Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 6, 2014

Original Article

EVALUATION OF THE ANTIEPILEPTIC ACTIVITY OF FELODEPINE IN ALBINO MICE

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Received: 08 May 2014 Revised and Accepted: 07 Jun 2014

ABSTRACT

Objective: To evaluate the antiepileptic activity of calcium channel blocker, felodepine in combination with different doses of valproic acid in animal models of epilepsy.

Methods: Albino mice(25-30gms) of either sex were randomly selected and divided into 2 main groups for MES and PTZ tests. Each of these groups further had 5 subgroups of 6 mice each, MES: Control – Propylene glycol(0.25mg/kg), Standard -Valproic acid (100mg/kg), T1- Felodepine(5mg/kg) + valproic acid (100mg/kg), T2 - Felodepine(5mg/kg) + valproic acid(75mg/kg), T3- Felodepine(5mg/kg) + valproic acid(50mg/kg). PTZ : T4 – Felodepine(5 mg/kg) + valproic acid(100mg/kg), T5 -Felodepine(5mg/kg) + valproic acid(75mg/kg), T6: Felodepine(5mg/kg) + valproic acid(50mg/kg). All drugs were administered orally 1 hr prior to induction of seizures. The anticonvulsant activity was screened using maximal electroshock (MES) and pentylenetetrazole (PTZ) models.

Results: The data was analysed by one way ANOVA followed by Bonferonni's multiple comparison test. In MES test, T3 does not produce tonic hind limb extension while tonic hind limb extension by T1 - 3 + 4.69 and T2 – 1.33 + 3.27. In PTZ model felodepine in combination with valproic acid produced significant antiepileptic activity in comparison to control. The seizure latency produced by T6 was 105 + 162.69secs in comparison to T4 – 88.33 + 98.06secs and T5 – 58.33 + 90.43 secs.

Conclusion: Felodepine in combination with lower dose of valproic acid produced significant antiepileptic activity.

Keywords: Felodepine, Valproic acid, Antiepileptic activity, Calcium channel blocker.

INTRODUCTION

Epilepsy is a common and disabling neurological disorder that can be especially gratifying to treat. It is a major health problem both in developing and developed countries.[1] It afflicts more than 50 million people worldwide, 5 million of whom have seizures more than once per month .[2]

A large number of promising compounds are currently undergoing preclinical and clinical evaluation and several of these will undoubtedly become meaningful additions to the neurologist pharmacological armamentarium.[3] Inspite of a vast number of drugs introduced for the treatment of epilepsy, there is still a need for an ideal antiepileptic agent with properties like broad spectrum activity, rapid onset of action, least side effects, good oral bioavailability and low cost.[4]

In recent years, calcium channel blockers have been found to suppress seizures induced by a variety of chemical or physical stimuli[5]. Physiological studies have emphasized the possible roles of calcium ion flux on the paroxysmal discharges associated with seizure activity[6]. Hence calcium channel blockade may be important in preventing seizure spread.

In neurons showing intrinsic burst firing, signalling epileptic activity there is massive influx of Ca2+ associated with the paroxysmal depolarising shift(PDS) and hence the influx of extracellular Ca2+ into neurons is considered to be an important feature in triggering epileptic activity. [7,8]Anticonvulsants such as phenytoin, barbiturates and benzodiazepines may act in part by inhibition of calcium influx and thus alter PDS.[9] The above findings suggest that in refractory epilepsy, treatment with conventional antiepileptic drugs combined with agents that modify calcium ion modulation, as add on therapy, may provide better seizure control.[10]

In the light of the development cited above an attempt has been made in the work to find out the effect of felodepine as an adjuvant along with the antiepileptic drugs in the prevention of experimentally induced seizures.

