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RESEARCH ARTICLE

Lacosamide Reduces Seizure Severity but Increases Seizure Frequency in PTZ-Kindled Rats

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Objective: This study evaluated the anticonvulsant action of lacosamide (LCS), a novel drug that was recently approved for the treatment of partial or secondarily generalized seizures, using an animal model of generalized epilepsy induced by repetitive pentylenetetrazole (PTZ) administration in rats. The main goal was to evaluate the behavioral pattern of lacosamide action by classifying seizures according to a modified Racine-scale. Furthermore, the reproducibility of the win-PTZ kindling model of epilepsy, a recently described variant of the standard PTZ-kindling model, was also assessed. **Methods**: Adult male Wistar rats (n=16) were divided into two groups and underwent the win-PTZ-kindling protocol in two independent trials. After finishing the kindling procedure, all animals, which presented stage 5 seizures were tested for the anticonvulsant action of lacosamide at three different doses (3, 10, and 30 mg/kg). **Results**: The maximal severity of seizures decreased and the latency to stage 3-5 seizures increased when the animals were treated with lacosamide at a single dose of 10 mg/kg compared to saline pretreatment ($p < 0.05$), both parameter reflecting an anticonvulsant action of the drug. Unfortunately, the number of stage 3-5 seizures also increased, but not significantly. The win-PTZ kindling model showed an adequate reproducibility between different trials, however, the number of fully kindled rats was lower than previously reported. **Conclusions**: Lacosamide showed a convincing anticonvulsant action in the win-PTZ kindling model of epilepsy by preventing the generalization of seizures. The win-PTZ kindling model was proved to be useful for studying epileptogenesis and the anticonvulsant action of drugs.

Keywords: lacosamide, anticonvulsant, kindling, epilepsy

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Introduction

Lacosamide is a so-called third generation antiepileptic drug (AED), which is claimed to act on voltage-gated sodium channels in a different way than other anticonvulsants have done so far, i.e., by enhancing the slow inactivation of the channels [1,2]. Furthermore, lacosamide was shown to bind to collapsing-response mediator protein 2 (CRMP-2), another unique mechanism of action proposed for this drug, which was not linked to epilepsy before, but since then several hypotheses appeared describing the role of CRMP-2 in neurite outgrowth [3,4].

The initial discovery that compounds having N-acetyl-alanine-N-benzylamide core structure exhibit a remarkable anticonvulsant activity in the maximal electroshock seizure (MES) test dates back in 1985, when Harold Kohn and colleagues synthesized and evaluated over 250 compounds by the Anticonvulsant Screening Project (ASP) [2]. Lacosamide, the (2R)-2-(acetylamino)-N-benzyl-3-methoxy-propanamide, also known as harkoseride, showed a similar potency to that of phenytoin, the ED50 (i.e. the dose at which 50% of animals experience efficacy) being 4.5 mg/kg when administered intraperitoneally in mice and 3.9 mg/kg after p.o. administration in rats. Several other animal models of seizures were used to characterize the anticonvulsant action of lacosamide before admitting it to clinical trials. Based on the algorithm used by the ASP, a compound showing anticonvulsant activity in the MES test was subject to a testing in the subcutaneous pentylenetetrazole (sc PTZ) seizure test. Lacosamide was incapable to reduce the clonic seizures induced by PTZ at doses of 85 mg/kg and 70 mg/kg in both mice and rats, respectively. However, it significantly increased the seizure threshold in the intravenous PTZ test, which involved a continuous infusion of a 0.5% solution of PTZ [5].

Pentylenetetrazole is a frequently used proconvulsant agent, a GABAA receptor antagonist, which induces acute seizures in laboratory animals after s.c. or i.p. administration at high doses (above 70 mg/kg) [6]. But it can also cause anxiety-like behavior at subconvulsive doses (from 15 to 30 mg/kg) [7]. Interestingly, repeated administration of PTZ at subconvulsive doses (between 35 and 40 mg/kg) induces seizures in rats, these seizures showing increasing severity and culminating in generalization [8]. The process is similar to the widely used kindling technique, where seizures are induced by repeated subconvulsive electrical stimulation [9]. Electrical kindling has been used as a chronic model of temporal lobe epilepsy and complex partial seizures, while chemical kindling models have had different purposes depending on the pharmacological nature of the convulsant agent. PTZ-kindling model is commonly used to study epileptogenesis and test the anticonvulsant action of different compounds [10]. It involves repeated administration of PTZ at a subconvulsive dose

