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ANTICONVULSANT EFFECT OF DILTIAZEM ON MAXIMAL ELECTROSHOCK (MES)-INDUCED CONVULSIONS AND COMPARISON OF LOW DOSE DILTIAZEM & PHENYTOIN ON MES-INDUCED CONVULSIONS IN EXPERIMENTAL ANIMALS.

Pharmacology

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ABSTRACT

Epilepsy is one of the most widespread neurological disorder affecting approximately 1% of world population. Many antiepileptic drugs have failed to provide satisfactory control of convulsions because of dose related adverse effects. Also research studies have shown that calcium ions are involved in the pathogenesis of convulsions. Hence the present study is aimed at evaluating the anticonvulsant effect of Diltiazem, a calcium channel blocker, in graded doses of 10mg/kg, 20mg/kg & 40mg/kg body weight intraperitoneal (i.p). and their effects are compared with standard drug Phenytoin. The test drug diltiazem produced significant anticonvulsant effect at doses of 20mg/kg & 40mg/kg body weight. Further low dose combination of Diltiazem 10mg/kg body weight + Phenytoin 10mg/kg body weight showed potentiation of anticonvulsant effect in MES-induced convulsions in wistar albino rats.

KEYWORDS

Anticonvulsant, MES, Diltiazem

INTRODUCTION

Epilepsy is one of the most widespread neurological disorder in the world[1]. Approximately 1% of the world population has epilepsy[2]. At least 20–30% of patients with temporal lobe epilepsy have resistant convulsions inspite of optimal Pharmacotherapy[3]. Various new drugs with their own unique advantages have been introduced, but many have failed to provide satisfactory control of convulsions because of dose related side effects hence limiting their use in clinical practice[4]. An ideal antiepileptic drug should correct the pathophysiology of epilepsy without interfering with physiological neurotransmission[5]. So there is ever increasing need for research and development of newer drugs for the treatment of epilepsy [6].

The initiation of convulsions is associated with high frequency burst of action potential, that is caused by long lasting depolarisation of the neuronal membrane triggered by a large influx of calcium ions into cells[6,7]. So there is a role of calcium channel blockers (CCBs) in the treatment of epilepsy[8].

The following study was conducted to analyze the anticonvulsant effect of diltiazem on MES-induced convulsions and comparison of combination of low doses of diltiazem & phenytoin on MES induced convulsions in Wistar albino rats.

MATERIALS & METHODS

The study was randomized controlled based on laboratory animal model with permission of Institutional Animal Ethics Committee (IAEC) (approval letter No. SNMC/IAEC/2022-23/01 dated 20/10/2022). It was conducted in the department of Pharmacology, S.Nijalingappa Medical college & HSK Hospital from November 2022 to October 2023. This was done to evaluate the anticonvulsant activity of Diltiazem in graded doses (10, 20, 40 mg/kg; i.p), Phenytoin (10, 25 mg/kg; i.p) and their low dose combination in MES-induced convulsions in Wistar albino rats.

i) Experimental Animals: Wistar Albino Rats of either sex weighing 100-200 grams, inbred animals from the Central Animal House, S.Nijalingappa medical college & HSK Hospital, Bagalkot were used for the study. The animals were acclimatized to laboratory conditions before the test and had free access to food & water. Experiment was done during light period between 9am & 1pm.Total number of animals used were- 70; Number of groups - 7; Number of animals in each group –10.

ii) Drugs: Phenytoin & Diltiazem

iii) Grouping of Animals: (N = 70)

Grouping of Rats (n=10): MES-induced convulsion model.