MATERIALS AND METHODS

Albino mice of either sex of average weight 25-30gms aged 3-4 months were bred in central animal house. They were housed in groups of three in clean polypropylene cages with 12 hour light/dark cycle at 25±2 º C and 65±5% humidity. They had access to food (standard pellet diet, Hindustan Lever Ltd) and water. All experiments were carried out between 11 AM and 3 PM. The ethical clearance was obtained from the Institutional Animals Ethical Committee.

Distilled water(Control group), Standard drug valproic acid(100mg/kg) were administered orally. Test drug, felodepine (5mg/kg) in combination with different doses of valproic acid (100mg/kg,75mg/kg, 50mg/kg) were administered orally 1 hour prior to MES and PTZ tests.

Evaluation of Antiepileptic activity

Maximal electroshock induced seizures

Electrical stimulation was applied via ear electrodes with a current strength of 50 mA for 0.2 sec .The resultant seizure passes through various phases; phase of tonic limb flexion (1.5 sec duration), phase of tonic limb extension (10 sec duration), finally followed by variable short clonic interval which may lead to asphyxial death in some animals. 24 hours before testing of anticonvulsants (to avoid any possible kindling effect), the animals were pre-screened for their ability to develop full tonic extension in the maximal electroshock test. Suppression of tonic hind limb extension was taken as measure of efficacy in this test.

Pentylenetetrazole (PTZ) Induced Convulsion

1 hour after test drugs and standard drug administration, 70 mg/kg pentylenetetrazole was injected intraperitoneally. Each animal was placed into an individual plastic cage for observation lasting 30 minutes. Within 30 minutes they developed a sequence of excitement, myoclonic jerks, clonic seizures, one or more maximal tonic seizures followed by recovery or some animals may succumb

to death. Seizure latency and abolition of clonic seizures with loss of righting reflex was taken as index of protection.

Statistical analysis

The effect of felodepine in combination with valproic acid on MES and PTZ models of seizure induction were expressed as mean ± SD. Percentage inhibition of seizure was calculated respectively. Data was analysed using one-way ANOVA followed by Bonferroni's multiple comparison tests. P values <0.05 were considered significant.

RESULTS

Maximum electroshock induced seizures

Felodepine in combination with valproic acid produced significant antiepileptic activity in comparison to control. In MES test, T3 does not produce tonic hind limb extension while tonic hind limb extension by T1 - 3 + 4.69 and T2 – 1.33 + 3.27. All the animals recovered from the MES induced seizure. The duration of seizure and duration of tonic hind limb extension by the groups is shown in table 1.

Table 1: Comparison of mean duration (in seconds) and standard deviation values of different parameters in MES method (mean + SD)

Parameters	Control	Standard	T1	T2	T3
Hind limb tonic flexion	1.5 ±0.55	-	-	-	-
Hind limb tonic extension	12.83 ± 1.47	1.83 ± 4.491***	3 ± 4.69***	1.33± 3.27***	-
Clonus	10.5 ± 1.05	2.67 ± 0.82***	3 ± 5.16**	1.33 ± 3.27***	2.33 ± 3.67***
Post ictal depression	94.67 ± 48.61	-	_	18.33 ± 44.91*	13.33 ± 32.66*
Recovery/ Death	R	R	R	R	R

n = 6, *p<0.05, **p< 0.01, ***p<0.001, Values are expressed as mean + SD. Statistical analysis of data was carried out by one way ANOVA.p < 0.05 is considered significant, Abolition of hind limb tonic extension considered to be protective end point

