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Combined Anticonvulsant Effect of Nifedipine and Pentazocine in Experimentally Induced Seizures by Maximal Electro Shock Method in Mice

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ABSTRACT

Objective: The aim of the study was to determine the combined anticonvulsant effect of nifedipine (calcium channel blocker) and pentazocine (opioid analgesic) in experimentally induced seizures by Maximal Electro-Shock (MES) method in mice.

Methodology: The swiss albino mice weighing 20-40g of either sex were obtained from National Institute of Nutrition, Hyderabad after obtaining ethical approval. The pretreated mice are subjected to MES stimulation by electro convulsometer with alternate current at intensity required to produce tonic hind limb extension response. The animals showing positive response are divided into four groups (6 animals per group). Group I received distilled water, group II treated with nifidipine (10mg/kg/bw), group III pentazocine (30mg/kg/bw) and group IV combination of nifidipine (10mg/kg/bw) and pentazocine (30mg/kg/bw). Intraperitoneal is the route of administration. The parameters like duration of convulsions, Tonic Hind Limb Extension (THLE) and duration of recovery recorded. $P<0.05$ was considered as significant and $P<0.001$ was considered as highly significant.

Results: The duration of convulsions, duration of THLE and duration of recovery has been significantly reduced in combination treatment wih nifedipine 10mg/kg and pentazocine 30mg/kg compared to nifedipine 10mg/kg and pentazocine 30mg/kg individually.

Conclusion: The results obtained in this study provide supporting pharmacological evidence of efficacy, possible potential benefit of combining nifedipine with pentazocine in the treatment of epilepsy.

Keywords: Seizures, Maximal electroshock, Electroconvulsometer, anticonvulsant, Nifedipine, Pentazocine.

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INTRODUCTION

Epilepsy is a common and chronic neurological disorder characterized by apparently unprovoked recurrent paroxysmal events or seizures that are associated with a sudden alteration in motor activity and behaviour, with or without alteration in conscious awareness.[1] The alteration in state is the result of an abnormal and excessive hypersynchronous firing within a group of epileptic neurons in the brain.[2]

Epilepsy affects at least 50 million people worldwide. Although antiepileptic drugs are the mainstay of epilepsy treatment, less than 70% of those afflicted with epilepsy achieve satisfactory seizure control with the available antiepileptic drugs.[3] Based on the total projected population of India in the year 2001, the estimated number of people with epilepsy is 5.5 million. The prevalence rate of epilepsy in India is 5.35 / 1000 population. This is the percentage of persons with active epilepsy who are not receiving treatment as per the standard guidelines.[4]

Epilepsy is one such disorder and the beneficial effects of calcium channel blockers (CCBs) in some experimental epileptic models have been reported already.[5-6] Studies have shown that CCBs could offer a new hope in epilepsy by potentiating the effect of classical antiepileptic drugs.[7] It has been shown that calcium ion is an important factor for the induction of epilepsy. Therefore, calcium channel blockers

such as nifedipine may be considered as therapeutic option in the management of seizures.[8]

Many studies demonstrated that opioid peptides with pharmacological electivity for mu, delta, Kappa, binding sites are anticonvulsants. It is interesting to note that mu, delta, kappa, opioids selectively hyperpolarize neurons throughout the CNS. Therefore it seems possible that opioid peptides may indeed form as endogenous anticonvulsant in the CNS modulating the underlying mechanisms of seizures arrest and refractoriness which are critical to the suppression of convulsions. Pentazocine is known to act as an agonist at kappa opioid receptors and a weak antagonist or a partial agonist at mu receptors.[9]

Mouse models have been largely used in epilepsy research because the genetic/molecular manipulations done in these animals offer great advantages in mechanistic investigation.[10]

In view of this present study was carried out to investigate combined anticonvulsant effect of nifedipine (CCBs) and pentazocine (opoid analgesic) in experimentally induced seizures by Maximal Electro-Shock (MES) model using electro-convulsometer in mice. The MES is probably the best-validated method for assessment of antiepileptic drugs in generalized tonic-clonic seizures.[11]

MATERIAL AND METHODS

Chemicals and Drugs

Anticonvulsant drugs used in the study are nifedipine (Depin) 5mg capsule by Zydus Cadila Healthcare Limited and Pentazocine (Fortwin) 1ml ampoule by Ranbaxy Laboratories was used to treat Maximal Electro-Shock (MES) seizures in mice. Tween 80 (Ranbaxy Laboratories) used to dissolve nifedipine and diluted with double distilled water to a required concentration of 5mg/ml.

