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Inverted-U response of lacosamide on pilocarpine-induced status epilepticus and oxidative stress in C57BL/6 mice is independent of hippocampal collapsin response mediator protein-2

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ABSTRACT

Objective: Currently, lacosamide (LCM) is not approved for use in status epilepticus (SE) but several shreds of evidence are available to support its use. The present study was, therefore, undertaken to evaluate the effect of LCM on pilocarpine (PILO) induced SE and neurodegeneration in C57BL/6 mice and to ascertain the involvement of CRMP-2 in mediating above effect.

Methods: Pilocarpine-induced SE model was developed to explore the effect of LCM 20, 40 and 80 mg/kg in mice. We assessed the seizure severity, seizure latency, spontaneous alternation behavior (SAB) and motor coordination by behavioral observation. Histopathological evaluation and measurement of the levels of CRMP-2, reduced glutathione (GSH) and malondialdehyde (MDA) were carried out in mice hippocampus.

Results: LCM exhibited a biphasic effect i.e., protection against SE at 20 mg/kg and 40 mg/kg dose whilst aggravated seizure-like behavior and mortality at 80 mg/kg. Further, it increased percentage alternation (i.e., restored spatial memory) in SAB and elevated motor impairment with increasing dose. Histologically, LCM 20 mg/kg and 40 mg/kg (but not 80 mg/kg) reduced neurodegeneration. LCM 20 mg/kg and 40 mg/kg reversed the elevated MDA and GSH levels while 80 mg/kg showed a tendency to increase oxidative stress. In contrast, LCM (at all doses) reversed the pilocarpine-induced elevation of collapsin response mediator protein-2 (CRMP-2).

Conclusion: LCM protected against pilocarpine-induced SE, associated neurodegeneration and improved pilocarpine-associated impairment of spatial memory. The study reveals that CRMP-2 may not be mediating the inverted-U-response of LCM at least in pilocarpine model. Therefore, the anti-oxidant effect of LCM (and not its ability to modulate CRMP-2) was anticipated as the mechanism underlying neuroprotection.

1. Introduction

Lacosamide (LCM), chemically known as (R-*N*-benzyl 2-acetamido-3-methoxypropionamide), was approved by the US-FDA in 2008 for adjunctive treatment in partial-onset epilepsy. It has been reported to act by two mechanisms of actions: a) by enhancing the slow inactivation of voltage-gated sodium channel and (Errington et al., 2008) by modulating collapsin response mediator protein-2 (CRMP-2) (Beyreuther et al., 2007). Though the ability of LCM to reduce neuronal excitability via slow inactivation of voltage-gated sodium channel is responsible for its anti-seizure effects, it has been proposed that its role in modulating CRMP-2 might be responsible for its neuroprotective and/or disease modifying effects. CRMP-2 is an intracellular phosphoprotein involved in axon guidance and neurite outgrowth (Wang and Strittmatter, 1996). It has been reported that chronic depolarization by KCl enhances the CRMP-2 level by inhibiting its phosphorylation by glycogen synthase kinase 3β (GSK3β) by the reduction in cyclin-dependent kinase (Cdk5). This, in turn, enhanced the binding of CRMP-2 to tubulin, thereby promoting neurite outgrowth (Brown et al., 2004). This neurite outgrowth is suppressed by LCM through inactivation of CRMP-2, which furthers impairs tubulin polymerization (Wilson et al., 2014a). Lately, the SE-induced mossy fibers sprouting (MFS) in the hippocampus was found to be associated with CRMP-2 and hence it was proposed that LCM may have efficacy in the prevention of epileptogenesis (Lee et al., 2012a).

LCM has been proved to be efficacious in various animal models, such as cobalt/homocysteine SE model, electrical SE model and perforant path model of self-sustaining SE, showing reduced seizure

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duration, an abolition of tonic-clonic seizures and neuroprotection, respectively (Beyreuther et al., 2007; Stohr et al., 2007; Licko et al., 2013). However, the effect of LCM on pilocarpine-induced SE and associated neurodegeneration remains undefined. In the present work, we sought to investigate the effect of LCM on pilocarpine-induced seizures and associated neurodegeneration in mice and to ascertain the involvement of CRMP-2 in mediating these effects. Further, pilocarpine-induced SE is known to be associated with oxidative stress (Freitas et al., 2004) and LCM has been found to increase endogenous antioxidant enzymes against transient ischemic damage in the brain (Choi et al., 2016). Therefore, we investigated the effect of LCM on SE-induced oxidative stress. In view of the impairment in spatial memory and learning (Giovagnoli and Avanzini, 1999) associated with pilocarpine-induced SE and the reported effects of LCM to restore the learning abilities of rats during PTZ-induced kindling (Shishomanova, 2014), we evaluated the effect of LCM on spatial memory and motor impairment following pilocarpine-induced SE.

2. Material and methods

2.1. Animals

Male C57BL/6 mice of age 9 ± 1 weeks old, weighing between 25 ± 5 g were used in all experiments. Animals were procured from Central Animal House Facility, Jamia Hamdard, New Delhi, India and were housed in polypropylene cages, bedded on rice hulls. Mice were maintained at 25 ± 2 °C temperature, 50–55% humidity in 12 h light/dark cycle plus free access to food and water. All experimental procedures were carried out in accordance with the guidelines of 'The Committee for the Purpose of Control and Supervision of Experiments on Animals' (CPCSEA), New Delhi, India. The proposal for conducting experiments on animals was approved by the Institutional Animal Ethics Committee of Jamia Hamdard University (File number 1299, 2016). All possible efforts were made to minimize the pain and sufferings to animals. The purpose behind the selection of C57BL/6 mice strains is their high sensitivity for pilocarpine-induced SE (Shibley and Smith, 2002).

