Print ISSN: 2319-2003 | Online ISSN: 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

DOI: http://dx.doi.org/10.18203/2319-2003.ijbcp20173685

Original Research Article

Anticonvulsant effect of lercanidipine against pentylenetetrazole induced kindling in mice

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Received: 01 August 2017
Accepted: 08 August 2017

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ABSTRACT

Background: Emerging evidence has demonstrated the role of high-voltage - sensitive activated dihydropyridine (L-type, CaV1.x) channels in the development of epilepsy. Based on that we hypothesized that lercanidipine, a dihydropyridine calcium channel blocker, would protect against Pentylenetetrazole (PTZ) induced kindling in mice model of epilepsy.
Methods: Kindling was induced in Swiss albino mice with PTZ in subconvulsive dose (30 mg/kg i.p.) thrice a week for nine weeks and the effect was scored using '4 point scoring system'. Rechallenging on the 3rd and 10th day with the same dose of PTZ was carried out after the last chronic dose.
Results: The data of the present study demonstrated that pretreatment with lercanidipine (½ h before PTZ, in doses of 1 and 3 mg/kg i.p. daily) alone and in combination with diazepam (2mg/kg i.p.) had decreased the incidence and severity of seizure as well as prolonged the onset of kindling in a dose-dependent manner ($p < 0.05$). On rechallenging, lercanidipine resulted in reduction of seizure score ($p < 0.05$) and increased the seizure latency.
Conclusions: The present study suggested that lercanidipine offered neuroprotection against PTZ induced kindling in mice.

Keywords: Anticonvulsant, Kindling, Lercanidipine, Pentylenetetrazole

INTRODUCTION

Epilepsy, being second most common neurodegenerative disease, encompasses broad range of conditions that result in dysfunction of brain, spinal cord and nerves.[1] The region of seizure generating tissue or the epileptogenic focus can be due to structural abnormalities that disrupt normal neural circuitry. Epilepsy is one of the most common neurodegenerative disorders affecting approximately 0.3% -0.5% of the population globally. Patient with epilepsy experiences major limitations in family, social, educational, and vocational activities and these limitations have profound effects on the patient's quality of life as well as on other family members.[2] The preferential treatment for epilepsy is administration of antiepileptic drugs. Even with the advent of new strategies in the management of epilepsy no drug has been shown to alter the underlying epileptogenic process.[3] Moreover many patients are refractory to the available pharmacotherapies and continue to experience seizures inspite of regular treatment.[4] Hence in order to prevent epilepsy or alter the

disease course, there is an ever increasing need for the development of a newer anticonvulsant agent.

Influx of extracellular calcium ion in the neuronal tissue causes neurotransmitter release and membrane excitability and these alterations probably exert a profound influence on the cellular events underlying epileptiform activity.[5] Recently many molecular targets have been explored based on several neurobiological techniques. One of the significant approach explored through many invitro and invivo studies was the inhibition of calcium (Ca^{2+}) entry via high-voltage activated dihydropyridine-sensitive (L-type, $Ca_V1.x$) channels.[6] Our literature survey had evidenced the anticonvulsant effect of several dihydropyridines in various animal models of epilepsy.[7-9] But the drawbacks with these dihydropyridines are shorter duration of action\ and or adverse effects that occur with their treatment. The newer generation dihydropyrine calcium antagonist lercanidipine is found to overcome these drawbacks and already had been proven for its effect on acute models of epilepsy.[10]

Comparing the acute animal models of epilepsy, the data obtained from chronic models gives the more predictive validity of the drug tested and henceforth false positive errors are minimized.[11] Moreover chronic models reflect the processes underlying human epilepsy. One such chronic models of epilepsy is kindling which is widely accepted as an experimental model of epileptogenesis.[12] On repeated and intermittent stimulation with PTZ, over a period of time there is an increased excitability and the neurons become pathological thus generating epileptic crisis and mimics human epilepsy. With the above evidence, the present study was done to evaluate the antiepileptic activity of lercanidipine against pentylenetetrazole induced kindling in albino mice followed by rechallenging on third and tenth day after the last chronic dose.

METHODS

The animal experiment was carried out in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Animals were handled with utmost care and all efforts were taken to minimize their suffering.

