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Original Article

The Combined Anti-epileptic Effect Of Phenytoin And Cilnidipine In Experimentally Induced Convulsions In Mice

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ABSTRACT

Context: Cilnidipine, an N-type and L-type calcium channel blocker has been reported to have antihypertensive, renoprotective and neuroprotective effect. In this present study we evaluated anti-epileptic effect of cilnidipine in experimentally induced convulsions in Mice. Aims: To evaluate the anti epileptic effect of cilnidipine. Materials and Methods: Cilnidipine, phenytoin sodium, 18 Albino mice weighing between 20-30 grams and Electroconvulsive meter was used. The animals were divided into three groups of 6 each, phenytoin group, Cilnidipine group and combined Phenytoin+ cilnidipine group. The onset of convulsions or their inhibition, nature of convulsions, duration of tonic hind limb extension (THLE), period of post ictal depression (when present) and recovery were observed and noted in all groups of animals and compared with the standard drug, Phenytoin Sodium. Statistical Analysis used. ANOVA test was used. P value <0.05 was considered to be statistically significant. Results. Durations of tonic hind limb extensions, clonus phase and recovery were decreased in combined Phenytoin+Cilnidipine group when compared to individual Phenytoin group. Combined group showed statistically significant decrease in duration of tonic hind limb extensions, clonus phase and recovery. Conclusion: It was observed that anti-convulsant effect of combination of cilnidipine and phenytoin is superior to phenytoin alone. The combined effect was comparable to that of standard drug in electrically induced seizures

1. Introduction

Epilepsy is a syndrome of brain dysfunction is characterized by recurrent and unpredictable spontaneous seizures. Epilepsy is also the second most common neurological disorder, after stroke, affecting 1% of the world's population. Epilepsy is the second most common neurological disorder in India. The prevalence of epilepsy is 0.7% in India1. The WHO has estimated that approximately 80% of the people with epilepsy are living in developing countries and most of them do not get adequate medical treatment 1. The first generation AEDs like phenobarbitone, phenytoin, carbamazepine, ethosuximide are very effective drugs but have limitations such as sedation, major drug interactions and toxic effects especially at higher doses, warranting therapeutic drug monitoring. The second generation AEDs that have invaded the therapeutic scenario since 1990,lamotrigine,topiramate, levetiracetam, zonisamide to name few, have a better safety profile but are not as effective as the older drugs. Most of them are useful as add on drugs when the initial drug fails to control the seizures. The existing drugs control seizures in only 70% of Seizures in spite of drug therapy (refractory epilepsy). The mounting number of drugs, the additional adverse effects, drug interactions and other limitations contribute to cause decreased patient compliance, especially if epilepsy coexistent with other chronic diseases like hypertension. In fact epileptic form bursts are often associated with influx of Calcium ions into the nerve cells and a decrease in extra-cellular concentration of Calcium precedes the onset of seizures in many experimental models of epilepsy.

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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.

A new group of drugs with antiepileptic activity, without sedative properties is an interesting prospect. The results from experimental animal models of epilepsy and theoretical considerations suggest that calcium antagonist may form such a group. The initiation of epileptogenic activity in the neuron is connected with a phenomenon known as intrinsic burst firing which is activated by inward calcium current. Calcium is described as the primary mediator of excitotoxic neuronal damage during seizure activity. There is a decrease in the extra cellular calcium concentration prior to the onset of seizure activity followed by an increase in the intracellular calcium concentration. Considering the crucial role played by calcium, calcium channel blockers can be used in the treatment of epilepsy 2. Present work is based upon the production of convulsions by maximal electric shock method and evaluation of anti-epileptic effect of the Cilnidipine (N-type and L-type calcium channel blocker) and combined anti-epileptic effect of Phenytoin and Cilnidipine on duration of seizures, clonus, duration of tonic hind limb extension and recovery.

2. Materials and Methods

For conducting the present study, the following drugs, chemicals, animals and equipment were used.

