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Acute Anticonvulsant Activity of Diltiazem, Nimodipine and Flunarizine in Wistar Albino Rats by Maximum Electroshock-Induced Seizure

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ABSTRACT

Background and objectives: In spite of growing armamentarium of antiepileptic drugs, many patients continue to have seizures. This propels for search of novel and safe drugs for resistant and recurrent convulsions. Calcium channel blockers have been ascribed with anticonvulsant actions. The objective of the present study was to evaluate the anticonvulsant actions of diltiazem, nimodipine and flunarizine among Wistar albino rats in Maximum Electroshock-induced Seizure (MES) model.

Methodology: Thirty inbred Wistar rats weighing 150-200 grams of either sex, divided into five groups containing six rats in each. Group 3, 4 and 5 were pre-treated with diltiazem (20mg/kg), nimodipine (20mg/kg) and flunarizine (10mg/kg) respectively. Group 2 was standard group, received phenytoin (25mg/kg). The groups pre-treated with calcium channel blockers were compared with this standard. Group 1 was negative control and were given normal saline. All groups were subjected to MES. During and after the MES, the duration of flexion, duration of tonic hind limb extension and duration of clonus (in seconds) were noted. Abolition of hind limb extension and reduction (or absence) of the clonus duration after the drug administration were considered as anticonvulsant effect of the test drug.

Results: Rats pre-treated with diltiazem, nimodipine, flunarizine showed statistically significant reduction in duration of hind limb extension phase and clonic seizures. Total duration of the seizures was also significantly lower and comparable to phenytoin pre-treated rats. All rats in all groups survived the experiments indicating the doses used during the study were not lethal

Conclusions: Diltiazem (20mg/kg), nimodipine (20mg/kg) and flunarizine (10mg/kg) have anticonvulsant action among Wistar rats in MES model.

Keywords: *Calcium channel blockers; anticonvulsant; seizure; epilepsy; diltiazem; nimodipine; flunarizine; maximum electroshock model*

Introduction

In spite of availability of considerable number of anti-epileptic drugs, many patients continue to have seizures that are refractory to treatment defying our understanding and approaches of epilepsy[1]. In the search for a novel anticonvulsant drugs, many plant extracts and old drugs are evaluated for newer indications. With increasing understanding of epileptogenic molecular mechanisms, more avenues are opening.

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Many of the currently used anti-epileptic drugs are shown to inhibit calcium channel activity[2,3]. Theoretical considerations and few animal model studies have suggested that calcium channel antagonists may play a role as anticonvulsants[4]. These drugs are postulated to inhibit the positive inward burst firing activating wide range of neurons leading to seizures. To support such theoretical considerations, few animal model studies and clinical studies have shown that nimodipine has anticonvulsant property[5,6]. Combination of calcium channel blockers was shown to have mixed effects. Diltiazem enhances the nimodipine's antiseizure effects. Flunarizine inhibits nimodipine's effects[7]. During last decade of twentieth century, there was heightened interest in evaluation of calcium channel blockers for epilepsy at least in animal model. There were series of research reports pertinent to this area[8–12]. After the introduction of gabapentin, topiramate, tiagibine, levetiracetam and zonisamide, the evaluation of monotherapy for epilepsy has veined[13–15]. However, the evaluation continued as add on therapy both in animal model and in clinical trials[16–19].

There are many animal models have been developed over the previous two decades for evaluation of the novel anti-epileptic drugs. The maximal electroshock (MES) model remains as an important gatekeeper for such evaluation, in spite of the fact that it failed in levetiracetam efficacy[20]. MES model has been successfully used to prove the anticonvulsant action of plethora of plant extracts[21]. However, the evaluation of calcium channel blockers alone was done in limited number of studies. Sahadevan has shown the anticonvulsant action of nimodipine and flunarizine in mice MES model[22]. Diltiazem anticonvulsant effects were hitherto unevaluated in Indian set up in rat MES model.

The objective of the study was to evaluate the anticonvulsant effect of diltiazem, nimodipine and flunarizine in Wistar albino rats using MES model.

Methodology

Experiments were conducted with 36 inbred Wister rats, in 3–4 weeks old, weighing 150-200 grams, of either sex, were used in this study. All rats were obtained from animal house, BLDEU's Shri B M Patil Medical College, Vijayapura, Karnataka state. Animals were group housed in cages of six with water and food supplied ad libitum. The temperature was maintained at 25° ± 1 °C and relative humidity of 41.55%. A 12:12, light: dark cycle was following during the experiment. The experiment was carried out during 1200-1400 hr. Animals had free access to food and water. However, food but not water was withdrawn 8hr before and during the experiments.

Institutional animal ethics committee, BLDEU's Shri B M Patil Medical College, Vijayapura, Karnataka state, (with CPCSEA, India registered) (approval letter number: 32/16, dated-16.01.2016) and also Institutional animal ethics committee, KMCH College of Pharmacy, Coimbatore, Tamil Nadu, (approval letter number: KMCRET/PhD/05/16-17, dated-22.02.2016) approved the study before the start of the study

Evaluation of anticonvulsant activity-Maximum electroshock (MES) model: The rats were pretested prior to the drug administration for the electroshock sensitivity. Convulsions were induced by using electro-convulsiometer (Techno India Ltd). MES stimulation were given using trans-auricular (ear-clip) electrodes from the apparatus. Intensity of MES was at 150mA for 0.2 seconds, with constant voltage stimulators of 250 V. At this intensity and duration all the control group rats exhibited tonic hind limb extension. Only those rates that consistently exhibited the tonic hind lime extension in three trials on three separate days were used for the study[21].

