ANTICONVULSANT ACTIVITY OF FLUPIRTINE IN ALBINO MICE

Naveen Kumar M,[1] Gokul CG,[1] Somashekar HS,[2] Prabhakar Adake,[1] Abhishek Acharya,[1] Santhosh R.[1]

1. Post graduate, Department of Pharmacology, J.J.M.Medical College, Davangere - 577004, Karnatkaka, India.
2. Professor and Head of the Department, Department of Pharmacology, J.J.M.Medical College, Davangere-577004, Karnatkaka, India.

Summary

The present study has shown that flupirtine 79 mg/kg has protection against MES induced seizures in mice but lower dose of the flupirtine did not give protection. The protection was not comparable to standard Phenytoin. A total of 24 mice (N=24) with a weight ranging from 20g-25g were used in this study with 6 (n=6) animals in each of the 4 groups. Group I (Standard) received phenytoin sodium 20mg/kg po (per oral). Flupirtine dissolved in di-Methyl Sulphoxide (DMSO). Group II (Test) received flupirtine 39mg/kg po. Group III (Test) received flupirtine 79mg/kg po. Group IV (Control) received DMSO 1ml/kg po. Convulsion was induced by stimulus of 45mA for 0.2sec by ear electrodes. The animals were observed closely for 2 min and duration of hind limb extension was recorded. Hind limb tonic extension (HLTE) (absent or present) was taken as primary efficacy end point and decrease in duration of HLTE were taken as secondary end point. Results were analyzed by ANOVA followed by Post hoc Tukey's test. Selective neuronal potassium channel opener and functional antagonist of N-methyl-D-aspartate (NMDA) receptor Flupirtine at the dose of 79mg/kg has significantly reduced duration of hind limb tonic extension in comparision with control (DMSO) group. In phenytoin group all the animals were protected from MES induced seizures but in flupirtine 79 mg/kg group only 33% of the animals were protected and in other flupirtine 39 mg/kg group no animals were protected from seizures.

Key words: Flupirtine, N-methyl-D-aspartate, Potassium channel opener, Maximal Eletroshok induced seizures.

Introduction

Epilepsy is one of the most common neurological disorders. Worldwide, the prevalence is estimated to be 0.5 – 1%, and there is a life time incidence of 1 – 3%.[1] It has important medical, social and psychological consequences.[1]

Epilepsy is a heterogeneous symptom complex, a chronic disorder characterized by recurrent seizures. Seizures are finite episodes of brain dysfunction resulting from abnormal discharge of cerebral neurons.[2] It is estimated that in India (with population more than 1 billion), there will be 6- 10 million people with epilepsy, accounting for nearly 1/5 of global burden.[3]

In 1912, Phenobarbital was first used for epilepsy and in the next 25 years, 35 analogs of Phenobarbital were studied as anticonvulsants. In 1938, phenytoin was found to be effective against experimental seizures in cats. Between 1935 and 1960 tremendous strides were made both in the development of experimental models and in methods for screening and testing new antiepileptic drugs. During that period 13 new antiepileptic drugs were developed and marketed. Following the enactment of requirements for proof of drug efficacy in 1962, antiepileptic drug development slowed dramatically and only a few new antiepileptic drugs were marketed in the next 3 decades. However, a series of new compounds became available in the 1990's.[2]

Despite the introduction of several new therapeutic options in the 1990s, a significant fraction of the patients with epilepsy continue to live with uncontrolled seizures.[1] 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] Contemporary anticonvulsant therapy, however, is neither universally effective nor invariably safe. Their important adverse effects include central nervous system depression, ataxia, megaloblastic anemia, cardiac arrhythmias, hepatic dysfunction and teratogenicity.[4]

Flupirtine (ethyl-N-[2-amino-6-(4-flurophenylmethyllamino) pyridine-3-yl] carbamate) has been in clinical use for many years as a centrally active analgesic with muscle – relaxant properties. In preclinical and preliminary clinical studies, neuroprotective, antiepileptic and antiparkinsonian effects were additionally found.[5] by activating cerebral inwardly rectifying K^+ channels flupirtine stabilizes the resting membrane potential(RMP) and also decrease the excitability of neuronal membrane. This may have anti seizure potential.

So this study has been undertaken to evaluate the effect of flupirtine in MES induced seizure and compare its efficacy with currently used antiepileptic drug phenytion sodium in mice.

OBJECTIVES

1. To evaluate the effect of flupirtine in MES induced seizure in albino mice.
2. To check for dose dependent effect of flupirtine in MES induced seizure.
3. To compare the anticonvulsant effect of flupirtine with that of phenytion sodium.

Materials and Methods

Animals:

The study was done in male Albino mice (20–25 g). The mice were housed under standard conditions with food and water ad libitum, which was bred in the central animal house J.J.M. Medical College, Davangere. They were used to induce convulsions by Maximal electroshock method after obtaining Clarence from Institutional animal ethics committee.