Experimental animals

Healthy male Swiss albino mice (Mus musculus) (4-6 weeks old, weighing 20-30g) were procured from Tamil Nadu Veterinary and Animal Sciences University, Madhavaram Milk Colony, Chennai, Tamil Nadu 600051, India. Animals were acclimatized to laboratory conditions for a period of 7 days in the Animal house at Department of Pharmacology at a tertiary care hospital. They were housed in groups of six in polypropylene cages bedded with paddy husk under controlled room temperature (24-27°C) in a 12 hour light dark cycle and relative humidity (55±5%). The mice were fed with standard pellet diet and water ad-libitum. The experiment was conducted throughout during the light period between 10.00 and 12.00 hours. All attempts were done to minimize the number of animals.

Chemicals and drugs

All chemicals used in this study were of analytical reagent grade and were purchased from reliable producers: Lercanidipine (Sigma aldrich, U.S.A.), Pentylenetetrazole (Sigma aldrich, U.S.A.) and diazepam (Ranbaxy labs, India). Appropriate vehicle was used for control animals. Dose calculation for lercanidipine and diazepam were based on earlier published reports. [10] Fresh solution of lercanidipine was made in 99.9% HPLC grade methanol. Diazepam and pentylenetetrazole were dissolved in normal saline and distilled water respectively. All drugs and chemicals were administered in a volume of 10 mg/ kg by intraperitoneal route. Each mouse received only single type of treatment and was not reused.

It was randomized controlled experimental study.

Sample size

Thirty six mice were randomized into six groups (n = 6 in each group) to study the anticonvulsant effect against pentylenetetrazole. Mice were randomized using random tables.

Grouping

The experimental design is summarized below:

- Group 1- Normal saline (vehicle)
- Group 2- Diazepam 4mg/kg i.p. daily (standard drug)
- Group 3- Lercanidipine 1mg/kg i.p. daily
- Group 4- Lercanidipine 3mg/kg i.p. daily
- Group 5- Lercanidipine 1mg/kg + Diazepam 2mg/kg i.p. daily
- Group 6- Lercanidipine 3mg/kg + Diazepam 2mg/kg i.p. daily

The experimental animals were treated in the same chronological order throughout the study procedure. Seizure assessments were carried out by an experimenter blind to animal and drug information.

PTZ induced chemical kindling

For kindling induction PTZ was administered in a subconvulsive dose of 30mg/kg i.p. thrice a week till nine weeks, for a total of 27 injections. Animals received vehicle and drugs in respective groups thirty minutes prior to PTZ. After each injection of PTZ, central nervous system excitation was noted over 10-15 min. The intensity of behavioral seizures was evaluated using a 4 point scoring system as follows:

- no effect

- straub's tail
- <25 jerks
- >25 jerks
- clonic convulsions

This scoring was done throughout the nine weeks study period and tabulated.

Rechallenging with PTZ

The chronically treated mice were then challenged with the same subconvulsive dose of PTZ (30mg/kg i.p.) on the 3rd and 10th day of the last chronic dose. Different phases of CNS excitation and convulsions were observed in these experimental animals. All groups were compared with vehicle treated animals.[13,14]

Statistical methods

The data were analyzed using SPSS (v16.0) software. The cumulative kindling scores were expressed as mean±standard deviation (SD) and were analyzed by one-way Analysis of Variance (ANOVA). When significant differences were obtained, post-hoc comparisons within logical sets of means were performed using Tukey's test at following periods:

- Different weeks of treatment
- 3rd day of rechallenge dose and
- 10th day of rechallenge dose

P values less than 0.05 were considered statistically significant.

RESULTS

Effect of lercanidipine on mean seizure score in kindled mice

Mice pretreated with diazepam (4mg/kg i.p. daily) had shown significant reduction in mean kindling score ($p < 0.05$ using One way ANOVA followed by Tukey test) throughout the nine weeks compared to control animals (maximum and minimum mean kindling score 4.66±1.63 at ninth week and 0.5±0.55 at third week respectively) (Figure 1). Repeated treatment with lercanidipine at higher dose (3 mg/kg i.p. daily) exhibited statistically significant ($p < 0.05$) reduction of the mean kindling score throughout the period of nine weeks. Lercanidipine at lower dose (1mg/kg i.p. daily) did not show reduction in the mean kindling scores ($p > 0.05$) throughout the period of observation. Combination of lercanidipine 1 and 3mg/kg i.p. with the standard drug diazepam (2mg/kg i.p.) had resulted in statistically significant reduction ($p<0.05$) in seizure score in a dose-dependent manner. No adverse effect was noted in the lercanidipine treated group in our study.

