ANTI-CONVULSANT EFFECT OF NIFEDIPINE, DIAZEPAM AND IN COMBINATION ON MES INDUCED EPILEPSY IN RATS

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ABSTRACT

Background: The role of calcium in the process of epileptogenesis and neurotoxicity and excitotoxicity during status epilepticus is involved. In fact, epileptiform bursts are often associated with influx of calcium ion into the nerve cells and a decrease in the extracellular concentration of calcium precedes the onset of seizures. **Aim:** To investigate the activity of nifedipine, the dihydropyridine calcium channel blocker, diazepam, the benzodiazepine anti- convulsant of established e□cacy and their combinations against rat models of MES induced tonic seizure. **Method:** Wister albino rats of either sex, weighing between 150-220 gm were used for maximal electroshock method. Rats were divided into 10 groups, in each group n=6 total N=60.**Group 1:** Control, **Group 2-4:**Nifedipine 2, 4 and 8 mg/kg accordingly, **Group 5-7:** Diazepam 1,2 and 4 mg/kg accordingly, **Group 8:**Nifedipine (3.3mg) + Diazepam (3mg), **Group 9:**Nifedipine (6.6mg) + Diazepam (3mg)**, Group 10:** Phenytoin (25mg/kg). The Controls in the central panel was set at 120 mA current. The Start-Reset knob was switched in the reset position with time of 0.2 seconds was set. The following parameters were observed. Latent period of convulsions, duration of flexion, tonic hind limb extension, posttictal sleep**. Results:** Nifedipine in doses of 4 and 8 mg / Kg., diazepam in all doses (1, 2 and 4 mg / Kg.) and their combinations were significantly reducing the duration of tonic hind limb extension (THLE). Only Nifedipine in doses of 2 mg / Kg. had no significant change in duration of THLE in comparison to Normal saline. The e□ect of Nifedipine 8 mg / Kg. on reducing the duration of THLE, indicative its anti-seizure activity on MES seizure was comparable to Diazepam in the dose of 1 mg / Kg. The MES-induced anti-seizure activity of Diazepam in doses of 1 mg / Kg. It was significantly less than Diazepam in doses of 2 and 4 mg / Kg or from the combination doses. Diazepam in 4 mg / Kg. dose was equally e□ective as the combination. But its e□ect was also equi-e□ective with Phenytoin. **Conclusion:** Nifedipine alone is inferior to diazepam in generalized tonic-clonic seizure and their combination is not having superadditive e□ect.

Keywords: Calcium channel blockers, Diazepam, Epilepsy, Maximum electrical shock

INTRODUCTION

Epilepsy is one such CNS disorder where role of calcium in the genesis, spread of seizure and in neuronal injury caused by repeated seizure are established [1]. World Health Organization (WHO) estimated that approximately 80% people with epilepsy live in developing countries and most of them do not get adequate medical treatment [2]. In many patients, the presently available antiepileptic drugs (AED) such as phenobarbital, phenytoin, benzodiazepines, sodium valproate, carbamazepine, ethosuximide, trimethadione etc., are unable to control seizures e□ciently and the problem of adverse e□ects has also not been circumvented completely and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy [3,4]. Hence, search should continue to develop newer, more e□ective, and safer neuroprotective agents for treatment of epilepsy. The role of calcium in the process of epileptogenesis and neurotoxicity and excitotoxicity during status epilepticus is established under the scanner of intensive research [5]. Calcium currents have established role by undergoing bursting in pacemaker cells, enhancing post-synaptic excitatory responses in dendrites and somatic nerve cells and providing post-burst re-excitement [6]. In fact, epileptiform bursts are often associated with influx of calcium ion into the nerve cells and a decrease in the extracellular concentration of calcium precedes the onset of seizures in many experimental models of epilepsy [5,7,8].

Calcium channel blockers, especially diahydropyridine type of calcium channels blockers have been shown to block various aspects of epileptogenesis and are e□ective anti-convulsants in a number of in vivo models [9].

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Many non-cardiac tissues are functionally dependent upon the influx of extracellular calcium through various channels of cell membrane. So it is expected that calcium channel blockers may play a crucial role in those pathological states where calcium channel over activity is the underlying cause [10,11].

The suitability of selecting phenytoin as the reference standard drug was because it selectively abolishes the tonic extensor phase, the experimental model of generalized tonic-clonic (grandmal) seizure, by inhibiting the spread of seizure discharge[3].

Aim: The present study was undertaken to investigate the activity of nifedipine, the dihydropyridine calcium channel blocker, diazepam, the benzodiazepine anti-convulsant of established e□cacy and their combinations against rat models of MES induced tonic seizure.