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(30-40 mg/kg) every 48 h until achieving the fully-kindled state. Recently, Davoudi et al. demonstrated that a simplified protocol (named by them "win-PTZ model"), which involves a reduced number of PTZ injections according to a specific dosing schedule over a certain number of days, leads to the same results. The similarity between the win-PTZ kindling model and the standard PTZ-kindling was confirmed by the results of a combined behavioral, electrophysiological and molecular biology experiment [11]. Thus, the win-PTZ kindling model can become a more convenient and more preferable approach because it is less labor-intensive than standard PTZ kindling, but further studies are needed to replicate the initial results in independent studies [12].

Therefore, this study evaluated the action of lacosamide on generalized seizure induced by PTZ using the novel approach for kindling protocol. The main objective was to characterize the behavioral pattern of lacosamide action by classifying seizures according to a modified Racine-scale. The second objective was to evaluate the reproducibility of the win-PTZ kindling model by performing two independent experiments.

Methods

Animals

Male adult Wistar rats (n=16) weighing from 350 to 550 g were kept singly in transparent Plexiglas cages in a laboratory with a temperature of 23±2°C and a relative humidity of 60±10% in natural light/dark cycle. Animals were adapted to laboratory conditions for at least 2 days before the experiments. Throughout the experiment, animals had free access to standard chow and tap water. First of all, all animals underwent win-PTZ kindling protocol without other intervention until reaching fully kindled state. After PTZ injections, each animal was put back into the home cage and observed for one hour. In the post-kindling state, the anticonvulsant action of lacosamide was tested after administering a challenge dose PTZ (Figure 1).

All procedures involving animals and their care were conducted after approval by the local ethics committee for animal experimentation (approval no. 34/2016) and conformed to institutional, national and European Union guidelines (Directive 2010/63/EU).

Drug preparation and administration

PTZ (Sigma Aldrich, St. Louis, USA) was dissolved in 0.9% saline in a concentration of 37.5 mg/ml and injected intraperitoneally (i.p.) at a subconvulsive dose of 37.5 mg/kg every alternate day. After each injection of PTZ, the rats were monitored for one hour and the behavioral severity of seizures was rated by two experienced observers according to a modified Racine scale as follows [8]: stage 0 – no response; stage 1 – ear and facial twitching; stage 2 – myoclonic jerks without rearing or convulsive waves through the body; stage 3 – myoclonic jerks with rearing; stage 4 – turn over into side position, clonictonic seizures; stage 5 – turn over into back position, generalized tonicclonic convulsions. An animal was considered fully-kindled when it have had stage 4-5 seizure score on two consecutive trials.

The anticonvulsant action of lacosamide in three different doses was tested on fully-kindled animals only, from either of the two groups. In this phase, each dose of PTZ used for seizure induction was administered on every alternate day for a period of 10 days (days 0, 2, 4, 6, 8 and 10). Lacosamide (3, 10, 30 mg/kg) was dissolved in 0.9% saline and were administered i.p., a single dose 15 minutes before the PTZ injection (according to LCS pharmacokinetics this is the time of peak effect). Lacosamide was granted by Hetero Drugs Ltd., India. The severity and the latency to the onset of seizures were noted. In order to exclude an unexpected modification of seizure pattern as a possible source of confounding only those animals were included which showed stage 5 seizures at the first PTZ challenge dose (T0) administered in the post-kindling period. Furthermore, at the end of the experiment each animal was retested with another challenge dose of PTZ (T10) and those animals which did not have stage 5 seizures were excluded (n=1). A detailed timeline of this experiment can be found in Figure 1.

Statistical analysis

Data were analyzed with GraphPad Prism 5 (GraphPad Software, San Diego, CA). The results are expressed as the mean ± SEM. The maximal seizure severity observed during the kindling period was compared between the groups using a two-way ANOVA test. In the testing period, the analysis of maximal seizure severity and latency to the onset of stage 3-5 seizures was performed with repeated

