seizures by raising brain GABA levels.

To date, the only approved drug that acts as an inactivator of GABA-AT is the rationally designed ASD vigabatrin. Vigabatrin is approved as add-on therapy for adult patients with refractory complex partial seizures and monotherapy for pediatric patients with infantile spasms, a severe form of epilepsy. However, the antiseizure effect of the irreversible GABA-AT inhibitor vigabatrin has been associated with severe adverse effects during long-term use, which led to its limited approval. Due to the relatively poor GABA-AT inhibition and ability to cross the blood–brain barrier (cerebrospinal fluid/plasma ratio is only about 0.1), vigabatrin is generally taken in large doses (1–3 g/day) that may cause visual field defects resulting from retinal damage in 25%–50% of patients and limit its clinical utility.

To increase therapeutic potency and to eliminate retinal toxicity, the vigabatrin analogue CPP-115 was designed. CPP-115 was described to be 187 times more potent in inactivating GABA-AT than vigabatrin in vitro and suppressed spasms at 1/100th the mass dose of vigabatrin in a rat model of infantile spasms. Meanwhile, CPP-115 has completed a Phase I clinical trial without revealing adverse effects, and markedly reduced seizures with no evidence of retinal dysfunction in a patient with refractory infantile spasms. Recently, the structure of CPP-115 has been further refined, leading to the development of OV329, a novel drug candidate for the treatment of pharmacoresistant epilepsies that was shown in vitro to be 10 times more potent as an inactivator of GABA-AT than CPP-115. Moreover, it has recently been demonstrated that pretreatment with OV329 reduces the severity of N-methyl-D-aspartate (NMDA)-induced seizures in mice.

We previously demonstrated that systemic administration of high doses of vigabatrin increases seizure thresholds in the timed intravenous pentylentetrazole seizure threshold (ivPTZ-ST) test in rats. Vigabatrin has also been shown to increase seizure thresholds and to reduce seizure severity (SS) and seizure duration (SD) in amygdalakindled rats. However, systemic treatments with high doses of vigabatrin were associated with marked adverse effects, such as sedation, ataxia, and weight loss. Because OV329 appears to be substantially more potent as a GABA-AT inactivator than vigabatrin, we hypothesized that OV329 produces pronounced anticonvulsant effects in the two different rat models mentioned above, namely, the ivPTZ-ST model and the amygdala-kindling model. We therefore examined the effects of OV329 on the seizure thresholds of rats, using the ivPTZ-ST model as a seizure test particularly sensitive to GABA-potentiating drugs, and amygdala-kindled rats as a highly predictive model of drug efficacy against difficult-to-treat types of seizures as in temporal lobe epilepsies. Based on previous studies showing pharmacoresistance to vigabatrin in a subset of patients and animal models, we additionally determined the OV329 responder rate in both rat models. Finally, drug-induced behavioral and physiological side effects were assessed. Our study helps to characterize the antiseizure potential and tolerability of the novel GABA-AT inactivator OV329 and to determine whether it shows improved efficacy compared to previous findings with vigabatrin.

2 | MATERIALS AND METHODS

2.1 | Animals

As in our previous studies with vigabatrin, adult female Wistar Unilever rats (~220–360 g) were used. The animals were obtained from Envigo and housed in groups of up to four. To consider estrous cycle-linked effects on brain...
physiology and behavior, females were housed without males to keep them acyclic or asynchronous with respect to their estrous cycle. We have previously shown that the PTZ seizure threshold is not affected by the estrous cycle in rats. Rats were kept under controlled ambient conditions (23–24°C, 45%–50% relative humidity, 12 h/12 h light/dark cycle, lights on at 7 a.m.). Standard laboratory chow (Altromin 1324 standard diet) and water were available ad libitum. Nesting material and tubes served as environmental enrichment. All procedures complied with the European Union directive 2010/63/EU and were formally approved by the animal subjects review board of our institution, “Lower Saxony State Office for Consumer Protection and Food Safety” (file number 14/1727).

### 2.2 | Drugs

OV329 was donated by Ovid Therapeutics as a hydrochloride salt. All OV329 doses refer to the free base. OV329 and PTZ (Sigma-Aldrich) were freshly prepared on each test day and dissolved in sterile 0.9% saline.

