# EFFECT OF ONDANSETRON ON CONVULSIONS AND

# ITS INTERACTIONS WITH PHENYTOIN.

[1] **G.J. Puthuran, [2]P.A. Patil*, [2]Suneel.I.Majagi**

[1]KLE College of Pharmacy, Belgaum-590010, [2]Department of Pharmacology, J.N. Medical College, Nehru Nagar, Belgaum-590010. Karnataka- India.

## Summary

The present study was planned to elicit the anticonvulsant activity of ondansetron in maximum electroshock seizure (MES), minimum electroshock threshold (MET) and phenylenetetrazol (PTZ) induced seizures. For interaction studies subanticonvulsant dose of phenytoin was combined with ondansetron in MES induced seizures.

Findings of the present study show that ondansetron has a anti-seizure activity on MET and PTZ models of convulsion but not in MES model. However it potentiated the anticonvulsant activity of phenytoin in MES models.

The anticonvulsant activity showed by ondansetron, possibly could be due to inhibition of $Na^+$ and $K^+$ conductance mediated through 5-HT$_3$ receptors and enhanced release of NE in cerebellum and cortex.

Use of ondansetron as an add on anticonvulsant, needs to be clinically confirmed.

**Key words:** Convulsions, Diazepam, Interaction, Ondansetron, Phenytoin.

*Corresponding author: Dr. P.A.Patil., Professor,

Department of Pharmacology, J.N. Medical College,

Nehru Nagar, Belgaum-590010. Karnataka- India.

E-mail: drpapatil@yahoo.co.in

Phone: 0831-24091828,Fax: 08312470759.

## Introduction

Epilepsy is the most common neurological disorder characterised by sudden, transient alteration of brain function usually with motor, sensory, autonomic or psychic symptoms often accompanied by loss of, or altered consciousness[1].

In spite of intensive investigations, the pathophysiology of epilepsy is poorly understood. Studies with various animal models have provided ample evidence for heterogeneity in the mechanisms of epileptogenesis[2].Logical biochemical hypothesis anomaly is epilepsy could be decreased activity of inhibitory GABAergic sytem and/or increased activity of excitatory amino acids(glutamate and aspartate system) in epilepsy[3].

Till today, the drug therapy of epilepsy is not satisfactory probably due to incomplete understanding of exact biochemical anomaly for its pathogenesis. Presently available antiepileptic drugs are unable to control seizures effectively in as many as 25% of the patients[4].Limitations with the conventional antiepileptic drugs highlight the need for developing newer agents for epilepsies. Apart from the excitatory and inhibitory amino acids, prostaglandins[5,6] and female sex hormones[7,8] have also been implicated with seizure disorder. Similarly serotonin which acts on its several receptors (5 $HT_1$-5 $HT_7$) has been proposed to play inhibitory role in audigenic seizures[9] and is also implicated with behavioural functions, sleep wakefulness cycle, aggression, impulsivity, anxiety and depression etc[10].If so, 5 HT blockers could be expected to possess proconvulsant activity.

Moreover, ondansetron, a 5 HT $_3$ receptor blocker has been reported to suppress GABA activated current and induce seizure in experimental animals[11], indicating its proconvulsant activity. On the other hand ondansetron has been reported to possess anxiolytic [12] and local anaesthetic [13] action. Since, local anaesthetic activity is shared by some commonly used antiepileptic drugs, ondansetron could also possess anticonvulsant activity. Accordingly it has been shown to elevate MET (minimum electric threshold) suggesting its potential anticonvulsant action and involvement of 5-$HT_3$ receptors in the genesis of seizures [14]. However there is paucity of information about its effect on MES (maximum electroshock seizures).

In view of these controversial reports, the present study was undertaken to find out the possible anticonvulsant activity of ondansetron in different seizure models, viz., maximum electroshock seizures (MES), minimum electroshock threshold (MET) and pentylenetetrazole (PTZ) induced convulsions in male Wistar rats. Since there is paucity of information regarding interaction of ondansetron with commonly used anticonvulsants like phenytoin, it was also planned to elicit the possible interaction between them.

## Materials and methods

**Animals:** The complete course of experiments were carried out using healthy male rats of Wistar strain, weighing between 100-150 grams. The animals were acclimatized to normal laboratory conditions with 12-hr natural light-dark cycle and were maintained on standard laboratory diet with free access to water.