MATERIALS

I. Drugs and Chemicals

Phenytoin sodium

Cilnidipine

Normal Saline

Double distilled water

II. Animals

18 Albino mice weighing between 20-30 gms

III Equipments

Electro Convulsiometer (Techno) with accessories
Animal weighing balance
Animal cages
Stop watch
Electronic weighing balance
Measuring jars and glass beakers
Tuberculin syringes
Disposable syringes and needles
Cotton and spirit

2. Methods

Electrical Method – Maximal Electro Shock Method (MES test)

In the present study, anticonvulsant activity of Cilnidipine and combined effect of phenytoin and cilnidipine is evaluated using MES method in mice. The onset of convulsions or their inhibition, nature of convulsions, duration of tonic hind limb extension (THLE), period of post ictal depression (when present) and recovery were observed and noted in all groups of animals and compared with the standard drug, Phenytoin Sodium.

Preparation of Solutions of Drugs:

1) Phenytoin sodium (epsolin injection): Each ml contains 50 mg.

Given dose of 70mg/kg i.p (intraperitoneal)

2) Cilnidipine (Cilcar 10 mg —J.K PHARMA)

given dose1mg/kg i.p.

The instrument provides alternating current stimulus of 50 cycles per second. The electronic timing circuit contained in the apparatus automatically passes stimulus, current for a period which can be varied from 0.1 to 1 second in steps of .01 sec.

The current variable is from 0.25 to 360 mA. The centre panel of convulsiometer consists of 3 controls, besides the on off switch and pilot lamp.

1. On-Off switch – switches the instrument or or off.

2. Pilot lamp lights up when the instrument is switched on.

3.Stimulus Current Control – Situated at the extreme left, indicates values of stimulus current. It has twelve positions (A, B, 3, 6, 9, 12, 15, 18, 21, 24, 27 & 30).

4. The Central Control – has 2 scales

a) Inner Scale B for 0.5 – 3 mA

b) Outer Scale A for 0.25 to 0.5 mA

The scales of this centre control are effective only when the left hand stimulus current control is at A or B.

5. Multiply by control – This is situated at the extreme right & it multiplies the stimulus current control by the figure it is set to.

Combination setting of these 3 controls gives stimulus from 0.25 to 360 mA.

6. Time Control – Stimulus is delivered for 0.2 secs in mice.

7. The Start Reset Switch: It should normally be in reset position. When all is ready and the stimulus has to be given, the switch is pressed down to start. The stimulus is automatically delivered for the preset time after which it switches off.

Experimental Animals

18 adult albino mice were used for the experiment. The animals were kept in cages with free access to food and water. They were kept under standardized housing conditions i.e. natural light dark cycle, temperature under 30°C and relative humidity of 55 ± 5%. After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups. Each animal was used only once and tests were performed between 9.00 and 15.00 hours. The experimental protocol was approved by the Institutional Animal Ethics Committee. All efforts were made to minimize animal suffering and use only the number of animals necessary to produce reliable scientific data.

I. Experimental Methods
Supra maximal Electroshock or Maximal Electro Shock (MES test)

The animal was subjected to electro shock through corneal electrodes with an intensity of: 50 mA for 0.2 sec. in mice.

This resulted in almost immediate onset of convulsions, preceded by tonic hind limb extension (THLE) and followed by post ictal depression and recovery.

The following parameters were recorded.

A. Duration of THLE

B. Duration of clonic convulsions

C. Recovery period.

PROCEDURE - FOR MICE

18Swiss Albino Mice were taken for the experiment

MES Method – 18 MICE

They were divided into 3 groups, each group contained 6 mice.

Group 1– Standard group – administered Phenytoin Sodium 70 mg/kg. i.p.

Group 2 – cilnidipine group – administered 1mg/kg i.p.

Group 3 – phenytoin+cilnidipine Group – administered both

Cilnidipine 1mg/kg i.p. and phenytoin 70mg/kg i.p, separately.

All the 3 groups of mice were subjected to MES method at 50 mA intensity, 60 minutes after the administration of the above i.p. injections. The results were tabulated under the parameters mentioned above.

3. Results

Data was analyzed and all descriptive statistics are expressed as mean, standard deviation and standard error. To find out the difference among the drugs (cilnidipine, phenytoin and Cilnidipine+phenytoin with MES method), ANOVA test was used. P value <0.05 was considered to be statistically significant.

The parameters that are considered for comparison of anti-convulsant activity among three groups are

I. For MES method:

A. Duration of tonic hind limb extension (in seconds)

B. Duration of clonic phase (in seconds)

C. Duration of recovery phase (in seconds)

Table 1, 2 and 3 show the duration of THLE(tonic hind limb extension), duration of clonic phase and duration of recovery phase in mice following MES seizures respectively.