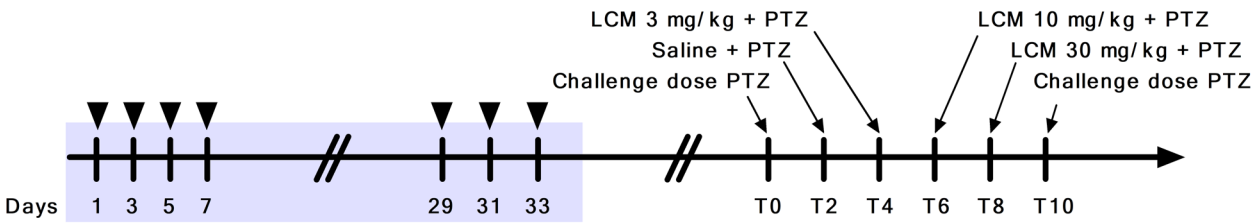

Fig. 1. Schematic timeline of the study design, highlighting the PTZ-kindling procedure and the drug testing in the post-kindling period

Legend: The shaded part of the timeline indicates the win-PTZ kindling procedure, and the double lines crossing the timeline represent latent periods, when no interventions were being performed. Arrowheads show the time of PTZ administration, and arrows show the time of drug or saline administration followed by a challenge dose of PTZ.

measures one-way ANOVA test; the number of stage 3-5 seizures were compared using Friedman test followed by a Dunn's post-hoc analysis. Means were considered to differ significantly if $p<0.05$.

Results

Win-PTZ kindling model

Two independent trials were performed in drug-naïve animals randomly assigned to each group (n=8/group). The kindling procedure had been started after two days of acclimatization of the animals to laboratory conditions. All animals that showed stage 4-5 seizures on two consecutive PTZ administrations at the beginning of the kindling procedure (on days 1, 3, 5, and 7) were excluded from further studies: one animal in the first group and three in the second group. Furthermore, one animal from the first group died before finishing the kindling procedure, having repetitive stage 5 seizures after the third dose of PTZ. After all, the number of animals achieving fully-kindled state, according to the predefined criteria of having stage 4-5 seizures on two consecutive PTZ administrations, was not different between groups (2/6 vs 3/5, $p > 0.05$). The severity of the observed seizures was also similar (Figure 2), and a significant increase with time was detected in both groups ($F(6,63)=2.927$, $p < 0.05$).

Anticonvulsant action of lacosamide

The maximum seizure score and the latency to the onset of generalized seizures reflected a convincing anticonvulsant action of lacosamide in case of 10 and 30 mg/kg dose. Although a decrease of the maximum seizure score (mean ± SEM) from 4.50 ± 0.27 (after saline administration) to 3.90 ± 0.28 was observed even after administering a small dose of 3 mg/kg LCS, a statistically significant difference was observed only at the dose of 10 mg/kg LCS (a decrease to 3.30 ± 0.15, $F(5,47) = 12.37$, $p < 0.001$), a reduction from stage 5 to 3 of the maximal seizure severity being observed in 7 of 8 animals. Interestingly, further increasing the dose to 30 mg/kg did not cause a more evident decrease of the seizure severity compared to the dose of 10 mg/kg (3.6 ± 0.22 vs 3.30 ± 0.15, $p > 0.05$) (Figure 3). Acute LCS administration at a dose of 10 mg/kg also prolonged the latency to the onset of stage 3-5 seizures to a mean ± SEM value of 19.1 ± 4.5 minutes; a significant difference between saline and lacosamide treatment was observed ($F(5,47) = 2.889$, $p < 0.05$; Figure 4).

On the other hand, the number of stage 3-5 seizures increased when the animals were administered LCS. The highest frequency of stage 3-5 seizures was observed at the dose of 10 mg/kg, but the difference between groups did not reach statistical significance (Figure 5). As the number of stage 3-5 seizures showed an up to 3 fold increase after LCS administration, in a few cases, the monitoring period was extended to 90 minutes in order to assure that all relevant seizures were registered. However, all animals exhibited stage 3-5 seizures during the first hour after PTZ challenge only.

Discussions

The PTZ-kindling model of epilepsy has been used for a long time to study the pathophysiology of epileptic syndromes and to test anticonvulsant drugs. In general, the kindling models more closely resemble human epilepsy syndromes and they are capable to reveal the disease modifying potential of drugs [13]. However, they are time-consuming and labor intensive. The time frame of the kindling model cannot be shortened, supposing that the molecular and cellular modifications, which were caused by an initial insult and resulted in an alteration of neuronal excitability, need time to evolve. But, Davoudi et al. observed that during this critical time interval no further interventions (i.e., PTZ injections) are needed for achieving the fully kindled state in rats [11]. They proposed a method, called win-PTZ model, which uses a reduced number of PTZ injections, a minimum of 7 instead of the 17 injections needed in the standard PTZ-kindling protocol. But reducing the number of injections can increase the interindividual het-