**Drugs used and their doses:** The drugs used were ondansetron as the investigational drug and diazepam and phenytoin as standard anticonvulsant drugs. The clinical doses of the drugs were converted into rat equivalent doses with the help of converting table [15] and the doses were 2.9 mg/kg for ondansetron (Emset,Cipla Ltd), 1.35 mg/kg for diazepam (Campose, Ranbaxy Laboratories Ltd) and 25 mg/kg for phenytoin (Dilandin, Cadila Healthcare Ltd) and purchased from local market. Ondansetron and diazepam were given one hour prior, while phenytoin was given intraperitoneally two hours prior to the experiment. To study drug interactions, sub-anticonvulsant dose of phenytoin was determined by trying different doses and the dose just failed to provide significant protection against MES was taken as sub-anticonvulsant dose and was found to be 12.5 mg/kg.

**Maximum Electricshock Seizures(MES)**[16]**:** MES were induced as described by Toman etal.(1946) with an alternating current of 150 mA delivered through the ear clip electrode for 0.2 seconds with help of convulsiometer. Only the animals which showed hind limb extension (HLE) during screening procedure on the earlier day were included in the study and absence of HLE was taken as protection against seizures.

**Minimum Electroshock Threshold (MET)**[17]**:** The experiments were carried out as per method described earlier by swinyard etal(1952) with slight modification in initial stimulus. The METs were determined by giving the shock with 0.25 mA current through ear electrodes, with an increment of 0.01 mA for a duration of 0.2 seconds and the appearance of head jerk was taken as indication of convulsion. The animals with baseline threshold (appearance of head jerk) ranging between 0.25 mA and 0.5 mA were included in the study.

**Pentylenetetrazol(PTZ) induced seizures**[18]**:** In the present study the chemical convulsant, PTZ was used in the dose of 50 mg/kg to induce convulsions, as described earlier by Louis etal (1982).Control animals within 30 min developed a sequence of excitement, myoclonic jerks, clonic seizures (some times leading to death). The anticonvulsant efficacy is determined by noting the number of animals protected from seizures, number of seizures, duration of seizure and delay in onset of seizures.

All the procedures were performed in accordance with the CPCSEA guidelines and the study was approved by IAEC.

**Statistical Analysis**: Data were expressed as Mean ± SEM. Significant protection against seizures in various treated groups in contrast to control was assessed by Fisher's exact test in MES, by ANOVA followed by Dunnet's post-hoc test in case of MET and by ANOVA followed by Dunnet's test PTZ induced seizures. $p \leq 0.05$ was taken as significant.

## Results

**MES studies:** Pretreatment with phenytoin alone (25 mg/kg) and combined (12.5 mg/kg) with ondansetron (2.9 mg/kg) provided significant ($p<0.001$) protection (100% and 66.6% respectively) against MES (Table I).

**MET studies:** Pre treatment with diazepam (1.35 mg/kg) and ondansetron (2.9 mg/kg) individually showed significant ($p<0.001$) protection against the seizures, at 30,60 and 90 minutes (Table II).

**PTZ induced seizures:** Diazepam (1.35 mg/kg) treatment showed significant (p<0.01) reduction in number of convulsions and significant (p<0.001) reduction in duration of seizures as well as significantly (p<0.001) delayed the onset of seizures. Ondansetron (2.9 mg/kg) treatment showed significant (p<0.001) reduction in duration of seizures as well as significantly (p<0.001) delayed the onset of seizures (Table III).

**Table I: Effect of ondansetron and phenytoin on MES.**

| Groups | Drugs and Dose mg/kg | Number of animals protected | % protection |
|---|---|---|---|
| 1(n=6) | Normal saline | 0 | -- |
| 2(n=6) | Phenytoin 12.5 | 0 | -- |
| 3(n=6) | Phenytoin 25 | 6* | 100 |
| 4(n=6) | Ondansetron 2.9 | 0 | -- |
| 5(n=12) | Phenytoin 12.5 with Ondansetron 2.9 | 8* | 66.6 |

Fisher's exact test, p<0.001*.