Graphs 1, 2 and 3 show Bar charts comparing the 3 drug groups by MES method in mice.

Graph 1 – Comparison of duration of THLE (in seconds) by MES method among the 3 groups of mice.

Graph 2 – Comparison of duration of clonic phase (in seconds) by MES method among 3 groups of mice.

Graph 3 – Comparison of duration of recovery period (in seconds) by MES method among 3 groups of mice.

P<0.05 is considered significant.

P<0.01 is considered very significant.

P<0.001 is considered highly significant.

Comparison of duration of tonic hind limb extension (in seconds) by mes method among 3 groups of mice table --- 1

GROUP	(SIZE OF SAMPLE)	MEAN (IN SECONDS)	STANDARD DEVIATION	STANDARD ERROR
GROUP 1	6	1.16	0.364	0.148
GROUP 2	6	17	1.414	0.577
GROUP 3	6	0.5	0.5+	0.2

GROUP 1 --- Phenytoin sodium 70mg/kg i.p

GROUP 2 --- cilnidipine 1mg/kg i.p

GROUP 3 --- phenytoin sodium i.p +cilnidipine 1mg/kg i.p

The observed difference among the 3 groups (as calculated by ANOVA test) is statistically significant at 95% confidence intervals. (p <0.05)

Graph 1 – Comparison of duration of THLE (in seconds) by MES method among the 3 groups of mice.

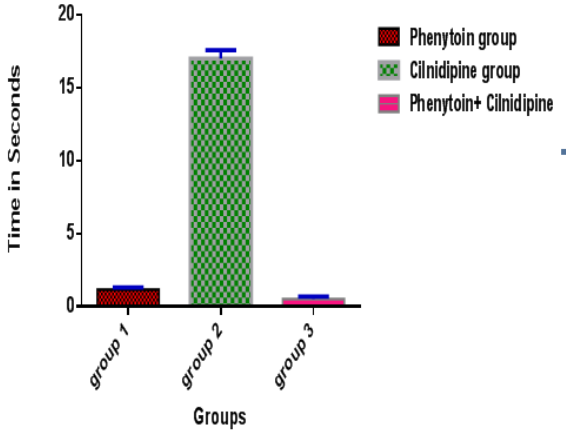


TABLE –2

GROUP	(SIZE OF SAMPLE)	MEAN (IN SECONDS)	STANDARD DEVIATION	STANDARD ERROR
GROUP 1	6	36.833	3.702	1.511
GROUP 2	6	53.5	1.892	0.772
GROUP 3	6	26.33	2.05	0.838

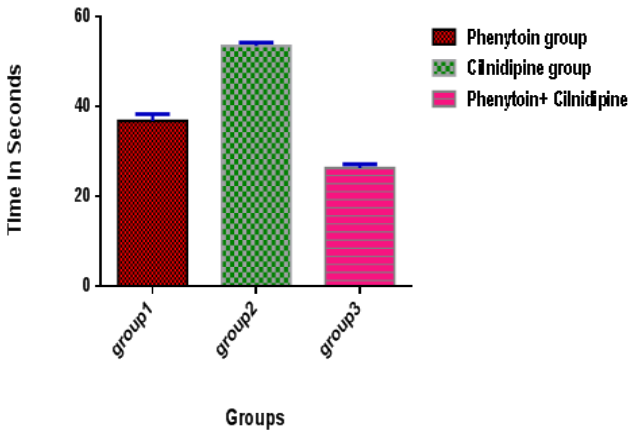
GROUP 1 -- Phenytoin 70 mg/kg i.p

Group 2 --- Cilnidipine 1mg/kg i.p

Group 3 --- Phenytoin 70 mg/kg i.p + Cilnidipine 1mg/kg i.p

The observed difference among the 3 groups as calculated by ANOVA test is statistically significant at 95% confidence intervals ($p < 0.05$).