MATERIAL AND METHODS

Study design: Experimental animal based study

Ethics approval: The study was approved by the institutional animal ethics committee of our institute

Sample size: In each group n=6 total N=60

Rearing of animal: Rats were kept at the animal house in polypropylene cages at controlled temperature of 23°±2C° and humidity of 50% with standard 12 hour light-dark cycle beginning at 6.00 AM. They received standard diet and water ad libitum

Inclusion criteria: Wister albino rats of either sex, weighing between 150-220 gm were used for maximal electroshock method. Rats were screened before the experiment first with Rota rod test. Those who were successfully completed the Rota rod test were subjected to maximal electroshock with an alternating current of 150 mA intensity for 0.2 sec through ear electrodes. Only those rats showing characteristic course of convulsions were selected for the experiment [12].

Drugs preparation:

Nifedipine: (Himedia Laboratories): 25 ml of standard solution of Nifedipine was prepared by dissolving 25mg of Nifedipine in a solution containing 9 ml of polyethylene glycol, 14.5 ml of glycerine, and 1.5 ml of distilled water in the ratio of 6:10:1 making the concentration of Nifedipine 1mg/ml. Dose of Nifedipine given to rat is 2mg, 4 and 8 mg/Kg of body weight. Diazepam: Stock solution of diazepam in the concentration of 1 mg / ml was prepared by adding 8 ml of distilled water to one ampoule of diazepam containing 10 mg / 2ml. It was administered to the rats in the dose of 1, 2 or 4 mg/Kg of body weight.

Phenytoin Sodium: To one ampoule of Phenytoin sodium containing 50 mg of phenytoin in 1 ml, 9 ml of distilled water was added to prepare a stock solution of 5mg / ml concentration. It was administered to the rats in the dose of 25 mg/Kg of body weight.

Dose and route of administration: Individual doses were calculated for each rat according to their body weight, combination was selected as ED25 and ED50 of Nifedipine (3.3 and 6.6 mg) with ED50 of Diazepam(3mg) and injected intraperitonelly using insulin syringe before subjecting them to maximal electro-shock seizure.

Grouping: Group 1: Control, Group 2-4: Nifedipine 2,4 and 8 mg/kg accordingly, Group 5-7:

Diazepam 1, 2 and 4 mg/kg accordingly, Group 8: Nifedipine (3.3mg)+ Diazepam (3mg), Group

9: Nifedipine (6.6mg) + Diazepam (3mg), Group 10: Phenytoin (25mg/kg)

Methodology:

Animals successful with screening tests were selected randomly using random number table.41

Initially 60 rats were selected for the study and they were randomly divided into 10 groups each containing 6 animals. The groups were kept in separate cases in the laboratory to condition them to the laboratory environment for 3 days and to avoid any possible kidding e□ect.

MES: The Controls in the central panel was set at 120 mA current. The Start-Reset knob was switched in the reset position with time of 0.2 seconds was set.

Parameters studied [12,13]: following parameters were studied.

Latent period of convulsions, duration of flexion, tonic hind limb extension, post ictal sleep Statistical analysis: SPSS statistical software was used for analysis. Post hoc test and one way ANOVA were applied for comparison between the groups. Data was presented as Mean ±SE.

RESULTS

Nifedipine in doses of 4 and 8 mg / Kg., diazepam in all doses (1, 2 and 4 mg / Kg.) and their combinations were significantly reducing the duration of tonic hind limb extension (THLE). Only Nifedipine in doses of 2 mg / Kg. had no significant change in duration of THLE in comparison to Normal saline. It indicated that Nifedipine in dose of 2 mg / Kg. was not having any e□ect on duration of THLE and its e□ect was comparable with normal saline. The insignificant e□ect of nifedipine on the duration of THLE in dose of 2 mg / Kg. was confirmed. Nifedipine in doses of 8 mg / Kg., all the three doses of Diazepam (1, 2 and 4

Group	Body weight (gm)	Time duration in seconds				
		Latent period of Convulsions	Duration Of Flexion	Duration of Tonic Hind limb Extension	Duration of Clonic Convul-sions	Post-ictal sleep
Group 1	180±6.41	4.33±0.33	4.83±0.48	11.33±0.61	3.67±0.33	135±1.83
Group 2	172.8±3.59	4.17±0.31	4.50±0.43	12.00±0.52	9.00±0.97	153.8±2.52
Group 3	173.3±4.6	6.17±0.48	5.67±0.67	7±0.58	10.17±0.7	172.3±12.08
Group 4	178.50±6.32	7.00±0.37	5.33±0.33	5.00±0.52	8.00±0.58	200.3±6.23
Group 5	172.83±4.93	4.33±0.33	5.67±0.42	4.83±0.40	4.50±0.56	1002.8±61.32
Group 6	181.00±4.9	4.33±0.33	4.83±0.31	3.00±0.63	4.33±0.42	1563.5±60.87
Group 7	177.83±4.35	7.00±0.37	4.83±0.31	2.17±0.48	3.50±0.43	2477.2±201.25
Group 8	182.50±3.07	6.00±0.58	5.17±0.48	3.00±0.93	5.50±0.43	1072±146.15
Group 9	187.17±2.96	7.00±0.37	7.33±0.49	2.17±0.70	10.83±0.60	2093.8±245.43
Group 10	167.83±4.13	7.00±0.37	5.17±0.48	1.17±0.48	4.17±0.6	158.3±6.79

*comparison with Control group

mg / Kg.) had significantly reduced the duration of THLE when compared to Nifedipine in dose of 4 mg / Kg.