A total of 24 animals (N=24) with a weight ranging from 20g-25g were used in this study with 6 (n=6) animals in each of the 4 groups. Group I (Standard) received phenytoin sodium 20mg/kg po (per oral). Flupirtine dissolved in Di Methyl Sulphoxide (DMSO). Group II (Test) received flupirtine 39mg/kg po. Group III (Test) received flupirtine 79mg/kg po. Group IV (Control) received DMSO 1ml/kg po. Convulsion was induced by electroconvulsiometer with the stimulus of 45mA for 0.2sec by trans auricular electrodes. The animals were observed closely for 2 minutes, abolision of HLTE and duration of hind limb tonic extension was recorded. Flupirtine was obtained from Lupin Pharmaceuticals, dissolved in DMSO and phenytoin is obtained from Abott Pharmaceuticals

Maximal Electroshock-Induced Seizures:

MESs was induced using a electroconvulsometer with a current of 45-mA intensity for 0.2-second duration via earclip electrodes. In mice maximal seizures consisted of initial tonic flexion, tonic hindlimb extension (HLTE), and terminal clonus. The endpoint of efficacy was taken as the inhibition of HLTE. This was defined as protection against MES induced seizures and expressed as percentage.

Statistical analysis:

Protection in MES seizure was recorded as a percentage and compared using the chi square test. Other values were expressed as means +/- SE and statistical significance was calculated by ANOVA with post hoc Tukey' s test. P < 0.05 was taken as significant.

Results

From table 1 & 2, graph 1 the mean duration of HLTE in flupirtine 79 mg/kg group was 7.167±5.60 which was statistically significant when compared standard Phenytoin group (7.167±5.60 vs 0) and DMSO control group (7.167±5.6 vs 14.2 ± 3.4) ($p<0.05$) but there was no significant difference observed between the control (14.2 ±3.4) and other flupirtine 39 mg/kg group (16.7 ±2.94 vs 14.2± 3.4) ($p>0.05$). There was significant difference in mean duration of HLTE between the Flupirtine 39 mg/kg and 79 mg/kg (16.7±2.94 vs 7.17±5.6) ($p<0.05$). In phenytoin group all the animals were protected from MES induced seizures but in flupirtine 79 mg/kg group only 33% of the animals were protected and in other test groups no animals were protected from seizures (table 1).

Above results demonstrated that flupirtine 79 mg/kg has some protection against MES induced seizures in mice but lower dose of the flupirtine did not give any protection. The protection given by flupirtine 79mg/kg was not comparable to standard Phenytoin.

Table 1: Percentage of Protection against MES seizure and Mean duration of HLTE

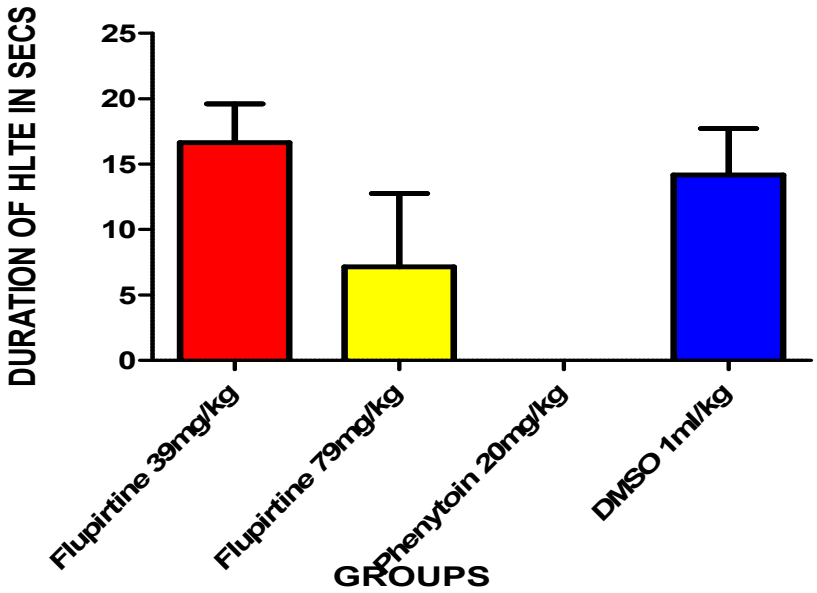
Drug	% of protection	Mean duration of HLTE (sec)
DMSO	0	14+/- 3.03
Phenytoin	100	0
Flupirtine 39 mg/kg	0	20.33+/- 2.58
Flupirtine 79 mg/kg	33	7.33+/-5.8

Table 2: Post hoc Tukey' s comparison test between groups (duration of HLTE).