2.2. Drugs, dosing schedule and study design

Drugs utilized in our research are Pilocarpine (PILO); (Everon life sciences Pvt Ltd), Lacosamide (LCM); (Torrent Pharmaceuticals Ltd), Sodium valproate (SVP); (Cayman Chemicals), Atropine and Diazepam (Neon Laboratories Ltd). The sample size was calculated using previous pilot study using G* power version 3.0.10 software, the effect size (d) = 3.9260, α = 0.05 (type 1 error), 95% confidence interval, power = 0.95, allocation ratio 1 and sample size = 18 (3 animals per group) which was raised to 5 animals per group. Mice were divided into six groups (five animals per group) by employing a simple randomization method. All treatments were given by intraperitoneal (i.p.) route in a volume not exceeding 10 ml/kg. (1) NC (0.9% normal saline);(2) TC (PILO, 260 mg/kg, i.p.); (3) LCM 20, LCM (20 mg/kg, i.p.) +PILO; (4) LCM 40, LCM (40 mg/kg, i.p.) + PILO; (5) LCM 80, LCM (80 mg/kg, i.p.) + PILO; (6) SVP, SVP (300 mg/kg, i.p.) + PILO. LCM dosing was continued for the next two days (48 h), followed by SAB and rota-rod test performed on the 3rd day of PILO administration. The animals were then sacrificed for histopathological analysis and estimation of the levels of CRMP-2, GSH, and MDA were assessed in the hippocampus. Animal Research Reporting of In-vivo Experiments (ARRIVE) guidelines were adopted for reporting of data.

2.3. Induction of status epilepticus and pilot study to determine pilocarpine dose

The dose of pilocarpine in the literature ranges from 250 to 350 mg/kg in mice (Shibley and Smith, 2002; Muller et al., 2009; Turski et al., 1984). During dose standardization process, two mice were injected with 300 and 280 mg/kg of pilocarpine respectively, and it was found that the seizure severity was excessively high, and the mice experienced post-ictal coma phase followed by death. Then, we administered a lower dose of 250 mg/kg, but it was insufficient to develop all the seizure stages required for SE induction. Considering above observations, we selected 260 mg/kg dose, which allowed us to develop most stages of SE in experimental mice.

In order to control the PILO induced peripheral side effects, atropine sulphate (2 mg/kg) was injected 15 min prior to PILO (260 mg/kg). LCM (test drug) and SVP (standard drug) were administered 30 min before PILO injection. Animals were video monitored for 2 h in order to assess the seizure severity or seizure scoring. Seizures scoring was performed using modified Racine scale, (1972) with slight modifications by Borges (2003) as follows: Stage 0, normal activity; Stage 1, rigid posture or immobility; Stage 2, stiffened, extended, and often arched (Straub's) tail; Stage 3, partial body clonus, including forelimb or hind limb clonus or head bobbing; Stage 3.5, whole body continuous clonic seizures while retaining posture; Stage 4, rearing; Stage 4.5, severe whole body continuous clonic seizures while retaining posture; Stage 5, rearing and falling; Stage 6, tonic–clonic seizures with loss of posture or jumping. SE was defined by continuous seizure activity for at least 2 h involving stage 3.5 seizures and one stage 5 or 6 seizure or several stage 4.5 seizures. Latency to first convulsion and SE were recorded along with seizure scoring. The mortality rate in each group (if any) was observed for 24 h. After 2 h of video monitoring, the animal survival was improved by terminating the seizures with diazepam (10 mg/kg, i.p.).

2.4. Spontaneous alternation behavior (SAB)

Spatial learning and memory of mice are measured by spontaneous alternation behavior test. It aims at testing the rodent's willingness to explore new surroundings. This test is grounded on the innate tendency of rodent to explore new arm rather than selecting the previously visited arm. Poor alternation in arm entries indicates the deficit spatial memory and increased percentage alternation signifies the repeated alternation across cross maze arms. The assessment of SAB in the cross maze was performed following the method of Vohora et al., 2005 for mice (Vohora et al., 2005). A four-arm wooden cross maze (height: 50 cm; arms: length 24.5 cm, breadth 8 cm, wall height 11 cm) with a squared central platform (12 × 12 cm) was used. The mice were positioned on the central platform and allowed to traverse the maze freely for 6 min. The total numbers of entries were recorded along with the sequence of entries into each arm. An alternation was defined as the entry into four different arms on an overlapping quintuple set. The quintuple set was represented by the five consecutive arm choices within the total set of arm choices. For instance, a quintuple set consisting of arm choices B, A, C, D, B comprised an alternation, whereas the set with B, A, D, B, A did not. Percentage alternation was calculated as actual alternations/possible alternations × 100, where possible alternation was the number of arm entries minus four.

2.5. Rota-rod test

Fine motor coordination and learning were assessed by following the original rota-rod testing method given by Dunham and Miya (1957) and Wahlsten et al. (2003), with some modifications given by Oliveira et al. (2015). The task comprised of two training sessions and one testing session. Animals experienced post-ictal coma after PILO administration and mice were not active in performing the rota-rod test. Therefore, we performed two-day training session prior to the PILO administration. At each training session, the mice were trained to stay on a rotating wheel of rota-rod at a minimum speed of 4 rpm. After this, a constant speed of 8 rpm was set and each trial starts with the mouse being placed in the apparatus and ends when the mouse falls off the rod