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RESEARCH ARTICLE

EFFECT OF NIMODIPINE AND DICLOFENAC IN EXPERIMENTALLY INDUCED CONVULSIONS USING INH AND ELECTRO CONVULSOMETER IN RATS AND MICE

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ABSTRACT

Aim: The study aims to evaluate the anticonvulsant effect of Nimodipine and combined effect of Nimodipine and Diclofenac in experimentally induced seizures in Rats and Mice taking distilled water as control.
Material and methods: Male wistar rats weighing 150-180gms and albino mice of average weight 20-30gms were used.All the preparations were administered intraperitoneally. Convulsions were induced electrically by electroconvulsometer and chemically by INH. The following parameters with electrically induced convulsions are recorded. a)duration of convulsions, b)duration of hind limb extension, c)post ictal depression followed by recovery. With chemically induced convulsions using INH the following parameters are recorded a)onset of convulsions, b)type of convulsions, c)duration of convulsions. Distilled water is used as control. Drugs are given 30 minutes before the electrically and chemically induced convulsions. Effect of Nimodipine and combined effect of Nimodipine and Diclofenac were studied.
Results: Nimodipine has reduced the duration of convulsions, hind limb extension, delayed the onset of convulsions and recovery from postictal depression was fast from electric shock. Combined effect of Nimodipine and Diclofenac has an improved effect in duration of convulsions, hind limb extension and recovery when compared with that of Nimodipine alone.
Observation: Statistic ANOVA test show significant P value <0.0001 in electrical method and chemical method .The results obtained in this study provide supporting pharmacological evidence of improved efficacy possible potential benefit of combining Nimodipine with Diclofenac in epilepsy.
Keywords: Isoniazid(INH), Hind limb extension (HLE)

INTRODUCTION

Epilepsy is one of the common afflictions of human with a prevalence of approximately 1% of the total population. Epileptic seizures have been known to represent an occasional discharge in the nervous tissue characterised by recurrent paroxysmal changes in the neurological function caused by abnormalities in the electrical activity of the brain. Epilepsy is the second most common neurological disorder in India. .[1] A large number of promising compounds are currently undergoing preclinical and clinical evaluation and several of these will undoubtedly become meaningful additions to neurologist's pharmacological armamentarium. [2]Infact Epileptiform bursts are often associated with influx of calcium ions into nerve cells and a decrease in extracellular concentration of calcium precedes the onset of seizures in many experimental models of epilepsy.

Since the entry of calcium into neurons seems to play an important role in epileptogenesis the use of calcium channel blockers in treatment of seizures may be successful.[3]Based on the findings that levels of Prostaglandins (PG's) the Cyclooxygenase (cox) metabolites of Arachadonic-acid are increased in brain during experimentally induced seizures, a role for PG's and their synthesis inhibitors in convulsive behaviour have been suggested.[4]

Present work is based upon the production of convulsions by maximum electric shock and convulsion induced by isoniazid and evaluation of effect of Nimodipine which is a Calcium Channel Blocker and combined effect of Nimodipine and Diclofenac a COX inhibitor on the onset of seizure, duration and type and duration of Tonic Hind Limb extension in rats and mice.

Aims and Objectives:

To evaluate the anti convulsant effect of Nimodipine.

To evaluate the combined effect of Nimodipine & Diclofenac in experimentally induced seizures in rats and mice

MATERIAL AND METHODS:

The standard solution of Nimodipine was prepared by dissolving 30 mg of Nimodipine tablet in 10 ml of double distilled water at room temperature. The solution was freshly prepared each time, while the experiments were done. This solution has a concentration of 3 mg/1ml. The standard solution of diclofenac was prepared by dissolving 50 mg of diclofenac tablets in

double distilled water and using Suspension tween -80 (0.2%). The solution has a concentration of 5mg/ml. The standard solution of INH was prepared by dissolving 300mg tablets in 10ml of double distilled water at room temperature. The solution is prepared freshly. This solution has a concentration of 30mg/ml Albino male mice &Wistar rats of average of 20-30 gms & 150-180 gms respectively were used to induce convulsions by electroshock and chemo shock (INH).

The above test animals were divided into two groups such one group was pretested with convulsions induced by maximal electroshock with an alternating at 50 mA intensity for 0.2 Sec. for mice and 150 mA intensity for 0.2 Sec. for rats through pinnal electrolytes (a drop of normal saline was applied to ear before using pinnal electrodes). Majority of animals showed tonic flexion & tonic extension of both fore limbs and hind limbs, clonus and postictal depression followed by recovery or death.