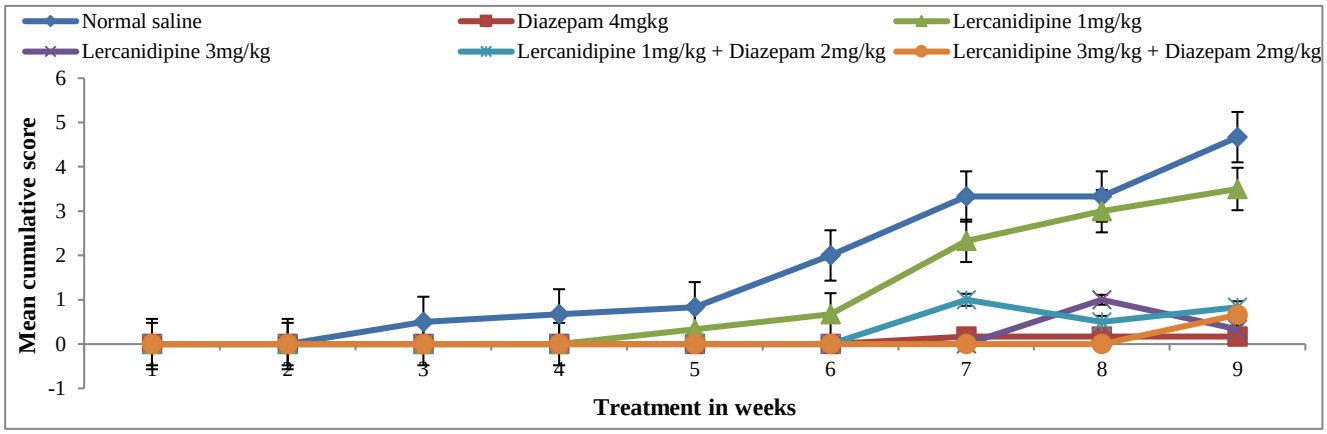


Figure 1: Effect of lercanidipine on kindling score by PTZ in mice.

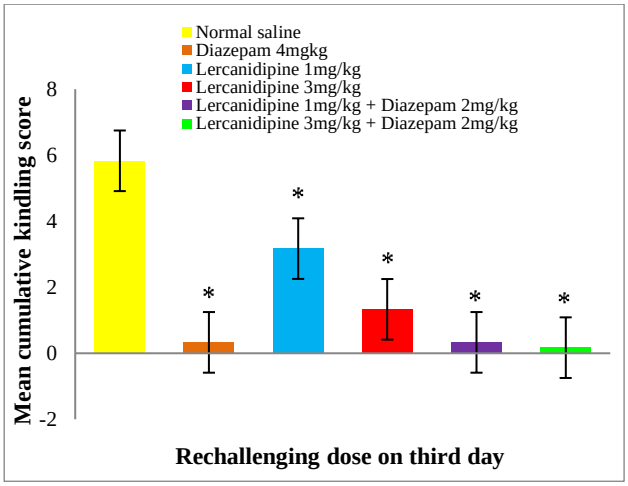
Effect of lercanidipine on third day of rechallenging with PTZ

All the six animals in each group were subjected to rechallenge test on third day (Figure 2). The mean kindling score of diazepam (0.33±0.52) was statistically significant ($p < 0.05$) compared to the vehicle treated animals (5.83±2.04) on the third day after the end of chronic kindling in mice. Lercanidipine when given alone and in combination with diazepam (2mg/kg) at two different doses (1 and 3mg/kg respectively) had produced statistically significant ($p < 0.05$) reduction in seizure score compared to the vehicle treated group.