Group I: Distilled water (Control); 5ml/kg body wt i.p.
Group II: Phenytoin (standard); 25mg/kg body wt i.p.
Group III: Diltiazem (test drug); 10mg/kg body wt i.p.
Group IV: Diltiazem (test drug); 20mg/kg body wt i.p.
Group V: Diltiazem (test drug); 40mg/kg body wt i.p.
Group VI: Phenytoin (standard); 10mg/kg body wt i.p.
Group VII: Diltiazem + Phenytoin (low doses); 10mg + 10mg/kg body wt i.p

iv) Experimental Model and Drug Treatment[9,10]:

Maximal Electric Shock method (MES): This method was developed by Merritt & Putnam. It is mainly used as model to elicit grand mal epilepsy and the end point is Tonic Hind Limb Extension [THLE], evoked by electric stimuli[11]. The agents tested through this model are considered to be having anticonvulsant effect if they suppress THLE. The electric stimuli are given by electrodes [corneal or ear]. When MES is used for Rats electroshock of 150mA, 50Hz intensity is given for 0.2sec.

Procedure [12,13]

- Screening of rats was done 1 week prior to experiment. Above rats were subjected to maximal electroshock through auricular electrodes (covered in cotton wool & saline moistened) and observed for tonic flexion of fore & hind limbs with tail erection, tonic extension of both fore & hind limbs, clonus, stupor followed by postictal sleep & recovery. Those rats which showed all phases of convulsions with MES were taken into study.
- Rats were selected by the process of randomization and were placed into 7 different groups.
- After half an hour of drug administration, rats were subjected to MES and the parameters recorded were onset & duration (in sec) of tonic hind limb extension (THLE) and duration (in sec) of postictal sleep.

v) Statistical Analysis: Data was analyzed by using Analysis of Variance (ANOVA) with drug treatment as independent factor. p value < 0.05 was considered as statistically significant.

OBSERVATIONS & RESULTS

Table No.1 Comparison Of Results Of Different Groups In MES Model (N=70)

Group (n=10) in each group	Drug	Dose in mg/kg (i.p.)	Onset of THLE in sec (mean ± SEM)	Duration of THLE in sec (mean ± SEM)	Duration of postictal sleep (mean ± SEM)	Recovery/ mortality
I control	Distilled water	1ml/kg	3.5 ± 0.29	13.5 ± 1.44	56 ± 0.58	Recovery

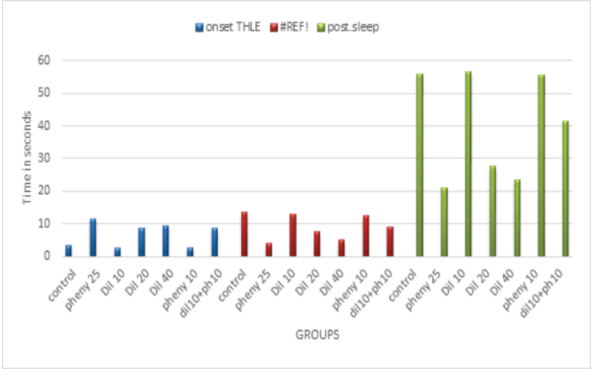
II std	Phenytoin	25	11.5 ± 0.86**	4 ± 0.58**	21 ± 1.73**	Recovery
III test	Diltiazem	10	2.5 ± 0.29	13 ± 1.15	56.5 ± 0.86	Recovery
IV test	Diltiazem	20	8.5 ± 0.29**	7.5 ± 0.29**	27.5 ± 0.86**	Recovery
V test	Diltiazem	40	9.5 ± 0.86**	5 ± 0.58**	23.5 ± 0.86**	Recovery
VI std	Phenytoin (low dose)	10	2.66 ± 0	12.5 ± 0.29	55.5 ± 1.44	Recovery
VII combination	Diltiazem + Phenytoin (low doses)	10+10	8.5 ± 0.29**	9 ± 1.15**	41.5 ± 0.29**	Recovery

Note: All values are in Mean ± SEM * $p<0.05$ (significant) & ** $p<0.001$ (highly significant) in comparison to control group. (SEM-standard error of mean)

Table No. 2 Intergroup Comparison By One Way ANOVA Test

Parameters		Sum of Squares	df	Mean Square	F	Sig.
Onset of THLE	Between Groups	376.62	6	62.77	36.02	0.001**
	Within Groups	61.00	35	1.74		
	Total	437.62	41			
Duration of THLE	Between Groups	671.14	6	111.86	35.16	0.001**
	Within Groups	111.33	35	3.18		
	Total	782.48	41			
Duration of postictal sleep	Between Groups	7456.00	6	1242.67	117.13	0.001**
	Within Groups	371.33	35	10.61		
	Total	7827.33	41			

Note: All values are in Mean ± SEM * $p < 0.05$ & ** $p < 0.001$ in comparison to control group.