Only those animals showing hind limb tonic extension response were used for the experiment and divided into 6 groups of 6 each. Remaining number of animals were divided into 6 (3 groups of mice + 3 groups of rats) groups of 6 each for chemo shock or INH induced convulsions method (3 groups of mice and 3groups of rats with 6 each).

All the test animals which are tested for standard convulsive responses were subjected to further experiment of this study after 24 Hrs(to avoid any possible kindling effect). All the test animals were allowed food and water ad libitum both being withdrawn just prior to experimentation. All the preparations were administered intra-peritoneally. They were maintained under standard 12 hour light dark cycle experiments were carried out at around the same time each day.

The selected animals were grouped into 12 groups (6 groups mice & 6 groups rats) each group containing 6 animals. 6 groups of mice and 6 groups of rats were divided into 2 equal parts for 2 methods each comprising 3 groups. These animals were subjected to maximal electroshock and chemoshock. The results obtained were noted, timed and tabulated. Statistical analyses were done using unpaired T test and oneway ANOVA test.

Experimental Methods

Two commonly used screening methods for evaluation of anti epileptic drugs are.

1. Supra maximal electroshock or maximal electro shock.

Albino Mice & Wistar Rats were subjected to electro shock through pinnal electrodes applied to external ears with current strength of 50 mA for 0.2 Sec. for mice and 150 mA for 0.2 Sec. for rats. This resulted in seizures and the various phases of seizures i.e, onset, type to recovery were noted and timed.

The following parameters were recorded.

a) Duration of convulsions. b) Duration of hind limb extensions. c) Post ictal depression followed by recovery.

Abolition or decrease in duration of hind limb extension was taken as the index of protection.

Chemical method – INH induced seizures

Animal received an intra-peritoneal injection of INH – (300mg/Kg in Rats & 150 Mg/Kg/bw in Mice) and the resulted seizures with its various phases to recovery were noted and limited.

The following parameters are recorded;

1. Onset of convulsions.

2. Type of convulsions.3.Duration of convulsions.

Extent of decrease in duration of convulsions was taken as index of protection.

Note: A pilot study was done to select the ED 50 doses of the drugs (both standard as well as test drugs) used here.

Maximal Electro Shock Method

Control Group -These animals (both Rats &Mice) constitute the control group for MES Method which received 0.2 ml of distilled water intra peritoneally. After an interval of 60 minutes they were subjected to Electro Shock (MES) convulsion with an alternating current of intensity of 50mA for Mice & 150mA for Rats for 0.2 Sec. through pinnal electrodes.

T1 (Test 1 group) -6 Albino Mice & Rats received Nimodipine 30 mg/kg/bw intraperitoneally. After an interval of 60 min they were subjected to electro shock convulsion.

T2 (Test 2 Group)- 6 Albino Mice & Rats received Nimodipine 30 mg/kg/bw Intraperitoneally. After an interval of 20 min Diclofenac Sodium 10 mg/kg/bw is given intraperitoneally. They were subjected to electro shock convulsion after 1 hr.

Chemical Method

Control Group (C) – 6 Albino Mice & Rats constitute the control group for INH method. Each Mouse received 0.2 ml of distilled water intraperitoneally after an interval of 60 min they received INH. 150 mg/kg/bw intraperitoneally for Mice & 300 mg /kg/bw of INH for Rats. The duration of different parameters were noted.

Test Group T1 – 6 Albino Mice & Rats received 30 mg/kg/bw of Nimodipine intraperitoneally. After an interval of 60 min INH 150mg/kg for Mice and 300 mg/kg for rats given intraperitoneally.

Test Group T2 – Animals are given Nimodipine 30 mg/kg/bw followed 20 min later 10 mg/kg/bw Diclofenac given intraperitoneally. After an interval of 1hr INH 150 mg/kg/bw& 300 mg/kg/bw given intraperitoneally.

OBSERVATIONS AND RESULTS:

Data were analyzed using SAS® V8e for windows. All Descriptive Statistics are expressed as Mean, Standard Deviation and Standard Error. To find out the difference between the Drugs (Control, Nimodipine and Nimodipine + Diclofenac) within each method (MES method and Chemical method), ANOVA was used. P-value<0.05 was considered to be significant. Further, Unpaired t-test was used to find out the different groups that are differing statistically significant. Here also, P-value<0.05 was considered to be significant.

