

## ORIGINAL ARTICLE

# Anticonvulsant Action of Flupirtine and its Interaction with Antiepileptic Drugs in Rats

Dudhe BG<sup>1</sup>, Bhore AS<sup>2</sup>

1- Assistant Professor, 2- Professor & Head, Department of Pharmacology, Dr. Panjabrao Deshmukh Memorial Medical College, Amravati (MS), India

### Abstract

**Aims & objective:** To evaluate the effect of flupirtine in PTZ induced seizures in rats and study the interactions of flupirtine with some antiepileptic drugs, by using subtherapeutic doses.

**Methods & Material:** The effects were assessed by methods of chemoshock (pentylene tetrazol) seizures. **Results:** Flupirtine alone showed protection against chemoshock seizures. In chemoshock method combined treatment of flupirtine and diazepam exerted a much stronger protective effect than used alone. But, protection was not significant when sodium valproate, phenobarbitone, phenytoin were given together in subtherapeutic doses and compared with control, and addition of flupirtine and AED alone. **Conclusions:** Flupirtine has anticonvulsant activity and has synergistic activity with diazepam drugs in PTZA model. Extrapolation of these combinations in clinical practice is needed.

**Keywords:** Antiepileptic drugs, chemoshock, electroshock, Flupirtine

**Address for correspondence:** Dr. B. G. DUDHE, Assistant Professor, Department of Pharmacology, Dr. Panjabrao Deshmukh Memorial Medical College, Panchwati, Amravati (MS), India.

**E-mail:** [drbhushandudhe@gmail.com](mailto:drbhushandudhe@gmail.com) **Phone no:** +919970846747

Received on :27/07/2016 Revised :02/08/2016 Accepted : 03/08/2016

### Introduction

Epilepsy and Seizures are widely prevalent medical disorders all over in the world and there is a life time incidence of 1 – 3%.<sup>[1]</sup> The term seizure refers to transient alteration of behavior where as epilepsy means repeated occurrence of seizure that is periodic and unpredictable. It has important medical, social and psychological consequences. Around 50 million people worldwide have epilepsy. Majority (nearly 80%) of them are found in developing countries. Epilepsy is the most common neurological disorder in India. A recent report of prevalence of active epilepsy is 3.6-8/1000 that is nearly seven million people in India.<sup>[1, 2]</sup> The burden of epilepsy as estimated using the disability-adjusted life years (DALYs) accounts for 1% of the total burden of disease in the world, excluding that due to social stigma and isolation, that people with epilepsy (PWE) in India face.<sup>[26]</sup>

Despite the introduction of several new drugs in the 1990s, a significant fraction of the patients

with epilepsy continue to live with uncontrolled seizures.<sup>[3]</sup> There is no definite cure for epilepsy. Whatever drugs are available aim at achieving control attacks of epilepsy at an acceptable level of tolerable side effects. This greatly affects the quality of life of a patient in context with intellectual skills. There is still a need for an ideal antiepileptic agent with properties like broad spectrum activity, rapid onset of action, least side effects, and good oral bioavailability and low cost. The number of epileptologists and neurologists are very meager for rendering services to epilepsy patients in India.

Flupirtine is prototype of new class of drugs, the Selective Neuronal Potassium Channel Opener (SNEPCO). It is unique as a non opioid, non steroidal, non-NSAID analgesic. The present review addresses new and challenging data that have been published during recent years. In particular, it has been discovered that flupirtine, in a clinically relevant dosage range, has potent cytoprotective and neuroprotective potential as

well as anticonvulsant, myorelaxant and antiparkinsonian effects.<sup>[4]</sup>

So this study has been undertaken to evaluate the effect of flupirtine in Chemoshock seizure induced by pentylenetetrazol (PTZ) induced seizures and to study its interactions with currently used antiepileptic drug and also provide further information on neuropharmacological characterization of flupirtine in rats.