Groups	Mean difference	P value < 0.05, S/NS
Phenytoin 20mg/kg vs DMSO 1ml/kg	**-14.2**	**S*** **
Flupirtine 79mg/kg vs DMSO 1ml/kg	**-7.0**	**S***
Flupirtine 39mg/kg vs Flupirtine 79mg/kg	**9.5**	**S****
Flupirtine 39mg/kg vs DMSO 1ml/kg	**2.50**	**NS**
Flupirtine 79mg/kg vs Phenytoin 20 mg/kg	**7.17**	**S***

S=significant, NS=Not significant, S* = P<0.0001**

Fig -1: BAR DIAGRAM SHOWING MEAN DURATION OF HLTE IN SECONDS



Discussion

This study demonstrated that flupirtine at 79 mg/kg showed protection against MES induced seizure. In flupirtine high dose group 33% animals were protected from MES induced seizures and duration of HLTE was significantly reduced when compared to control and flupirtine lower dose (39 mg/kg). But the protection was not comparable with the standard phenytoin sodium.

Flupirtine (ethyl-N-[2-amino-6-(4-flurophenylmethyllamino) pyridine-3-yl] carbamate) has been in clinical use for many years as a centrally active analgesic with muscle relaxant properties. In preclinical and preliminary clinical studies neuroprotective, antiepileptic and antiparkinsonian effects were additionally found.[5] The mechanism of action of flupirtine has not been clear up to now. Although flupirtine does not have relevant affinity for any known recognition site on the NMDA receptor complex in binding studies,[6,7] antagonism of this receptor has recently been discussed at length as a possible mechanism of action of this compound.[8-12]

The profile of preclinical and clinical actions (analgesic, muscle relaxant, neuroprotective, antiepileptic and antiparkinsonian properties) suggests that the action of flupirtine is connected with the NMDA receptor.It has not been possible to convincingly demonstrate a direct action on the NMDA receptor to date. At a therapeutically relevant concentration, flupirtine activates neuronal inwardly rectifying G-protein-regulated K^+ channels. The spectrum of action of the available experimental K^+ channel openers, as far as they have been investigated to date, corresponds to that of flupirtine. These K^+ channel openers also display analgesic, neuroprotective and anticonvulsant properties.[5]

Flupirtine activates inwardly rectifying K^+ channels and thus stabilizes the resting membrane potential. The Mg^{++} block of the NMDA receptor remains in force; i.e. the NMDA receptor is indirectly inhibited. This mechanism provides an explanation for the analgesia, muscle relaxation and neuroprotection. The model on the mechanism of action of flupirtine presented here links neuronal K^+ channels with NMDA receptors via membrane excitability. This provides an understanding of the clinically observed profile of flupirtine's actions, with analgesic, muscle-relaxant and neuroprotective effects. Here in this study possible mechanism of anticonvulsant activity is by activation of inwardly rectifying K^+ channels along with indirect blockade of excitatory NMDA receptor.

Since flupirtine acts by blocking NMDA receptors prevent the exicitotoxicity induced by repetitive firing of neurons during episodes of seizures, it has been shown that flupirtine mainly acts on CNS K^+ channels compared to cardiac K^+ channels this may infer that flupirtine has less incidence of cardiac

arrhythmias and other peripheral side effects due to activation of K^+ channels may be minimal with flupirtine. But this study concentrated mainly on the acute models of seizures, the protection on long term administration of flupirtine in chronic models of epilepsy and PTZ model has to be determined.

But limitations of this study is, the study has been carried out only in one species of animals viz mice and needs to be extended to other animals as well, study has been done on MES model only, has to be evaluated in other models like PTZ, no attempt was made to establish exact mechanism of anticonvulsant activity.

Despite such a wide therapeutic armamentarium, it is currently estimated that about 30% of epileptic patients do not receive satisfactory treatment. Thus, to improve some of the well known limitations of current anticonvulsant treatment, one of the most ambitious goals in today‘s antiepileptic research is the identification of additional molecules targeting novel molecular mechanisms involved in neuronal excitability control. The one possible novel target may be neuronal K + channels. Flupirtine mainly acts through CNS K+ channels it can be considered new substance class, the selective neuronal potassium channel openers (SNEPCO).

To conclude flupirtine being cerebroselective K+ channel activator has shown some protection in MES induced seizures and also may prevent the excitotoxicity by blocking NMDA receptors. Flupirtine may be beneficial in patients with GTCS but data on its clinical efficacy in humans is lacking. Its efficacy in humans has to be proved with proper dose titration. Thus selective neuronal potassium channel openers (SNEPCO) have the potential to become novel target for developing newer antiepileptic dugs in future.

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Corresponding Author: Dr. Naveen Kumar. M, Post graduate,

Department of Pharmacology, J.J.M. Medical College, Davangere – 577004, Karnataka, India. E mail: dr.naveen.madalageri@gmail.com