The e□ect of Nifedipine 8 mg / Kg. on reducing the duration of THLE, indicative its anti- seizure activity on MES seizure was comparable to Diazepam in the dose of 1 mg / Kg as there was no significant ($P > 0.05$) di□erence between them. However, its anti-seizure e□ect was significantly less when compared with Diazepam in 2 or 4 mg / Kg., both of the combination doses and of course from phenytoin.

The MES-induced anti-seizure activity of Diazepam in doses of 1 mg / Kg. It was significantly less than Diazepam in doses of 2 and 4 mg / Kg or from the combination doses and from the reference drug phenytoin as proved from the significant less reduction in the duration of THLE.

Diazepam in the dose of 2 mg / Kg. was found to be equally e□ective as 4 mg / Kg. and the two combination doses. Of course its e□ect was significantly less ($P < 0.05$) than Phenytoin Diazepam in 4 mg / Kg. dose was equally e□ective as the combination. But its e□ect was also equi-e□ective with Phenytoin.

There was no significant di□erence of anti-seizure e□ect of the two combinations of doses i.e. ED50 dose of diazepam (3mg) and ED25 dose (3.3 mg) of nifedipine with that of ED50 doses of diazepam with ED50 doses of nifedipine (6.6mg). However the former combination less effective than Phenytoin ($P < 0.05$)

The combination of ED50 dose of Nifedipine (6.6 mg) and ED50 doses of Diazepam was found to have comparable anti-seizure e□ect with Phenytoin, as their mean THLE duration was not significantly di□erent.

DISCUSION

The e□cacy of CCBs to change the parameters in MES test in the present study correlates well with the ability to prevent partial and generalized tonic clonic seizures and it is said that this model evaluates the capacity to prevent seizure spread. Calcium ion influx may be involved in the origin of seizures. In this study both nifedipine was observed to prevent seizures in MES models of epilepsy. CCBs nifedipine have got primarily favourable e□ects as anticonvulsant drug [14].

The anticonvulsant activity elicited by nifedipine observed in our study is in keeping with previous studies. Clinical data, as well as experimental studies have demonstrated that nifedipine reduces the severity of convulsions. For instance, this calcium blocker decreased the duration of the tonic hindlimb extensor phase in maximal electroshock seizures, increased the latent period and decreased the duration of clonic convulsions in pentylenetetrazole (PTZ)-treated animals [15].

The fact that the central actions of voltage dependent CCBs show anticonvulsant activity, depending on the kind of experimental convulsions investigated,

the protection o□ered by nifedipine in kindled seizures is in accordance with the literature [16].

Intracerebroventricular injection of Bay k 8644 induced seizures were reversed by such as dihydropyridines (calcium channel blockers), excitatory amino acid antagonists such as 2-amino-

7-phosphonoeptanoate and CPPene, 2-chloroadenosine, magnesium valproate, diazepam and clonazepam and two kappa opioid agonists (anticonvulsant drugs). E□ects of antiepileptic drugs, calcium channel blockers and other compounds on seizures induced by activation of voltage- dependent L calcium channel in DBA/2 mice [17].

The protection of mice against MES induced seizures by the standard anticonvulsant drugs, phenobarbitone and diazepam is expected, since various authors have shown that they exert their anticonvulsant activities by enhancing GABA mediated inhibition [18].

In the present study Nifedipine in 2 mg / Kg. dose was found to be have no anti-seizure e□ect. Nifedipine 8 mg / Kg dose was found to be equally e□ective anti-seizure e□ect as that of Diazepam 1 mg / Kg. Diazepam 2 mg / Kg was found to have equal anti-seizure e□ect as 4 mg

/Kg. dose. The dose was also found equally e□ective as the combination of ED50 dose of diazepam (3.3 mg) with Nifedipine either 3.3 mg (ED25) or 6.8 mg (ED50) per Kg combination. However, its seizure protective e□ect was found less than Phenytoin 25 mg / Kg. Combination of Nifedipine either in the dose of 3.3(ED25 of Nifedipine) or 6.8 (ED50 of Nifedipine) did not improve the anti-seizure protection. Diazepam in the dose of 4 mg / Kg. was found to have seizure protective e□ect equal to phenytoin 25 mg / Kg.

CONCLUSION

From the present study it is concluded that Nifedipine alone is inferior to diazepam in generalized tonic-clonic seizure and their combination is not having super additive e□ect.

Conflict of interest: Nil

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