## Materials and Methods

### Drugs and Chemicals

Flupirtine {2-amino-6-[(4-fluorobenzyl) amino] pyridin-3-yl} carbamate is a prototype of new class of drugs, the selective neuronal potassium channel opener (SNEPCO). Capsule Flupirtine and Inj. Sodium Valproate purchased from Sun Pharmaceutical Ind. Ltd, Mumbai; Inj. Diazepam purchased from Ranbaxy Laboratories, Sikanderpur; Inj. Phenobarbitone purchased from Samarth Pharma Pvt Ltd. Mumbai; Inj. Phenytoin Sodium purchased from Vulcan Laboratories Pvt. Ltd. Kolkata; Pentylenetetrazole (PTZA) purchased from Himedia Labs. Pvt. Ltd, Mumbai; Dimethyl sulphoxide (DMSO) purchased from Sigma Aldrich, Mumbai. Flupirtine was dissolved in DMSO freshly and given intraperitoneally. Control group of animals received same volume of vehicle i.e. DMSO.<sup>[5]</sup> Other drugs used were injections of DMSO, diazepam, sodium valproate, phenobarbitone sodium, phenytoin sodium and pentylenetetrazol. Solutions of these drugs were prepared freshly in desired strength in water except for diazepam in which 1.5% v/v of 95% alcohol was added. All chemicals were of highest purity commercially available.

### Animals

The study protocol was approved by Institutional Animal Ethics Committee of registration number: 420/01/a/CPCSEA on date: 10/12/ 2012. Male albino rats weighing between 150-200 gm were procured from National Institute of Nutrition, Hyderabad. Rats were housed in colony cages with free access to food and water except 4 hours prior and during experiment and were maintained on natural light and dark cycle. The rats were randomly divided into multiple groups of 10 each (n=10) and convulsive tests were carried out between 12.00

to 14.00 hrs. Rats were repeated for convulsive tests after a gap of 7 days. Female rats were excluded to avoid variation in results because of hormonal effect. Female rats are known to eliminate several antiepileptic drugs less rapidly than male rats.<sup>[6]</sup> Drugs were injected intraperitoneally (I.P.) in a volume of 0.2ml/100 gms of rats in four groups of 10 rats each:

**Group I:** DMSO (control group)

**Group II:** Flupirtine alone (dissolved in DMSO)

**Group III:** Antiepileptic drug alone (D/V/B/P)

**Group IV:** Flupirtine + Antiepileptic drugs (D/V/B/P)

(D= Diazepam, V= Sodium Valproate, B= Phenobarbitone sodium, P= Phenytoin sodium)

For making groups for Chemoshock method (PTZA), we keep control and flupirtine group in common, make Diazepam, Sodium Valproate, Phenobarbitone sodium, Phenytoin sodium groups and make flupirtine + diazepam, Flupirtine + Sodium Valproate, Flupirtine + Phenobarbitone sodium, Flupirtine + Phenytoin sodium, hence there are in total 10 groups for chemoshock method.

To leave scope to assess potentiation as well as antagonism, all drugs were given in sub-therapeutic doses (showing antiepileptic response in 10-30% rats), which was decided by trial and error method. Convulsive tests were carried out by following methods 30 minutes after drug administrations. Sub-therapeutic dose of flupirtine by PTZA method was 18 mg/ kg.

### Chemoshock seizure induced by pentylenetetrazol (PTZ)

Seizure induced by chemo convulsant pentylenetetrazole is most useful in identifying drugs that are effective against absence seizures (petit mal epilepsy) in human. PTZ in a dose causing tonic-clonic convulsions in all animals without mortality (65 mg/kg) was injected i.p. and the animals were subsequently placed singly in cages and observed for tonic-clonic convulsions for a period of 30 minutes. Abolition of tonic clonic convulsions indicates protective (antiepileptic) effect of a drug.

**Statistical analysis:** Comparison of percentage protection was done by proportion test.<sup>[8, 9]</sup>  $p < 0.05$  was considered as statistical significance. Data was analyzed on STATA Statistical Software (Version 13.0).

## Results

With graded doses of PTZ (pentylenetetrazol) alone no convulsions were observed at a dose of 30, 40 and 50 mg/kg of PTZ. Convulsions were observed in 40% of animals at a dose of 60 mg/kg and 100% convulsions at a dose of 65 mg/kg without any mortality. We chose dose 65 mg/kg over 70 mg/kg due to less mortality.