Table 1: Unpaired t-test table showing the significant difference between different pairs of groups

Method	Variable	Groups	Mice P-value	Rats P-value
MES method	Convulsion	Control and T1drug	0.1965***	0.0237**
		Control and T2drug	<0.0001*	<0.0001*
		T1drug and T2drug	<0.0001*	0.0101**
	HLE	Control and T1drug	0.0147**	<0.0001*
		Control and T2drug	0.0011**	<0.0001*
		T1drug and T2drug	0.0649*	<0.0001*
	Recovery	Control and T1drug	0.0025**	0.0056**
		Control and T2drug	<0.0001*	0.0001*
		T1drug and T2drug	0.0024**	0.0442**

Table 2: Unpaired t-test table showing the significant difference between different pairs of groups

Method	Variable	Groups	Mice P – Value	Rats P – Value
Chemical method	Onset of Seizures	Control and T1drug	0.0003*	<0.0001*
		Control and T2drug	0.0001*	<0.0001*
		T1drug and T2drug	<0.0001*	0.0002*
	Duration	Control and T1drug	0.0287**	<0.0001*
		Control and T2drug	0.0007*	<0.0001*
		T1drug and T2drug	0.2031***	0.0026**

**** - Very Significant; ** - Significant and *** - Not Significant***

DISCUSSION

In a study described by N.Khanna[5] it was suggested that calcium channel blockers like Nifedipine & Nimodipine possess anticonvulsant activity. Nimodipine is a short acting dihydropyridine that inhibits the entry of calcium through slow channels (L-Type Channels)

Similarly other studies by J.Bhaduri[6] have suggested that Prostaglandins have a proconvulsant activity. Inhibition of Prostaglandin synthesis has been reported to increase the brain levels of dopamine & NA. beingstrom et al also showed that PGE2 causes reduction in the release of dopamine & NA in Rat brain slices.

The present study is based on the combined effect of Nimodipine & Diclofenac in experimentally induced convulsions (both MES & INH) induced taking distilled water as control.

In MES method both species (6 animals in each group) control, test drug Nimodipine and Nimodipine + Diclofenac).

The mean duration of convulsion was decreased, when Nimodipine was combined with Diclofenac than when given alone similarly the other 2 parameters (duration of HLE & Recovery) was also decreased when Nimodipine is given alone and much more decreased when Nimodipine was combined with Diclofenac.

To test the variance and statistical significance the Anova test was used as there are 3 groups for comparison. For convulsions and recovery from convulsion in both Rats & Mice the 'P value was < 0.0001', where as the duration of HLE was 0.002 in Mice and in Rat was <0.0001.

Unpaired 't' test showed. P-Value <.0001 for all the three parameters (duration of convulsions, duration of hind limb extension and recovery) with combined effect of Nimodipine plus Diclofenac when compared with control and to also check for carryover effects and baseline variance.

In chemical method the onset of seizures was delayed, the duration of convulsion was also decreased more when Nimodpine is combined with Diclofenac rather than when it is given alone as compared to control.

One way Anova showed significant P value <0.0001 for delayed onset of seizures in both species. Similarly the duration of convulsions was reduced significantly and P value in mice was 0.0007 whereas in Rats it was <.0001 which was significant. Based on above findings Nimodipine in combination with Diclofenac reduced duration of convulsions duration of hind limb extension and recovery was fast from electrically induced convulsions.

This combination therapy may have important potential as an non sedative antiepileptic drugs, especially in those patients who are refractory to anticonvulsant treatment, or in cases of in treatable epilepsy.

Thus on the basis of present data and other studies it can be said that Nimodipine combined with Diclofenac may be effective against partial and generalised tonic clonic seizures.

CONCLUSION:

Nimodipine drug has independently reduced the duration of Convulsion, HLE, delayed the onset of convulsions and recovery was fast from electric shock. But when combined with Diclofenac there was an

improved effect in Duration / HLE / Recovery when compared with that of control and Nimodipine alone.

The results obtained in this study provide supporting pharmacological evidence of efficacy, possible potential benefit of combining Nimodipine with Diclofenac in epilepsy.

However, studies with other models of epilepsy and in combination with other Calcium channel blockers and COX inhibitors on experimental animals and human beings would be needed to substantiate the present work.

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