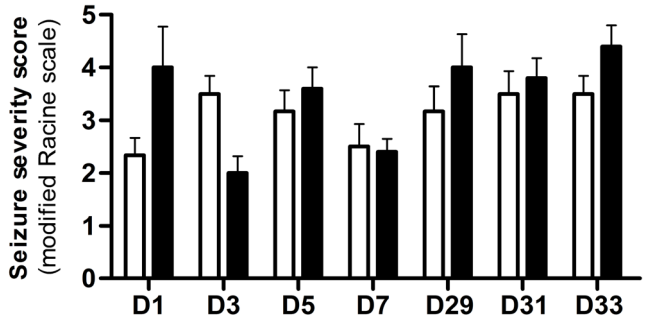

Fig. 2. The evaluation of the reproducibility of win-PTZ kindling model by comparing the intensity of seizures. After comparing the seizure stages between groups and between days using two-way analysis of variance (for repeated measures), there was no significant difference between the groups. Values are expressed as mean ± SEM (n = 8).

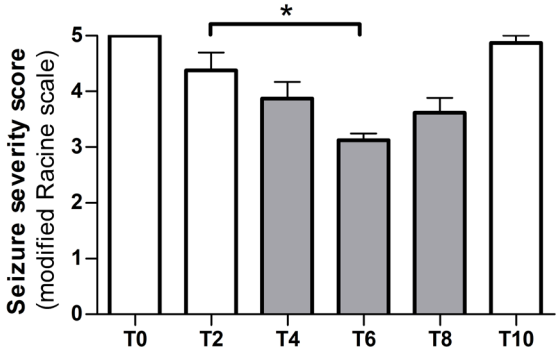

Fig. 3. Lacosamide reduced the maximal seizure severity after acute administration to fully-kindled rats.

Legend: The asterisk shows a statistically significant difference ($p < 0.05$). Values are expressed as mean ± SEM (n = 8). Grey bars indicate lacosamide treatment, and open bars show saline or no treatment. For detailed informations regarding each testing day (T0-T10) see Fig. 1.

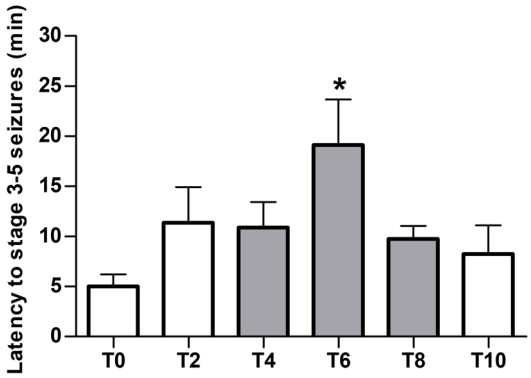

Fig. 4. The latency of the onset of stage 3-5 seizures increased after the administration of lacosamide at a dose of 10 mg/kg.

Legend: The asterisk shows a statistically significant difference ($p < 0.05$). Values are expressed as mean ± SEM (n = 8). Grey bars indicate lacosamide treatment, and open bars show saline or no treatment. For detailed informations see regarding each testing day (T0-T10) see Fig. 1.

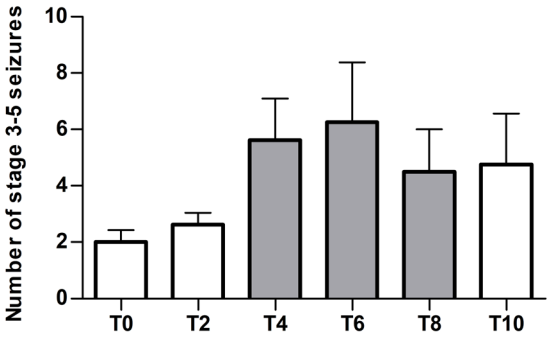

Fig. 5. Lacosamide administration to fully-kindled rats slightly increased the total number of stage 3-5 seizures but without statistically significant differences.