Flupirtine in a fixed subtherapeutic dose of 18 mg / kg in combination with graded dose of PTZ showed no convulsions at 30, 40, 50 mg/kg. Convulsions were observed in 30% of animals at a dose of 60 mg/kg and 80% convulsions were observed at 65. [Table 1]

**Table 1:** Seizure producing effect of combination of **flupirtine**, with **pentylenetetrazol**

Flupirtine (mg/kg)	PTZ (mg/kg)	Animals convulsing (%)	Onset of convulsion (min)	Mortality
--	30	00	--	00
--	40	00	--	00
--	50	00	--	00
--	60	40	12.5	00
--	65	100	11	00
--	70	100	9.5	02
18	30	00	--	00
18	40	00	--	00
18	50	00	--	00
18	60	30	14.5	00
18	65	80	12.5	00
18	70	80	10	01

**Table 2:** Effect of antiepileptic drugs against pentylenetetrazol (PTZ) in chemoshock method.

Drugs	Dose mg/kg	Number of Animals in Group	Percentage of animals protected, showing abolition of tonic clonic phase										
			Group I	Group II	Group III	Group IV	Vs.		Vs.		Vs.		
							Group I	Group II	Group I	Group III	Group I	Group IV	Group IV
Flupirtine	18	10	0	20	-	-	0.136	-	-	-	-	-	-
Diazepam	0.5	10	0	20	30	50	0.136	0.060	0.009**	1			
Valproate	50	10	0	20	20	20	0.136	0.136	0.136	0.273			
Phenobarbitone	5	10	0	20	30	30	0.136	0.060	0.060	0.297			
Phenytoin	6	10	0	20	20	20	0.136	0.136	0.136	0.273			

\*p value < 0.05 is significant; \*\*p value < 0.01 is highly significant as compared to control group.

In chemoshock methods of convulsion rats show 20% protection with flupirtine alone and 30% protection with diazepam alone. However, the protection was highly significant i.e. 50% when both drugs were given together in subtherapeutic doses when compared with control (p=0.0098\*\*). Protection by combination of flupirtine+ diazepam was not significant when compared to addition of flupirtine and diazepam alone. Rats show 20% protection with sodium valproate alone. However, the protection was not significant i.e. 20% when both drugs (flupirtine and valproate)

were given together in subtherapeutic doses and compared with control, and addition of flupirtine and sodium valproate alone. In case of PTZA induced convulsion rats show 30% protection with phenobarbitone alone. However, the protection was not significant i.e. 30% when both drugs (flupirtine & phenobarbitone) were given together in subtherapeutic doses when compared with control, and addition of flupirtine and phenobarbitone alone. Rats show 20% protection with flupirtine alone and 20% protection with phenytoin alone. However, the

protection was not significant i.e. 20% when both drugs were given together in subtherapeutic doses when compared with control, and addition of Flupirtine and phenytoin alone. [Table 2]

## Discussion

Results of present study are in agreement with previous studies that demonstrate anticonvulsants activity of Flupirtine.<sup>[10-14]</sup> The present study was carried out to study anticonvulsant effect of Flupirtine by using animal model of parameter- PTZ induced convulsions in sub-therapeutic dose of 18mg/kg respectively. According to Jakovlev V (1985) the anticonvulsive activity of Flupirtine observed in PTZ shock test occurs after high doses, probably cannot be considered to occur within the analgesic dose range.<sup>[15]</sup>

Objectives of using combination of Flupirtine with other anticonvulsants are to achieve synergism, to reduce the duration and severity of suffering of patients from seizure, to broaden the spectrum of anticonvulsant activity and to reduce the incidence of adverse effects by using low doses of drugs.

Thus, in the present study, the anticonvulsant effect of Flupirtine alone and in combination with other established anticonvulsant drugs in subtherapeutic doses was investigated. Co-administration of flupirtine with carbamazepine is not advisable as carbamazepine induces hepatic enzymes.<sup>[16]</sup> Hence study of this combination was not done.