### 2.3 | Timed intravenous PTZ infusion seizure threshold test

The ivPTZ-ST model is generally considered to be an acute screening model, which provides a sensitive parametric method for evaluating seizure thresholds in individual animals. It allows determining drug effects on different components of seizure behavior, such as myoclonic twitches and clonic seizures. For a fast screening of the anticonvulsant potential of OV329, rats received intravenous infusion of PTZ, a central nervous system (CNS) stimulant that induces convulsions by GABA_A receptor antagonism. Thus, the ivPTZ-ST test is particularly sensitive to drugs that increase GABAergic inhibition, such as GABA-AT inactivators, and can be used to determine the ability of drugs to alter seizure thresholds by applying the drug at the appropriate time before onset of PTZ infusion. To avoid a kindling effect, the number of ivPTZ-ST tests was limited to a maximum of five in the same individual, which has been proven not to change seizure thresholds.

To quantify the threshold doses of the different components of ivPTZ-induced seizures, a 0.8% PTZ solution was continuously infused (Pump 11 Elite, Harvard Apparatus; constant flow rate of 1 ml/min) via flexible polyethylene tubing (PE20 tubing, nonsterile; Instech Laboratories) connected to a 24-gauge needle inserted into the lateral tail vein of rats that were allowed to move freely inside a Makrolon cage type III. We measured two endpoints: the occurrence of the first myoclonic twitch and the subsequent occurrence of the first clonic seizure, the latter of which was set as end of infusion. The seizure thresholds were calculated in mg/kg PTZ based on the body weight of the animal (measured immediately before seizure threshold testing), the rate of infusion, the PTZ concentration, and the latency to induce the seizure.

We first determined the basal seizure threshold once for each rat (predrug control). Two days later, the rats (n = 8) entered the drug-testing phase, where they received intraperitoneal injections of three OV329 doses (5, 20, and 40 mg/kg) and saline as vehicle control at a volume of 3 ml/kg body weight in a randomized crossover design (Figure 1A). Because full GABA-AT recovery after irreversible inhibition by OV329 is expected to require resynthesis of the enzyme and full enzyme activity does not return within 48 h in vitro, ivPTZ-ST tests were done 7 days apart. We have previously shown that the maximum effect of vigabatrin on the PTZ-ST occurs 6 h postinjection. Therefore, we decided to conduct the ivPTZ-ST test 6 h after OV329 or saline injections in the present study. OV329 doses were based on previous studies and our own preliminary investigation (unpublished results).

Based on previous experiments with vigabatrin, we determined the OV329 antiseizure response/nonresponse rate. In our present study, responders were defined as showing at least 25% increase in seizure threshold (for the myoclonic jerk and clonic seizure separately) after OV329 treatment compared to saline control.

### 2.4 | Amygdala-kindling model

In addition to the acute seizure model described above, we used a chronic seizure/epilepsy model, namely, fully amygdala-kindled rats, to verify and extend our findings. Fully amygdala-kindled rats are characterized by permanent brain alterations reflecting epileptic brain networks. The amygdala-kindling model is highly predictive for detecting clinically effective drugs for the treatment of focal onset seizures as in temporal lobe epilepsies and is thus considered a model for difficult-to-treat types of seizures. We previously published kindling protocols comparable to the one used here, which is described in detail in the Supplement. Briefly, rats were electrically kindled via a chronically implanted electrode in the right amygdala. After establishing reproducible stimulation thresholds for eliciting afterdischarges (afterdischarge threshold [ADT]) in the EEG of fully kindled rats, the rats were alternately treated with vehicle (precontrol and postcontrol) and OV329 (refer to Figure 1B). The postcontrol for the previous dose served as precontrol for the subsequent dose. Thus, each rat served as its own control. During this
drug-testing phase, the ADT determination performed by an ascending staircase procedure (refer to Supplement) always started three steps below the previous vehicle ADT. Because OV329 is expected to induce an irreversible inactivation of GABA-AT requiring resynthesis of the enzyme,40 the vehicle postcontrol ADT determination was made not earlier than 1 week following the previous drug trial (equally to the ivPTZ-ST protocol). This was not necessary after vehicle injections, which is why the testing interval could be shorter after vehicle injections. As in the ivPTZ-ST test, saline at a volume of 3 ml/kg body weight served as vehicle. Because the lower OV329 doses of 5 and 20 mg/kg ip induced only subtle effects in the ivPTZ-ST test, whereas the higher dose of 40 mg/kg ip was clearly anticonvulsant (refer to results), we used doses of 30 and 40 mg/kg ip in the amygdala-kindling model. As in the ivPTZ-ST test, the pretreatment time was 6 h and responders were defined as showing at least 25% increase