Animals

Adult healthy swiss albino mice of either sex weighing 20-40g were obtained from National Institute of Nutrition, Hyderabad with the approval from Institutional Animal Ethics Committee (IAEC), Department of Pharmacology, Osmania Medical College, Koti, Hyderabad. The mice are housed in open cages having free access to food and water. The room temperature was maintained at 22°C ±2°C and 12hrs light and 12hrs dark. The mice are acclimatized to adopt the experimental conditions and only healthy animals used for the experiment.

Maximal Electro-Shock (MES) Method

Electro Convulsometer (Techno Electronics) was used to induce seizures by MES stimulation through transcorneal electrodes. A seizure is generally considered to be maximal if increments in current intensity do not alter the pattern or the duration of its various components. The conventional MES test has standardized parameters such as a 50-mA (mice) fixed current, a 50-60–Hz pulse frequency, a 0.6-ms pulse width and a 0.2 seconds stimulus.[12] Before induction of seizures the corneal electrodes are applied with normal saline (0.9% Nacl). The parameters recorded are Duration of convulsions, Duration of Tonic Hind Limb Extensions (THLE) and Post-ictal depression followed by recovery.

Experimental design

Animals that have shown convulsive responses were used for the experiment. The animals are divided into four groups, 6 animals per group.

Group I given distilled water as vehicle.

Group II treatment with Nifedipine at a dose 10mg/kg/bw.

Group III treatment with pentazocine at a dose 30mg/kg/bw.

Group IV treatment with both Nifedipine at a dose 10mg/kg/bw and pentazocine at a dose 30mg/kg/bw.

The animals received 0.2ml volume by intraperitoneal (IP) route. After 45 minutes of treatment the animals are again subjected to electroshock stimulation by MES method. The parameters recorded are duration of convulsions, duration of THLE and duration of recovery. Abolition or decrease of tonic hind limb extension was considered as positive response.

Statistical Analysis

The results are expressed as mean± SEM. The data was analysed using One-way analysis of variance (ANOVA) to determine statistically significant differences between the means of groups. Further data are subjected to multiple comparisons by post hoc Least Significant Difference (LSD). $P<0.05$ was considered as significant and $P<0.001$ was considered as highly significant.

RESULTS

The study was undertaken to explore combined anticonvulsant effect of nifedipine (calcium channel blocker) and pentazocine (opoid analgesic) by Maximal Electro-shock (MES) induced model in mice. The abolition or reduction of duration of tonic extension was considered as index for antiepileptic activity. The mean duration of the convulsions in the group IV treated with nifedipine (10mg/kg/bw) and penatzocine (30mg/kg/bw) has shown highly significant reduction $p<0.001$compared to group I, group II and group III (Table 1 & 2, Figure 1). The mean duration of tonic hind limb extension has shown highly significant reduction in group IV $p<0.001$ compared to group I and significant reduction $p<0.05$ compared to group II and group III (Table 1 & 2, Figure 1). Further mean duration of recovery in group IV also showed highly significant reduction $p<0.001$ compared group I which received double distilled water as vehicle and group III which are treated with pentazocine (30mg/kg/bw) but $p<0.05$ compared to group II which are treated with nifedipine (10mg/kg/bw) (Table 1 & 2, Figure 1).

On the basis of the above findings, the combined treatment with nifedipine (10mg/kg/bw) and pentazocine (30mg/kg/bw) has shown reduced duration of convulsions, duration of tonic hind limb extension and duration of recovery compared to animals treated with of nifedipine (10mg/kg/bw) and pentazocine (30mg/kg/bw) alone.

Table: 1 Combined anticonvulsant effect of nifedipine and pentazocine in animals subjected to MES seizures in mice.

Groups	Duration of Convulsions (time in seconds)	Duration of THLE (time in seconds)	Duration of Recovery (time in seconds)
Group I	42.17±1.046	16.33±0.760	7. 00±0.577
Group II	40.33±0.715	13.17±0.477*	5.66±0.333*
Group III	39.83±0.749	13.50±0.764*	6.17±0.307
Group IV	34.66±1.282**	10.83±0.946**	4.33±0.422**

The data are expressed as Mean ± SEM; No of animals 6/group; **statistically highly significant (P<0.001) compared to group I; *statistically significant (P<0.05) compared to group I.