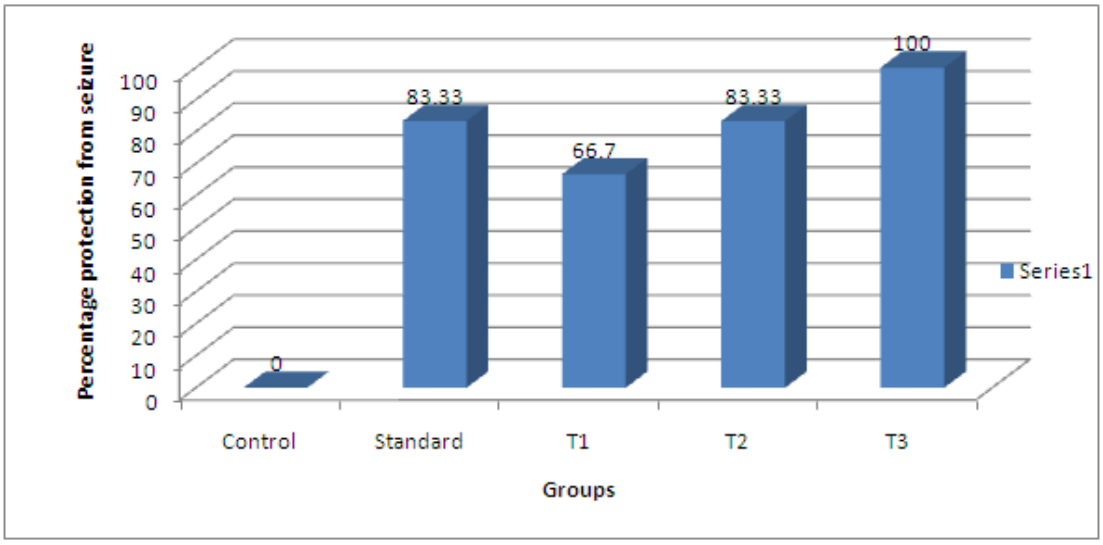

Fig. 1: Bar diagram showing percentage of protection rendered by different groups in MES method

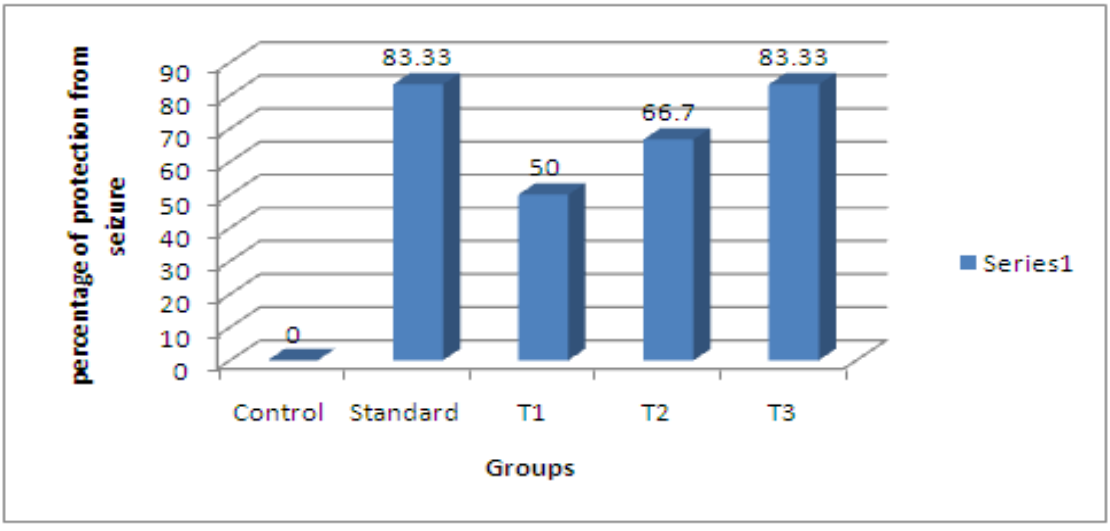

Fig. 2: Bar diagram sowing percentage of protection rendered by different groups in PTZ test.