FIGURE 1 Outline of the experimental design using two different seizure threshold models. (A) Seizure thresholds were determined by timed intravenous pentylentetrazole (PTZ) infusion. Following determination of basal seizure thresholds, rats (n = 8) received intraperitoneal (i.p.) injections of OV329 (5, 20, and 40 mg/kg) and saline as vehicle control (3 ml/kg) in a crossover design 3½ h before test battery for adverse effects and 6 h before PTZ infusion. For more details, refer to text. (B) Seizure thresholds were determined in fully amygdala-kindled rats. Rats were kindled via a chronically implanted electrode in the right amygdala until fully kindled. Determination of seizure thresholds (afterdischarge thresholds [ADTs]) was performed until reproducible before onset of drug and vehicle trials. Rats (n = 6) received i.p. injections of OV329 (30 and 40 mg/kg) and saline as vehicle control (3 ml/kg) in an alternating design starting with vehicle as indicated in the figure. The postcontrol for the previous dose served as precontrol for the subsequent dose. Thus, each rat served as its own control. Pretreatment time was 6 h. Adverse effects were assessed 30 min before ADT determination. In addition to the ADT, further seizure parameters (generalized seizure threshold, seizure severity, seizure duration, afterdischarge duration) were determined. EEG, electroencephalography; w, weeks
in seizure threshold (here, ADT and generalized seizure threshold [GST], respectively) following OV329 compared to saline.

Usually, fully kindled rats show generalized motor seizures (Stage 4 or 5 seizures according to Racine\textsuperscript{41}) at the ADT. [Correction added on 19 October 2021, after first online publication: The reference citation number was corrected in the preceding sentence.] Otherwise, the electrical current was further elevated up to the GST. Apart from the ADT, we recorded the seizure severity (SS), seizure duration (SD), and afterdischarge duration (ADD) at ADT and GST, respectively.

### 2.5 Assessment of adverse effects

The assessment of drug-induced behavioral alterations was conducted 30 min before each seizure threshold determination and is detailed in the Supplement.

### 2.6 Histological verification

Histological verification of the kindling site is detailed in the Supplement.

### 2.7 Statistical analysis

Normally distributed data were compared using one-way mixed-effects model analysis or one-way repeated measures analysis of variance. Nonparametric statistics were used in the case that data did not follow a normal distribution (for details, see Supplement).

### 3 RESULTS

#### 3.1 OV329 causes anticonvulsant effects in the ivPTZ-ST rat model

The basal (predrug) PTZ seizure thresholds (mean ± SEM) were 15.8 ± 0.97 mg/kg for the myoclonic twitch and 19.5 ± 1.34 mg/kg for the clonic seizure. As depicted in Figure 2, 40 mg/kg ip OV329 significantly increased the threshold for the myoclonic twitch (33.1 ± 2.54 mg/kg, \( p = .0026 \); Figure 2A) and for the clonic seizure (40.98 ± 3.04 mg/kg, \( p = .0027 \); Figure 2B) compared to the basal threshold. In contrast, vehicle injections did not alter myoclonic and clonic seizure thresholds (\( p > .05 \); Figure 2A,B). Considering interindividual differences in basal seizure thresholds, we further calculated the effects of vehicle control and OV329 as percent change from the basal seizure threshold for a more reliable quantification and better comparison between vehicle- and drug-treated rats. We found that OV329 (40 mg/kg ip) significantly increased the ivPTZ-induced seizure threshold for the myoclonic twitch (102.74\% ± 14.07\%, \( p = .0006 \); Figure 2C) and the clonic seizure (105.18\% ± 14.66\%, \( p = .0012 \); Figure 2D) compared to vehicle-injected animals (myoclonic twitch: \( -1.63\% ± 5.91\% \), clonic seizure: 4.58\% ± 6.66\%). The anticonvulsant effects of OV329 were dose-dependent, as we did not observe significant effects on seizure thresholds at lower doses, namely, 1 mg/kg (data not shown), 1 mg/kg (data not shown), 5 mg/kg (Figure 2), and 20 mg/kg OV329 (Figure 2). Higher OV329 doses, namely, 50 mg/kg, did not result in a more pronounced antiseizure effect, but were associated with severe adverse effects, for example, catalepsy, and therefore were excluded from further investigation. Dose–response analyses of OV329 on ivPTZ-induced seizure thresholds revealed \( ED_{50} = 25.83 \) mg/kg for the myoclonic twitch and \( ED_{50} = 20.8 \) mg/kg for the clonus.