Legend: Values are expressed as mean ± SEM (n = 8). Grey bars indicate lacosamide treatment, and open bars show saline or no treatment. For detailed informations see regarding each testing day (T0-T10) see Fig. 1.

erogeneity in response to the proconvulsant action of PTZ. In this study, two independent trials were performed to reproduce the win-PTZ kindling model. There were no significant differences between them regarding the number of fully kindled rats or the seizure severity. These results support the high reproducibility of the model, however, the number of fully kindled rats observed in this study was lower than that reported earlier. On the other hand, in the post-kindling period, the response of the animals to a challenge dose of PTZ was similar, all animals showing stage 5 seizures.

The anticonvulsant activity of lacosamide was tested on many different seizure models previously. It showed a convincing anticonvulsant effect in MES test and in the 6 Hz model of psychomotor seizure both in rats and mice, the calculated ED50 laying between 5 and 10 mg/kg [5]. However, the acute seizures provoked by s.c. PTZ were resistant to lacosamide up to a huge dose of 100 mg/kg. In kindling models of epilepsy, lacosamide was tested in the rapid hippocampal kindling model and exhibited a dose-dependent reduction in seizure severity, but it required higher doses than for the MES test [2].

In this study, lacosamide demonstrated a remarkable anticonvulsant effect in PTZ kindling model of epilepsy in rats at 10 and 30 mg/kg of dose, significantly decreasing the severity of seizures. In previous studies lacosamide showed no efficacy in PTZ induced seizures, but this study demonstrated that the acute administration of lacosamide reduced the severity of seizures by inhibiting their generalization using the win-PTZ-kindling model. At a dose of 10 mg/kg, lacosamide significantly decreased the severity of seizures. To our best knowledge, this is the first study that tested lacosamide on fully kindled rats in the PTZkindling model of epilepsy in rats.

The main difference between s.c. PTZ induced acute seizures and the PTZ kindling model is the dose of the PTZ used to evoke seizure: in the former case a high dose (85-100 mg/kg) is administered once, whereas in the latter case half of that dose (35-40 mg/kg) is administered repeatedly in order to sensitize the animals to the convulsive effect of PTZ [8,11]. Another important difference between acutely induced seizures by PTZ and PTZ kindling is that sensitization brings cellular and molecular remodeling of neuronal circuits in the brain. It was suggested that the background of the evoked seizures is different: in the early stage of PTZ kindling seizures appear as a result of thalamo-cortical changes, whereas in the late stage the generalized clonictonic seizures originate from limbic structures [10,14]. Thus, in the fully kindling state, the generalized seizures induced by a challenge dose of PTZ resemble seizures of temporal lobe epilepsy in humans, while the acute seizures have cortical origin, a different type of seizure.

Therefore, the anticonvulsant action of drugs in PTZ kindling model differs from that described in the s.c. PTZ seizure model [15]. Taken together, the anticonvulsant effect of lacosamide described in this study is not in contradiction with previously reported results. Presumably, if the type of seizures vary with dose and mode of administration of PTZ, the anticonvulsant action of the tested drugs should also be different.

Lacosamide exhibited a statistically significant anticonvulsant action characterized by a decrease of the seizure severity at 10 mg/kg dose, but interestingly, its efficacy to reduce seizure severity did not increase after administering a dose of 30 mg/kg compared to that observed at 10 mg/kg dose. Unfortunately, lacosamide increased the number of seizures with stage 3 score or above, which suggested an intensification of seizure activity. This observation is in accordance with previously reported data where lacosamide shortened the duration but increased the frequency of epileptiform activities in an in vitro model [16]. Moreover, at 30 mg/kg the latency of stage 3-5 seizures decreased and the maximal severity of the seizures increased, which also suggested that the proconvulsant action of LCS may be

dose-dependent. At this high dose, an increase in seizure susceptibility was described earlier, drug-induced seizures being observed in 50% of the kindled rats treated with 30 mg/kg LCS [17]. Nevertheless, it is commonly accepted that all AEDs can aggravate epilepsy at high, suprathera-peutic doses [18].

Conclusions

Lacosamide, a novel antiepileptic drug, which has recently been approved for use in clinical practice to treat partial and secondarily generalized seizures, demonstrated a remarkable anticonvulsant action in the PTZ kindling model of epilepsy in rats by suppressing the generalization of seizures. However, it seemed to increase the total number of stage 3-5 seizures. Another important finding of this study is that the win-PTZ kindling model of epilepsy, besides its advantages related to ethical aspects and labor intensity of experimental activities, has an adequate reproducibility and is suitable to study either epileptogenesis or the anticonvulsant action of drugs.

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Conflict of interest

None to declare.

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