Table 2: Comparison of mean duration (in seconds) and standard deviation values of different parameters in PTZ method(mean + SD)

Parameters	Control	Standard	T4	T5	T6
Seizure latency	46.67 ±15.67	53 ± 129.82	88.33 ±98.06	58.33 ± 90.43	105 ± 162.69
Mild myoclonic jerks	5.5 ± 1.87	1 ± 2.45**	8.83 ± 9.81	13.33 ± 21.60	2 ± 3.35*
Generalised clonic seizures with loss of righting reflex	11.83 ± 2.04	0	5.33 ± 5.89*	8.33 ± 16.02	1.33 ± 3.27***
Post ictal depression	185.33 ± 15.88	29.33 ± 71.85***	36.67 ± 57.15***	16.67 ± 40.82***	16.67 ± 40.82***
Recovery/ Death	2D	R	1 D	R	1D

n=6, *p<0.05, **p<0.01,***p<0.001

Values are expressed as mean + SD. Statistical analysis of data was carried out by one way ANOVA.p < 0.05 is considered significant.

PTZ induced seizures

In PTZ model felodepine in combination with valproic acid produced significant antiepileptic activity in comparison to control. The seizure latency produced by T6 is 105 + 162.69secs in comparison to T4 – 88.33 + 98.06secs and T5 – 58.33 + 90.43 secs. There was no mortality in T5 group and one mortality each in T4 and T6 groups. The duration of seizure latency and duration of generalised clonic seizure with loss of righting reflex by the groups is shown in table 2.

DISCUSSION

Epilepsy is a group of heterogenous neurological disorder characterized by spontaneous and recurrent seizures and is one of the most common disorder that affects 1% of the population. Contemporary anticonvulsant therapy is neither universally effective nor invariably safe. In the recent years a wide variety of targets have been found to be involved in seizure generation. In recent years investigators have reported that calcium channel blocker may prevent or suppress seizure induced by a variety of chemical or physical stimuli.Influx of extracellular calcium into neurons is considered to be an important feature in triggerring epileptic activity.

In the above study, percentage of protection provided by different groups in comparison to control in MES model are T1 – 66.7%, T2 – 83.33% and T3 – 100% while the percentage of protection provided by different groups in comparison to control in PTZ model are T1 – 50%, T2- 66.7% and T3 – 83.33%. The combination of felodepine(5mg/kg) with valproic -acid (50mg/kg) produced significant antiepileptic activity when compared to other test groups.

In MES method, felodepine when combined with different doses of valproic acid have conferred protection against MES induced hind limb extension. Felodepine in combination with valproic acid(50mg/kg) produced no tonic hind limb flexion and extension in MES model(as shown in table 1). In PTZ model, felodepine with valproic acid(50mg/kg) produces maximum seizure latency when compared to other test groups(as shown in table 2). Hence we can conclude that felodepine in combination with lower doses of valproic acid confers protection from seizure.

During epilepsy neurons show paroxysmal depolarisation shift(PDS) which corresponds to epileptic field potentials which is generated by neurons. These studies show that calcium and calcium dependent currents are responsible for this event and these PDS / EFP were depressed by calcium channel blockers.[11] Studies have shown that T type calcium channel play a role in human idiopathic generalised epilepsies.[12] Hence calcium channel blockers can be studied for their effect on epilepsy.

Calcium ion entry through voltage-activated calcium channels both depolarizes the cell membrane and regulates numerous intracellular signaling pathways. L-type Ca(2+) channels have been shown to be important in various epilepsy models. The L-type calcium channel antagonist nifedipine blocks spontaneous firing and γ-aminobutyric acid $(GABA)_A$–induced depolarization of single cells in HH tissue slices. Hence the calcium channel antagonist felodepine produces significant antiepileptic activity.[13]

Blocking or activating of L- and T-type Ca2+ channels in the S1po area (cortical focus area) can significantly control generation of spike wave discharges during absence seizures.[14]

CONCLUSION

Calcium channel antagonist felodepine has proved to have significant anticonvulsant activity when used as an adjuvant with standard antiepileptic drug in lower doses . However further studies needs to be done to determine the exact mechanism of antiepileptic action of felodepine .

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