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,<sup>[17, 18]</sup> antagonism of this receptor has recently been discussed at length as a possible mechanism of action of this compound.<sup>[17-21]</sup> 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<sup>+</sup> channels. The spectrum of action of the available experimental K<sup>+</sup> channel

openers, as far as they have been investigated to date, corresponds to that of flupirtine. These K<sup>+</sup> channel openers also display analgesic, neuroprotective and anticonvulsant properties. Flupirtine activates inwardly rectifying K<sup>+</sup> channels and thus stabilizes the resting membrane potential. The Mg<sup>++</sup> block of the NMDA receptor remains in force; i.e. the NMDA receptor is indirectly inhibited. Here in this study possible mechanism of anticonvulsant activity could be by activation of inwardly rectifying K<sup>+</sup> channels along with indirect blockade of excitatory NMDA receptor.<sup>[22]</sup>

Bajrić M, et al. (2012) showed that Flupirtine (30 μM) modulated GABA-induced currents in hippocampal neurons by reducing EC50 values for GABA threefold and maximal current amplitudes by 15%.

These results indicated that Flupirtine exerts antiepileptic activity, modulates tonic, but not phasic, GABAergic inhibition and blocks Kv7 channels in hippocampal neurons, and affects GABA<sub>A</sub> receptors in a subunit-dependent manner.<sup>[13]</sup>

Anticonvulsant action of Flupirtine may in some way be related to GABA mediated inhibition and antiepileptic drugs potentiate GABA mediated responses i.e both Flupirtine and antiepileptic drugs share same mechanism. This may be taken possible explanation for potentiation of anticonvulsant action of benzodiazepines by Flupirtine. The clinical significance of such potentiation needs to be studied further.

Present study results are in accordance with study by Naveen Kumar M, et al. (2011). He studied the anticonvulsant action of flupirtine, in comparison with phenytoin and DMSO as a control in albino mice by using MES method. Animal group with Flupirtine 79 mg/kg/po gives 33% protection in MES induces convulsions.

Unlike CNS depression, Flupirtine does not produce clinically significant respiratory or cardiovascular depression.<sup>[13]</sup> Flupirtine is free from abuse potential,<sup>[23]</sup> doesn't impair psychomotor performance in humans<sup>[30]</sup> and is antioxidant and Neuroprotective.<sup>[25]</sup>

Flupirtine alone showed anticonvulsant action in MES method. Flupirtine along with diazepam, in sub-therapeutic doses exerted significant protection against seizure induced by PTZ method. Significant anticonvulsant activity of

subtherapeutic doses of flupirtine with sodium valproate, phenobarbitone, and phenytoin was not seen when both drugs were used in subtherapeutic doses in PTZ method. So this combination is not likely to have clinical significance in absence seizures.

## Conclusion

Flupirtine thus seems to be capable of synergistic anticonvulsant with Diazepam against PTZ induced chemo convulsion in rats. Extrapolation of these combinations in clinical practice may suggest its utility in absence seizures as add-on drug. Conducting clinical trials in above different subsets will reveal more details.