#### 3.2 All rats are responsive to OV329

Because previous experiments have shown that not all rats respond to the treatment with vigabatrin in a high dose with tolerable adverse effects,\textsuperscript{22} we conducted an intraindividual response analysis between OV329 and saline as vehicle control. As shown in Figure 3, we found a dose-dependent increase in the responder rate. Whereas only one of eight (12.5\%) rats showed at least 25\% increase in ivPTZ-induced clonic seizure threshold after 5 mg/kg OV329 compared to saline, the responder rate increased to three of eight (37.5\%) for the myoclonic jerk and the clonic seizure at a dose of 20 mg/kg OV329. Importantly, all eight rats were responsive to 40 mg/kg OV329 with regard to the myoclonic twitch as well as the clonic seizure (100\% responder rate).

#### 3.3 OV329 causes anticonvulsant effects in amygdala-kindled rats

As for PTZ seizure thresholds, kindled seizure parameters were determined 6 h after drug or vehicle injection. For evaluation of ADTs, GSTs, and seizure parameters at both thresholds (SS, SD, ADD), mean drug values were compared with mean predrug and postdrug control values. During predrug control testing, the animals showed stable ADTs after saline (S) injection (mean ADT ± SEM = 143.3 ± 18.2 \( \mu A \) [range = 110–200 \( \mu A \)] at S1 vs. 130 ± 16.9 \( \mu A \) [range = 90–200 \( \mu A \)] at S2; Figure 4A).
OV329 significantly increased the ADT (Figure 4A) compared to predrug saline control at doses of 30 mg/kg (mean ± SEM = 170 ± 29.1 μA, range = 110–280 μA, \( p = .049 \)) and 40 mg/kg (398.3 ± 73.8 μA, range = 200–590 μA, \( p = .027 \)). The saline postdrug control ADTs (measured 1 week after OV329 treatment) were as low as predrug control values and thus also differed significantly from the ADTs after OV329 treatment (125 ± 19.8 μA, range = 60–200 μA, \( p = .027 \) for 30 mg/kg; 115.8 ± 11.4 μA, range = 75–160 μA, \( p = .023 \) for 40 mg/kg). Remarkably, OV329 (40 mg/kg) suppressed generalized seizures in all rats (Figure 4B). Following injection of vehicle or 30 mg/kg OV329, a secondarily generalized seizure (Stage 4/541) was always inducible in kindled rats. After injection of 40 mg/kg OV329, none of the animals expressed generalized motor seizures up to the highest current intensity tested (840 μA). In these cases, the GST was set at 1000 μA (the next theoretical 20% step in seizure threshold testing) for data evaluation (Figure 4B). Because 40 mg/kg OV329 prevented the animals from generalized seizure expression, further kindling parameters at GST (SS, SD) were set at zero. SS at ADT was significantly reduced after 40 mg/kg OV329 compared to predrug control (Figure 4C; \( p = .011 \)) and postdrug control (Figure 4C; \( p = .0086 \)).

OV329 (40 mg/kg) further significantly reduced the duration of motor seizures (SD1) and the duration of
motor seizures plus the adjacent time of immobility (SD2) at the ADT (Figure 5; SD1: 24.3 ± 4.6 s, range = 10–42 s, p = .0022; SD2: 24.3 ± 4.1 s, range 12–40 s, p < .0001) compared to predrug control (SD1: 65.5 ± 5.5 s, range = 46–81 s; SD2: 550 ± 36.1 s, range = 420–665 s). In OV329-treated rats, ADD1 was always equal to ADD2, that is, no distinguishable afterdischarge patterns occurred. At 40 mg/kg OV329, the ADD at ADT was significantly reduced compared to predrug control (Figure 5; ADD1 at ADT: 24.3 ± 4.1 s, range = 12–40 s, p = .0232) and post-drug control (p = .0021), the latter showing that we did not observe any carryover effects on kindled seizure parameters. Multiple comparisons between S1 and subsequent saline controls revealed no differences regarding kindled seizure parameters. OV329 (40 mg/kg ip) also significantly decreased the SD and ADD at GST (Figure 5), as generalized seizures were completely suppressed. That vehicle control injections given 7 days following an OV329 treatment did not alter seizure thresholds in the ivPTZ-ST model and in amygdala-kindled rats indicates the return of GABA-AT activity within 7 days in our rats.