Effect of lercanidipine on tenth day of rechallenging with PTZ

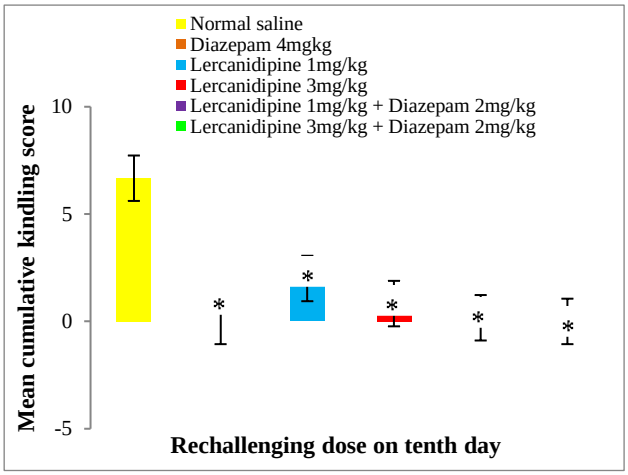
On rechallenging at tenth day, lercanidipine treated group had shown a significant ($p < 0.05$) reduction in seizure

score compared to vehicle group (2±1.1 and 0.83±1.17 with lercanidipine 1 and 3mg/kg respectively versus 6.67±2.06 in vehicle group) (Figure 3). During the study period, animals treated repeatedly with diazepam (4mg/kg) did not show seizures. Combined with diazepam, lercanidipine at different doses (1 and 3mg/kg) exhibited significant reduction ($p < 0.05$) of the mean kindling score (0.16±0.41 and 0 respectively).



Occurrence of clonic seizures was observed and scored. Comparison was done by One- way Analysis of Variance followed by Tukey test. *p<0.05 as compared to vehicle treated group was considered significant. Number of animals in each group was 6.

Figure 2: Effect of rechallenging on third day with PTZ (30mg/kg) in chronically kindled mice receiving vehicle, diazepam and lercanidipine daily throughout the study.



Occurrence of clonic seizures was observed and scored. Comparison was done by one way One- way Analysis of Variance followed by Tukey test. *p<0.05 as compared to vehicle treated group was considered significant. Number of animals in each group was 6.

Figure 3: Effect of rechallenging on tenth day with PTZ (30 mg/ kg) in chronically kindled mice receiving vehicle, diazepam and lercanidipine daily throughout the study.

Effect of lercanidipine on percentage of animals protected during rechallenging

The percentage of animals protected with diazepam (100%) and combination therapy (100%) was higher compared to animals treated with vehicle and lercanidipine (0%) given at lower dose (1mg/kg) (Table 1). With lercanidipine 3mg/kg the percentage of protection had dramatically increased on tenth day (33.33%) of rechallenging compared to the percentage of protection on third day (16.66%) (Table 2).

Table 1: Effect of lercanidipine on third day of rechallenging in chronically kindled mice with pentylenetetrazole.

Group	Percentage of protection (n = 6)
Normal saline	0
Diazepam 4mg/kg	100
Lercanidipine 1mg/kg	0
Lercanidipine 3mg/kg	16.67
Lercanidipine 1mg/kg + Diazepam 2mg/kg	100
Lercanidipine 3mg/kg + Diazepam 2mg/kg	100

Animals were treated with lercanidipine and diazepam everyday throughout the study period. Values are expressed as percentage. Number of animals in each group was six.

Table 2: Effect of lercanidipine on tenth day of rechallenging in chronically kindled mice with pentylenetetrazole.

Group	Percentage of protection (n = 6)
Normal saline	0
Diazepam 4mg/kg	100
Lercanidipine 1mg/kg	0
Lercanidipine 3mg/kg	33.33
Lercanidipine 1mg/kg+ Diazepam 2mg/kg	100
Lercanidipine 3mg/kg+ Diazepam 2mg/kg	100

Animals were treated with lercanidipine and diazepam everyday throughout the study period. Values are expressed as percentage. Number of animals in each group was six.