Graph No. 1 Showing Onset Of THLE (in sec), Duration Of THLE (In Sec) And Duration Of Postictal Sleep (In Sec) In MES Model

The results of MES-induced convulsion model indicated a significant increase in onset of THLE, significant decrease in duration of THLE and significant decrease in duration of postictal sleep in the treatment groups of Phenytoin 25mg/kg; Diltiazem 20mg/kg; Diltiazem 40mg/kg body wt compared to control group indicating anticonvulsant action. Further combination of low dose diltiazem & phenytoin showed potentiation by increasing the onset of THLE, decreasing the duration of THLE and decreasing duration of postictal sleep in comparison to control group. The results given are Mean ± SEM. Refer to table no. 1 & 2 and graph no. 1.

CONCLUSION

From this study it is concluded that Diltiazem 20mg/kg, Diltiazem 40mg/kg & low dose combination of Diltiazem 10mg/kg + Phenytoin 10mg/kg body wt possess anticonvulsant effect on MES-induced convulsions in Wistar albino rats.

DISCUSSION

Calcium channels, L & T type are commonly associated with the genesis of epilepsy[14]. Multiple studies postulate that influx of calcium into intracellular space cause intrinsic burst firing, considered as initial event in epilepsy[13]. At a cellular level, antiepileptic drugs were found to decrease neuronal excitability by interacting with voltage dependent calcium channels, by modulating GABA metabolism, transport, and by affecting inotropic glutamate receptors. Epileptic depolarization of neurons were found to be depressed by calcium channel blockers. So, there may be a role of calcium channel blockers in the treatment of epilepsy. Saniya K et.al. study 2019 showed that Diltiazem 20mg/kg, Nimodipine 20mg/kg and Flunarizine 10mg/kg have significant anticonvulsant action among Wistar albino rats in MES model[14]. Umesh G. Wari et.al study 2015 showed that diltiazem at 7.5mg/kg & 15mg/kg has no significant anticonvulsant effect among Wistar albino rats in MES & PTZ models[15]. Hence we evaluated the anticonvulsant effect of Diltiazem at graded doses of 10mg/kg, 20mg/kg & 40mg/kg body wt i.p.

Present study showed that Diltiazem 20mg/kg, 40mg/kg & low dose combination of Diltiazem 10mg/kg + Phenytoin 10mg/kg body wt. possess significant anticonvulsant effect on MES-induced convulsions which is comparable to Saniya K et.al. study 2019 which also showed significant anticonvulsant effect of Diltiazem at 20mg/kg body wt. among albino rats in MES model.

Hassan et.al, study 2014 showed anticonvulsant effect of amlodipine when used in combination with lamotrigine, topiramate and gabapentin[16]. The result of the study stated that CCBs can be used as potential adjuvant drugs. However studies on different species, epilepsy models and comparison with other antiepileptic drugs need to be assessed with more research activities for their use in epilepsy either as monotherapy or in combination with other antiepileptic drugs.

CCBs are potent blockers of the voltage dependent L and T type calcium channels but there is difference in the affinity of the various CCBs to block these channels. Patients continue to have recurrent episodes of convulsions and experience multiple side effects with commonly used antiepileptic drugs. This rises the need for novel, safe and effective therapy. Diltiazem blocks L type of calcium channels[17]. Hence diltiazem can be considered as a viable option in anticonvulsant therapy. Researches are still required to establish the efficacy and mechanism of action of diltiazem in this prospect.

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