3.4 | All amygdala-kindled rats respond to OV329

As in the ivPTZ-ST model, we also evaluated the efficacy of OV329 in individual amygdala-kindled animals and therefore again conducted an intraindividual analysis between drug and control treatment. We found a responder rate of 2/6 (33.3%) for the ADT and 3/6 (50%) for the GST at 30 mg/kg OV329. Strikingly, all rats were responsive to 40 mg/kg OV329 concerning the ADT and GST (100% responder rate; Figure 6).

3.5 | OV329 causes mild adverse effects

Low doses of OV329 were well tolerated, whereas higher doses were associated with decreased body temperature, moderate loss of body weight (Figure S1), and sedation in about one-third of rats. Half of the animals (3/8 ivPTZ-tested rats, 4/6 amygdala-kindled rats) did not pass the rotarod test, indicating moderate but not significant deficits in motor coordination compared to saline. The evaluation of toxic effects of OV329 on the rotarod performance yielded TD50 = 46.16 mg/kg (for details, see Supplement).

4 | DISCUSSION

This study sought to determine the anticonvulsant potential of the GABA-AT inactivator OV329 in two well-established seizure/epilepsy models. We discovered three main novel findings. First, OV329 increased the threshold of ivPTZ-induced acute seizures. Second, OV329 increased the threshold of amygdala-kindled chronic, difficult-to-treat seizures (focal and generalized). Third, all rats were responsive to OV329 in both seizure models. In addition,
**FIGURE 4** OV329 increases the afterdischarge threshold (ADT), suppresses generalized seizures, and reduces seizure severity in amygdala-kindled rats. Effects are shown of 30 and 40 mg/kg OV329 injected intraperitoneally on the ADT (A), the generalized seizure threshold (GST; B), and the seizure severity (SS) at ADT (C) and GST (D) in rats (n = 6). Saline (S) injections served as pre- and postdrug controls. Data are expressed as mean + SEM (A, B) and as box plots with whiskers (minimum to maximum; C, D). At 40 mg/kg OV329, none of the animals expressed generalized motor seizures, and the GST was set at 1000 µA (B) and the SS at GST was set at score = 0 (D) for data evaluation. Statistically significant differences between OV329 and saline as predrug control are indicated by asterisks, and differences between OV329 and saline as postdrug control are indicated by hashtags (A, B: one-way mixed-effects model analysis, post hoc Sidak correction; C, D: Friedman test for one-way repeated measures analysis of variance by ranks, with Dunn correction for post hoc multiple comparisons; *, # p < .05, **# p < .01, ****#, #### p < .0001)

**FIGURE 5** OV329 reduces seizure and afterdischarge durations in amygdala-kindled rats. Effects are shown of 30 and 40 mg/kg OV329 injected intraperitoneally on the duration of motor seizures (seizure duration 1 [SD1]; A, B) and the time of motor seizures plus the adjacent time of immobility (seizure duration 2 [SD2]; C, D) at the afterdischarge threshold (ADT) and the generalized seizure threshold (GST), and on the afterdischarge durations ADD1 (E, F) and ADD2 (G, H) in rats (n = 6). Saline (S) injections served as pre- and postdrug controls. At 40 mg/kg OV329, none of the animals expressed generalized motor seizures and the SD and ADD at GST were set at 0 s (B, D, F, G) for data evaluation. Data are expressed as mean + SEM. Statistically significant differences between OV329 and saline as predrug control are indicated by asterisks, and differences between OV329 and saline as postdrug control are indicated by hashtags (one-way mixed-effects model analysis, post hoc Sidak correction, *p < .05, **#p < .01, ****#, #### p < .0001)
OV329 showed a 30-fold greater anticonvulsant potency on ivPTZ-induced myoclonic jerks and clonic seizures compared to previous findings on vigabatrin (Bröer et al.16; see below). These results reveal an anticonvulsant profile of OV329 that appears to be superior in potency and efficacy to vigabatrin.