DISCUSSION

Our study results show the enhancing effect of lercanidipine with diazepam in the management of epilepsy against PTZ induced kindling in mice. Any disruption in the normal neuronal balancing mechanism between excitation and inhibition ultimately leads to seizure. Chemical kindling is a chronic model of animal epilepsy extensively used to understand the process of epileptogenesis and screen novel antiepileptic agents. Kindling is a phenomenon in which subconvulsive

stimulus of chemical or electrical current applied repetitively lead to generation of full blown convulsions which is spontaneous. Since recurrence and spontaneity is commonly observed with human epilepsy, kindling is usually preferred over acute models. Animals kindled with PTZ represent a model of limbic epilepsy with a focal onset and secondary generalization.[15]

Studies have demonstrated that calcium antagonist were able to reduce epileptic activity at the level of individual neurons as well as neuronal population.[16] In the present study, pretreatment with lercanidipine in the higher dose produced significant protection against the development of PTZ induced kindling compared to vehicle treated animals. The protection offered by lercanidipine with kindled seizures is in accordance with that of nifedipine against kindled seizures.[8] Moreover in our study with PTZ induced kindling, full blown convulsions were noted with the vehicle treated animals in later part of kindling procedure without any mortality. In addition, lercanidipine treated groups at higher dose and in combination with the standard drug were observed with statistically significant protection against PTZ induced kindling which is similar to a study done with BR-16A.[14]

Rechallenging with lercanidipine on third and tenth day after last chronic dose of PTZ produced statistically significant reduction of seizure score. The anticonvulsant activity of lercanidipine was further enhanced by its combination with diazepam. This was in accordance to that of Satyanarayana et al who demonstrated reduction in seizure score with nifedipine and not with nimodipine.[7] Similar result was observed with a study done in Italy who also demonstrated reduced seizures score.[17] From the above study it has been demonstrated that lercanidipine was found to increase the seizure latency following chronic PTZ administration. In addition, our test compound also decreased the development of kindling which showed the involvement of $GABA_A$ and benzodiazepine receptors.[12]

Pentylenetetrazole (PTZ) is a central and respiratory stimulant.[18] The mechanism by which PTZ acts is controversial. However certain proposed mechanisms are: Inhibition of the inhibitory function of GABA neurotransmitter; decreasing the recovery time by increasing potassium permeability of the axon; increase in membrane current of sodium and calcium leading to overall increase in excitability of neuronal membrane. Diazepam which enhances the GABA mediated synaptic inhibition protects animals against PTZ induced kindling. Moreover, the dose of standard drug was reduced to half when combined with lercanidipine which shows the possibility of minimizing the dose related adverse effect of conventional anticonvulsant if combined with lercanidipine.

Lercanidipine which possibly acts by blocking N and P/Q type calcium channel had enhanced the effect of diazepam related to increased release of GABA from the neurons. Thus, the effect observed with lercanidipine along with standard drug can be explained by two targets of actions of these drugs resulting in synergistic effect on GABA, GABA receptors and Ca^{2+} channels. It was suggested that amlodipine, a similar dihydropyridine calcium channel blocker, had enhanced the effect of lamotrigine by the possible blockade of N and P/Q type of calcium channels.[19] Experimental evidences also suggest the role of N type calcium channels in the glutamate release at the mammalian central nervous system.[20] The other member like nifedipine in the calcium channel blocker category was shown to block the glutamate receptors.[21]

Lercanidipine with high lipid solubility readily crosses the blood brain barrier inhibits the calcium ion entry into the neurons under certain conditions like seizures, Alzhemiers disease without affecting the normal calcium homeostasis.[22] Combined use of diazepam with lercanidipine had shown significant seizure reduction in PTZ induced kindling which substantiates the possible role of lercanidipine as add on therapy with conventional antiepileptic agents. The study demonstrates the beneficial role of lercanidipine in combination with diazepam in the management of epilepsy. Therapeutically, this enhancing profile for lercanidipine fosters a safer and more effective drug-combination regimen. The strength of our study was inducing kindling for a period of nine weeks which resulted in fully kindled animal model of epilepsy. However, our study was done only with mice involving smaller sample size and the molecular mechanisms were not studied. More detailed experimental and clinical studies may help to identify active role of lercanidipine in different types of epilepsy as add on therapy with conventional antiepileptic drugs in the management of epilepsy.

ACKNOWLEDGEMENTS

The authors would like to thank the management and Director for providing us with the support needed to do the study.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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Cite this article as: Selvaraj N, Kameswari D, Gandhi R, Rajamohamad MA. Anticonvulsant effect of lercanidipine against pentylenetetrazole induced kindling in mice. Int J Basic Clin Pharmacol 2017;6:2134-9.