## Acknowledgment

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

**Conflict of Interest:** None declared

**Source of Support:** Nil

**Ethical Permission:** Obtained

## References

1. Chopra JS, Sawhney IMS. Epilepsy in tropics. In Arturo.,Carpio, P R M.& de Bittencourt, editor. Neurology in tropics, New Delhi: BI Churchill Livingstone. Pvt. Ltd Publication;1999, 527-31.
2. Reddy DS. Pharmacotherapy of catamenial epilepsy. Indian J Pharmacol 2005; 37:288-93.
3. White HS. Preclinical development of antiepileptic drugs: Past, present and future directions. Epilepsia 2003; 44:2-8.
4. Kornhuber J, Bleich S, Wiltfang J, maler M and Parsons CG. Flupirtine shows functional NMDA receptor antagonism by enhancing Mg<sup>2+</sup> block via activation of voltage independent potassium channels. J Neural Transm 1999;106:857-67.
5. Vogel GH, Vogel WH. Antiepileptic activity. Hans Gerhard Vogel editor. Drug discovery and evaluation: Safety and Pharmacokinetic Assay, 2<sup>nd</sup> edition. Heidelberg: Springer Publishers; 2002.p.459-93.
6. Gupta YK, Malhotra J, George B, Kulkarni SK. Methods and considerations for experimental evaluation of antiepileptic drugs. Indian J Physiol Pharmacol 1999;43:25-43.
7. Swinyard EA, Brown WC, Goodman LS. Comparative assay of antiepileptic drugs in mice and rats. J. Pharmacol Exp Ther 1952; 106:319-30.
8. Hill AB, Hill ID. Principles of Medical Statistics, 12<sup>th</sup> ed. New Delhi: B.I. Publication Pvt Ltd; 1993.p.12-22.
9. Sunder Rao PSS, Richard J. Introduction to biostatistics and research methods, 4<sup>th</sup>ed. New Delhi: Prientes Hall of India Pvt Ltd; 2006.p.40-50.
10. Naveen Kumar M, Gokul CG, Somashekar HS, Adake P, AcharyaA,Santhosh R *et al.* Anticonvulsant activity of flupirtine in albino mice. Pharmacologyonline 2011;3:860-67.
11. Raol YH, Lapidés DA, Keating JG, Brooks-Kayal AR, Cooper EC. A KCNQ channel opener for experimental neonatal seizures and status epilepticus. Ann Neurol 2009;65:326-36.
12. Marylou VS, Adrian R, Steven LW, George FK. Activators of potassium M currents have anticonvulsant actions in two rat models of encephalitis. Eur J Pharmacol 2007;555:23-29.
13. Bajrić M, Klinger F, Schandl U, Kubista H, Boehm S. Antiepileptic activity and subtype-selective action of flupirtine at GABA<sub>A</sub> receptors. BMC Pharmacology and Toxicology 2012;13(Suppl 1): A90, Available at: <http://www.biomedcentral.com/2050-6511/13/S1/A90>
14. Jaideep Kapur. M Current-Based Therapies for Nerve Agent Seizures. University of Virginia, Department of Neurology, Charlottesville 2011, Form Approved OMB No. 0704.
15. Jakovlev V, Achterrath-Tuckermann U, von Svhlitegroll A. General pharmacologic studies on the analgesic flupirtine. Arzneimittel forschung 1985;35:44-5.
16. Harish S, Bhuvana K, Girish M, Bengalorkar K. Flupirtine: Clinical pharmacology. J Anaesthesiol Clin Pharmacol 2012;28:172-77.
17. Perovic S, Schleger C, Pergande G, Iskric I, Ushijima H, Rytic P, *et al.* The triamino pyridine flupirtine prevents cell death in rat cortical cells induced by Nmethyl-D-aspartate and gp 120 of HIV-1. Eur J Pharmacol Mol Pharmacol 1994;288:27-33.
18. Block F, Schwarz M, Pergande G. N-methyl-D-aspartate (NMDA)-mediated muscle relaxant action of flupirtine in rats. Neuroreport 1993;4:941-3.
19. Osborne NN, Pergande G, Block F, Schwarz M. Immunohistochemical evidence for flupirtine acting as an antagonist on the N-methyl-D-aspartate and homocysteic acid induced release of GABA in the rabbit retina. Brain Res 1994;667:291-94.
20. Schwarz M, Schmitt T, Pergande G, Block F. N-methyl-D-aspartate and  $\alpha$  adrenergic mechanism are involved in the depressant action of flupirtine on spinal reflexes in rats. Eur J Pharmacol 1995;276:247-55.
21. Timmann D, Plummer C, Schwarz M, Diener HC. Influence of flupirtine on human lower limb reflexes. Electroencephalogr Clin Neurophysiol.1995;97:184-88.
22. Banerjee TK, Ray BK, Das SK, Hazra A, Ghosal MK, Chaudhuri A, *et al.* A longitudinal study of epilepsy in Kolkota, India. Epilepsia 2010; 51:2384-91.
23. Sofia RD, Diamantis W, Gordon R. Abuse potential and physical dependence liability studies with flupirtine maleate in laboratory animals. Postgrad medical journal 1987; 63:35-40.
24. Muller LW. The effect of a new analgesic flupirtine on psychomotor performance in humans. Arzneimittel forschung 1985;35:1089-92.
25. Fawcett RJ, Osborne NN. Flupirtine attenuates sodium nitroprusside-induced damage to retinal photoreceptors, in situ. Brain Res Bull 2007;73:278-88.
26. Jain S, Satishchandra P. Organization of health care in different countries. India. In: Engel J, Pedley TA, Aicardi J (Eds). Epilepsy: A Comprehensive Textbook. Philadelphia: Lippincott Williams & Wilkins; 1998.pp.2885-9.