Rats were divided onto five groups of six each. The division and administration of the drugs are tabulated in the Table 1. All groups were subjected to MES.

Table 1: Description of groups and drugs administered during the study (n = 30)

Group	Description	Drug administered
I	Control group	Normal saline equivalent (PO)
II	Standard group	Phenytoin sodium 25 mg/ kg body weight
III	Diltiazem group	Diltiazem 20mg/kg (PO)
IV	Nimodipine group	Nimodipine 20mg/kg (PO)
V	Flunarizine group	Flunarizine 10mg/kg (PO)

During and after the MES, the duration of flexion, duration of tonic hind limb extension and duration of clonus (in seconds) were noted.

Abolition of hind limb extension and reduction (or absence) of the clonus duration after the drug administration were considered as anticonvulsant effect of the test drug.

Statistical analysis: The data obtained was expressed as mean ± standard deviation. Comparison of the data was done by one-way ANOVA, followed by Dunnett comparison. P value of less than 0.05 was taken as significant.

Results

MES induced seizures: Following the ear electrode stimulus, an immediate tonic seizure with hind limb extension was observed in all animals of group I and group II. There were no signs of toxicity in the control groups. The positive control group administered with phenytoin did not show the phase of clonic seizures. The latency of onset of flexion, extension and clonic seizures is tabulated in Table 2.

Table 2: Tabulation of latency and duration of seizures (expressed as mean ± standard deviation in seconds) in all the groups of rats in MES model (n = 36)

Time in seconds					Recovery/ mortality
Group	Flexion	Extension	Clonus	Duration	
Only MES	8.167 ± 0.47	11.167 ± 3.75	20.667 ± 4.35**	107 ± 23.84	Recovery
MES+ Phenytion 25 mg/kg	1.2 ± 0.68	4.83 ± 1.66*	0	43 ± 9.33*	Recovery
MES + Diltiazem 20mg/kg	1.16 ± 0.47	1.5 ± 1.5†	4 ± 2.543*	67.16 ± 14.06†	Recovery
MES + Nimodipine 20mg/kg	1.5 ± 0.67	3.5 ± 2.21†	5.5 ± 3.86*	69 ± 14.66†	Recovery
MES + Flunarizine 10mg/kg	1.02 ± 0.68	3 ± 1.91†	13.6 ± 6.28†	75.66 ± 5.40†	Recovery

Rats pre-treated with diltiazem, nimodipine, flunarizine showed statistically significant reduction in duration of hind limb extension phase and clonic seizures. Total duration of the seizures was also significantly lower and comparable to phenytoin pre-treated rats. All rats in all groups survived the experiments indicating the doses used during the study were not lethal.

Discussion

Calcium channels, both L-type and T-type has been increasingly implicated in epileptogenesis[2–4,23]. It is postulated that calcium channel antagonists have anti-seizure and neuroprotective roles. Many previous studies have demonstrated such anti-seizure actions of calcium channel blockers in rat MES model. Nifedipine (in doses of 10mg/kg), amlodipine (in doses of 1-4mg/kg)[11,12,16], felodepine (in doses of 5-10 mg/kg)[10], verapamil (in doses of 5 mg/kg)[5,15,19], flunarizine(in doses of 5 mg/kg)[17,18], nicardipine (in doses of 5 mg/kg)[17], nimodipine (in doses of 5-10 mg/kg) [5,6,17,18] and diltiazem (in doses of 5-10 mg/kg) have been shown to have anti-seizure actions in animal models. In the present study as well, diltiazem, nimodipine and flunarizine were proven to have anti-seizure actions.

Contrary to our findings, diltiazem was shown to have no anticonvulsant action even with increasing doses among mice MES model[22]. However, diltiazem was shown to have anticonvulsant actions in many other studies in the past[15,19]. In addition, diltiazem has been shown to enhance the actions of oxcarbazepine[14] and topiramate[19]. In the similar way, amlodipine has been shown to be anticonvulsant when used singly[9,11,15]. Amlodipine also enhances the anticonvulsant actions of lamotrigine, gabapentin and topiramate[13,16,19].

With patients continuing to have recurrent episodes of convulsions while on anticonvulsant therapy, the search for novel and safe effective in such resistant individuals endures. In this study an attempt was made to show the anticonvulsant effects of three calcium channel blockers – diltiazem, nimodipine and flunarizine. The effects in MES model, however, need not necessarily translate to the clinical effects in epileptic individuals. Further tests, both animal model and pre-clinical tests, evaluating the neurotransmitter levels in the specific areas of the brain, pathological alterations in the neuronal cells structure and functions as proven at least by histological and immunohistopathological studies shall add to the findings of the present study. The handling of free radicals in the brain and consequent brain inflammation has been postulated as potential epileptogenic mechanism[24]. Studies evaluating the oxidative stress, both in animal model and preclinical set up using these drugs shall make calcium channel blockers potential drugs for epilepsy.

Limitations of the study: Use of multiple animal models nullifies short comings of each other and achieves better clinical correlations in humans. Dose variations during MES model would have provided minimum effective dose (ED50) of the drugs. Use of standard anti-epileptic drug in each group would have evaluated the additive role the calcium blockers. Currently only calcium channel blockers are used – that are rarely clinically used as standalone therapy for epilepsy.

Conclusions

Diltiazem (20mg/kg), nimodipine (20mg/kg) and flunarizine (10mg/kg) have anticonvulsant action among Wistar rats in MES model

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