Graph 2 – Comparison of duration Clonus (in sec) by MES method in 3 groups of Mice



Comparison of duration of recovery (in seconds) by mes method among 3 groups of mice

GROUP	(SIZE OF SAMPLE)	MEAN (IN SECONDS)	STANDARD DEVIATION	STANDARD ERROR
GROUP 1	6	10.33	2.12	0.865
GROUP 2	6	32.3	2.15	0.88
GROUP 3	6	6.33	1.36	0.55

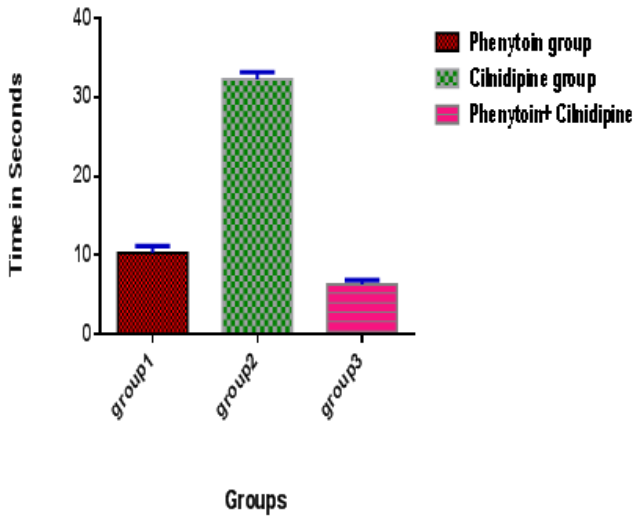
Group 1 -- Phenytoin 70mg/kg i.p

Group 2 --- cilnidipine 1mg/kg i.p

Group 3 --- Phenytoin 70 mg/kg i.p + Cilnidipine 1mg/kg i.p

The observed difference among the 3 groups as calculated by ANOVA test is statistically significant at 95% confidence intervals, i.e. ($p < 0.05$)

Graph 2 – Comparison of duration of Recovery (in sec) by MES method in 3 groups of Mice



4. Discussion

Overwhelming evidence indicates that calcium ions (ca2+) play an important role in the pathogenesis of epilepsy and Calcium channel blockers have been increasingly investigated in the treatment of refractory epilepsy. The aim of this study is to assess the anti-convulsant effect of cilnidipine alone and in combination with phenytoin in experimentally induced seizure models in mice.

The parameters considered are as follows (by MES method).

1. Duration of tonic hind limb extension (in seconds).
2. Duration of clonic phase (in seconds).
3. Duration of recovery phase (in seconds).

1. Duration of Tonic Hind Limb Extention (THLE) (in seconds)

In mice, the mean duration of THLE was 1.16 sec in Phenytoin group. Maximum with cilnidipine group (17 seconds). . The combined group has shown maximum decrease in THLE i.e., 0.5 seconds.

The observed difference among the three groups as calculated by ANOVA is statistically significant at 95% confident intervals ($p<0.05$).

2. Duration of clonic phase by MES method (in seconds)

The mean duration of clonus phase with phenytoin group is 36.8 seconds. It is increased with cilnidipine group to 53.5 seconds where as it has decreased to 26.3 seconds with combined group.

The observed difference among three groups as calculated by ANOVA test is statistically significant "($p<0.05$)

3.Duration of recovery phase by MES method (in seconds)

The average duration of recovery phase is 10.33 seconds with phenytoin group, it has increased to 32.3 seconds with cilnidipine group where as it has decreased to 6.33 with combined group.

The observed difference among the three groups as calculated by ANOVA test is statistically significant.

5. Conclusion

Durations of tonic hind limb extensions, clonus phase and recovery were decreased in combined (third) compared to individual Phenytoin group (first). Combined group showed statistically significant decrease in duration of tonic hind limb extensions, clonus phase and recovery. It was observed that anti-convulsant effect of combination of cilnidipine and phenytoin is superior to phenytoin alone. The combined effect was comparable to that of standard drug in electrically induced seizures. Hence anti-convulsant potential of this combination can be useful in the treatment generalized tonic clonic seizures. Further clinical investigation of these drugs is needed. Epilepsy being a chronic disease may be co-existent with other chronic diseases like hypertension. In these clinical settings the potentiating effect of Calcium channel blockers like cilnidipine which is cardio-protective, neuro-protective and reno-protective and anti-oxidant effect may prove to be useful. If Known epilepsy patient, who is on phenytoin, develops hypertension, then cilnidipine can be good choice.

Possible advantages of choosing cilnidipine as anti hypertensive in epileptic patient who is on phenytoin are

1. Phenytoin dose can be reduced.

2. Cilnidipine has neuroprotective cardio-protective, Reno protective and anti-oxidant effects which may be useful to patient. Further studies are needed to exploit this effect.

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