**Table II: Effect of ondansetron and diazepam on MET.**

| Groups (n=6) | Treatment groups Drugs and Dose mg/kg | MET (mA) Mean± S.E.M | | | |
|---|---|---|---|---|---|
| | | 0 minute | 30 minutes | 60 minutes | 90 minutes |
| 1 | Normal Saline 5 | 0.25±0.000 | 0.25±0.000 | 0.25±0.000 | 0.25±0.000 |
| 2 | Diazepam 1.35 | 0.25±0.000 | 0.63±0.036* | 0.63±0.036* | 0.65±0.022* |
| 3 | Ondansetron 2.9 | 0.25±0.000 | 0.49±0.053* | 0.58±0.016* | 0.55±0.020* |

ANOVA followed by Dunnet's post-hoc test, p<0.001*.

**Table III: Effect of various treatments on PTZ induced convulsions.**

| Treatment groups(mg/kg) (n=6 in each) | Number of convulsions | Durations of convulsions (sec) | Latency for convulsion (min) |
|---|---|---|---|
| | Mean ± SEM | | |
| Control | 2.30±0.22 | 197.50±18.73 | 8.66±2.33 |
| Diazepam 1.35 | 1.33±0.21* | 28.33±4.00** | 38.83±2.92** |
| Ondansetron 2.9 | 2.01±0.01 | 54.33±13.83** | 28.00±2.84** |

ANOVA followed by Dunnets,  $p < 0.01$* and $p < 0.001$**.

## Discussion

In the present study ondansetron was investigated for its influence on different seizure models in view of controversial reports regarding its effect on seizures. The results of the present studies clearly indicate that ondansetron has significantly elevated MET, which was almost comparable to that of diazepam. This observation of the present study confirms the earlier report that ondansetron in the dose of 3 mg/kg significantly elevated MET [14].

As expected phenytoin provided complete protection against MES, while ondansetron alone in the dose of 2.9 mg/kg though ineffective in providing protection against HLE, provided significant protection when coadministered with subanticonvulsant dose phenytoin (12.5 mg/kg). This observation of potentiation of anticonvulsant activity of phenytoin by ondansetron agrees with an earlier report [19].

In PTZ induced seizure model ondansetron significantly delayed the time for onset of seizures, decreased the duration of seizures similar to diazepam and decreased mortality as compared to saline treated group. However it failed to decrease the number of convulsions significantly. Information regarding the effect of ondansetron on PTZ induced convulsion could not be traced in the available literature.

It is difficult to propose the mechanism of antiseizure acitivity of ondansetron based on the findings of the present study. However based on the earlier literature about 5-HT and ondansetron several mechanisms can be postulated.

It is well known that 5-HT$_3$ receptors are legand gated channels for sodium and potassium cations and are widely distributed in CNS. Activation of these receptors leads to development of fast depolarisation by enhancing sodium and potassium conductance. Blockade of 5-HT$_3$ by ondansetron therefore can result in attenuation of sodium and potassium conductance thereby producing anti-seizure activity.

5-HT$_3$ receptors in the CNS have also been said to be involved in regulation of neurotransmitter release[20].Activation of 5-HT$_3$ receptors leads to decreased release of norepinephrine(NE) and its blockade enhances the release of NE[20].Anti-seizure (seizure attenuation) activity of NE in a wide variety of experimental models of epilepsy including genetically epilepsy prone rats(GEPR) is well established[21].This was supported by the fact that reserpine reduced the threshold for minimal electroshock seizures with parallel decrease in brain concentration of NE and other amines[22].Anti-seizure activity of NE could be due to enhanced inhibitory synaptic mechanisms in rat cerebellum and cerebral cortex[23]. Ondansetron, by blocking 5-HT$_3$ receptors could augment the release of NE, which contribute for its observed anticonvulsant activity, if not totally at least partially. However estimation of brain NE (which was not done in the present study) could have helped to establish the role of noradrenaline in antiseizure activity of ondnasetron.

The synergistic interaction between phenytoin and ondansetron appears to be pharmacodynamic rather than pharmacokinetic. Proposed mechanism of interaction include the membrane stabilising activity of phenytoin augmented by ondansetron through blockade of 5-HT$_3$ receptors, resulting in to decrease sodium and potassium conductance and synaptic inhibitory mechanisms mediated through ondansetron induced augmented release of NE[23].

The result of the present experimental study in rats, if could be extrapolated to man, suggest that, ondansetron could be a useful add on drug for the treatment of epilepsy. However further clinical trials can only say the final word in this regard.

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