As one mechanism, seizure control can be achieved by increasing brain GABA concentrations.3,5 Initial attempts to increase GABA levels in the brain directly by intravenous injection of the neurotransmitter were unsuccessful due to impermeability of the blood–brain barrier to GABA.42,43 Therefore, mechanism-based, selective inactivators of GABA-AT, like vigabatrin and OV329, were rationally designed to enhance GABAergic transmission in the CNS by suppressing GABA degradation.4,44 Vigabatrin demonstrated inhibition of GABA catabolism to be effective in decreasing seizures in both animal models and humans.44 In previous animal studies, systemic vigabatrin blocked generalized convulsions induced by subcutaneous PTZ with an ED$_{50}$ of 940 mg/kg and increased ivPTZ-ST at 490 mg/kg 6 h after intraperitoneal administration in mice.45,46 More recently, increased ivPTZ-ST 6 h after systemic vigabatrin (600 and 1200 mg/kg ip) was confirmed in rats.16 Our results reaffirm increased ivPTZ-ST by GABA-AT inactivation and further strengthen the concept of mechanism-based GABA-AT inactivators acting as effective anticonvulsant agents. Vigabatrin had long been the most effective of these mechanism-based GABA-AT inactivators as an anticonvulsant agent,47 until its analogues CPP-115 and OV329 showed higher in vitro efficacy.12,14 CPP-115 decreased infantile spasms in a rat model at considerably lower and better tolerated doses than vigabatrin.48 OV329 revealed pharmacokinetic properties superior to CPP-115 and reduced severity of NMDA-induced seizures in juvenile mice.14,15 Here, we demonstrated that systemic OV329 injections caused clear antiseizure effects at a dose of 40 mg/kg (the mean rise in seizure threshold was 103% above the basal threshold for the myoclonic
twitch and 105% above the basal threshold for the clonic seizure) in rats. In the same rat seizure model, a high dose of 1200 mg/kg vigabatrin was necessary to induce comparable seizure threshold elevations (59% and 87% above the basal threshold for the myoclonic twitch and for the clonic seizure, respectively), revealing OV329 is equieffective at 30-fold lower mass doses than vigabatrin (40 mg/kg OV329 vs. 1200 mg/kg vigabatrin) on ivPTZ-induced myoclonic jerks and clonic seizures.

In addition to the primary screening in our acute seizure model, we verified and further investigated the anticonvulsant activity of OV329 in the only clinically validated chronic seizure model, the kindled rodent model of mesial temporal lobe epilepsy.49 The amygdala-kindling model is highly predictive of drug efficacy against focal limbic seizures in humans.21 Furthermore, especially focal and to a lesser extent also focal-to-bilateral tonic–clonic seizures in amygdala-kindled rats are considered difficult to suppress, so that amygdala kindling was the first proposed animal model of drug-resistant focal epilepsy in humans.50 Using this model of difficult-to-treat types of seizures, we found that 6 h after its administration, OV329 (40 mg/kg) increased the threshold for electrographic seizures recorded from the stimulated amygdala. Furthermore, although the suppression of generalized motor seizures was more pronounced, we found that OV329 decreased the incidence not only of the generalized seizures, but also of the difficult-to-treat focal seizures. Although less predictive with regard to drug activity against epilepsies in humans, we additionally observed that OV329 decreased the duration of electrographic and motor seizures, and reduced SS in fully kindled rats. A similar dose of vigabatrin (50 mg/kg) failed to exert anticonvulsant effects in kindled rats 4 h after intraperitoneal administration, but rather increased the duration of motor seizures.19 Most studies on vigabatrin have observed antiseizure effects only at much higher doses (800–1500 mg/kg) in amygdala-kindled rats,17,51,52 again indicating the much greater potency of OV329 relative to vigabatrin. In comparison to the only study on vigabatrin, in which a lower dose (200 mg/kg) displayed anticonvulsant activity after acute intraperitoneal injection in kindled rats,19 OV329 resulted in a more than sixfold increase in ADT and more pronounced reductions in SS and SD. The decreased SS at increased ADT in the present study suggests that OV329 not only elevates seizure threshold but also reduces seizure propagation from the focus.