Table 2: Combined anticonvulsant effect of nifedipine and pentazocine in animals subjected to MES seizures in mice.

Variable	Groups		Mice (p Value)
Duration of Convulsion	Group I	Group II	0.199
		Group III	0.106
		Group IV	<0.000**
	Group II	Group III	0.721
		Group IV	< 0.001 **
	Group III	Group IV	<0.001**
Duration of THLE	Group I	Group II	<0.008*
		Group III	<0.015*
		Group IV	<0.000**
	Group II	Group III	0.758
		Group IV	<0.041*
	Group III	Group IV	<0.021*
Duration of Recovery	Group I	Group II	<0.038*
		Group III	0.179
		Group IV	<0.000**
	Group II	Group III	0.413
		Group IV	<0.038*
	Group III	Group IV	<0.006*

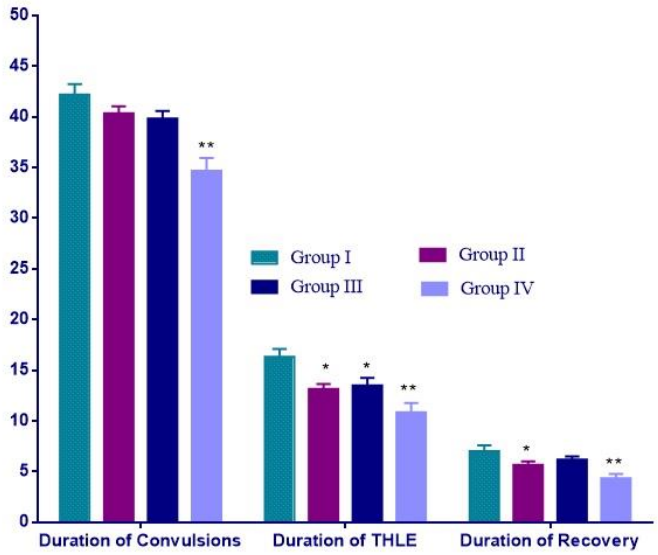

Figure 1: Combined effect of nifedipine and pentazocine on MES induced convulsions in mice.

**statistically highly significant (P<0.001) compared to group I; *statistically significant (P<0.05) compared to group I.

DISCUSSION

The study objective was to determine the combined anticonvulsant effect of Nifedipine, calcium channel blocker and Pentazocine, opioid analgesic by electro shock stimulation using Electro-Convulsometer through trans corneal electrodes at intensity sufficient to produce tonic hind limb extension (THLE) in mice.

The pretreated mice was subjected to MES stimulation with an alternating current of 50 mA intensity for 0.2 sec through corneal electrodes and various phases of seizures are recorded. The animals showing convulsive response were divided into groups and treated with nifedipine (10mg/kg/bw) and penatzocine (30mg/kg/bw) alone and in combination. The control animals received distilled water.

The route of administration is by intraperitoneal route. After 45 minutes of treatment the mice are subjected to MES and parameters like duration of convulsions, duration of THLE and duration of recovery were recorded.

Many of the currently used antiepileptic drugs have been shown to block the calcium channels and the present study also demonstrated that Nifedipine has anticonvulsant action, calcium channels are more commonly viewed as attractive targets for novel epileptic therapies.[13]

The pentazocine which is known to act as an agonist at k-opioid receptors and a weak antagonist or a partial agonist at u-receptors', has shown a significant anticonvulsant bioactivity in MES test at dose of 30mg/kg. [14,15]

From the results it was found that mice treated with combination of nifedipine and pentazocine significantly reduced the duration of convulsions, tonic hind limb extension and duration of recovery $p<0.001$ compared to nifedipine and pentazocine alone showing highly significant anticonvulsant activity.

Based on the above observations it can be suggested that combination of nifedipine and pentazocine may be effective against partial and generalized tonic clonic seizures.[16]

Conclusion

From the study we concluded that combined effect of nifedipine (10mg/kg/bw) the calcium channel blocker and pentazocine (30mg/kg/bw) the opioid analgesic are found to have significant anticonvulsant activity in MES induced animal model compared to nifedipine and pentazocine individually. The study outcome support pharmacological efficacy and potential benefit of combining nifedipine and pentazocine in epilepsy.

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DECLARATIONS

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Conflict of interest: No conflict of Interest

Ethical approval: The study was approved by the Institutional Animal Ethics Committee (IAEC), Department of Pharmacology, Osmania Medical College, Koti, Hyderabad

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