Although more than 20 new ASDs have been introduced in the past 30 years, there is continuing clinical demand for more efficacious and better tolerated ASDs, especially with regard to the large portion of about 30% drug-resistant epilepsy patients. Because vigabatrin is typically effective only in high doses that are often associated with serious side effects, more potent and less toxic GABA-AT inhibitors are desired. The favorable pharmacokinetic profile of OV329, including a higher binding affinity and a larger inactivation rate constant for GABA-AT compared to previous GABA-AT inhibitors,14,36 becomes manifest in vivo in the present study. Here, we prove for the first time the potent anticonvulsant properties of the new GABA-AT inactivator OV329 in two different rat models. Several vigabatrin studies have revealed pharmacoresistance in a subset of patients and rodents in PTZ tests and kindling models.23,25,51,52 Therefore, despite of its tremendous efficacy as an inactivator of GABA-AT, we presumed not all rats would respond sensitively to OV329. Remarkably, however, all rats were responsive to the highest (and tolerable) dose of OV329 in both seizure models. OV329 (40 mg/kg) not only increased the thresholds for ivPTZ-induced myoclonic twitch and clonic seizure but also elevated the ADT and completely suppressed generalized seizures throughout all kindled rats. Together with the above-described characteristics of amygdala-kindled rats being considered as a model for difficult-to-treat types of seizures, our data indicate that OV329 may be a promising candidate for the treatment of drug-resistant epilepsies.

Systemic administration of OV329 was associated with tolerable adverse effects, including decreased body temperature, reduced body weight, and impaired motor coordination in a few animals, most probably due to sedation. The highest dose decreased body temperature and weight by about 7–9°C and 6%, respectively, within 6 h after administration. This is in line with previous studies, where GABA-AT inhibition by vigabatrin resulted in comparable or greater reductions in body temperature and weight.16,52 Comparison of median effective doses of OV329 on ivPTZ-induced seizure thresholds (ED50 = 25.83 mg/kg and 20.8 mg/kg for the myoclonic twitch and the clonus, respectively) and median toxic doses on rotarod performance indicate a narrow therapeutic index for OV329 regarding PTZ-induced myoclonic twitches and clonic seizures. However, previous studies demonstrated that vigabatrin doses (1000–1200 mg/kg) required for an anticonvulsant effect equivalent to what we observed here with OV329 cause significant motor deficits in rats,16,54 whereas OV329 did not significantly impair rotarod performance across the animals. Moreover, we previously observed the anticonvulsant effects of vigabatrin (1200 mg/kg ip) being accompanied by ataxia and sedation in each rat,16 whereas in the present study, only one rat displayed ataxia along with increased seizure thresholds. These findings suggest that the improved (30-fold) in vivo potency of OV329 is also coupled to an improved tolerability compared to vigabatrin, although future studies are needed to comparably analyze the therapeutic indices of vigabatrin and OV329 more specifically. Because this study focused
on the acute effects of OV329 and its antiseizure potential, we did not determine the retinal toxicity of OV329. However, previous studies with CPP-115 indicate that the retinotoxicity of vigabatrin does not appear to be coupled to the GABA-AT activity.\textsuperscript{13,14}

In summary, we collected important data that could help to define the clinical potential of OV329. We provide proof-of-concept evidence that OV329, a novel GABA-AT inactivator, is highly active against ivPTZ-induced myoclonic twitches and clonic seizures as well as difficult-to-treat amygdala-kindled focal and focal-to-bilateral tonic–clonic seizures. OV329 showed robust anticonvulsant efficacy with only mild adverse effects in the two well-established animal seizure and epilepsy models. Together, the present findings highlight OV329 as a highly promising anticonvulsant agent and a promising candidate for the treatment of drug-resistant epilepsies.

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**CONFLICT OF INTEREST**

R.B.S. is an inventor on the patent that covers OV329, which has been licensed to the biotechnology company Ovid Therapeutics by Northwestern University. M.J.D. is a consultant to Ovid Therapeutics. None of the other authors has any conflict of interest to disclose. 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.

**ORCID**

Malte Feja  
https://orcid.org/0000-0002-8670-5495

Manuela Gernert  
https://orcid.org/0000-0001-6980-2640

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