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Original Research Article

Evaluation of the seizure modifying potential of ondansetron in mice

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ABSTRACT

Background: The aim of the study was to evaluate the seizure modifying potential of Ondansetron in experimental models of seizures in mice.
Methods: Mice were treated with three different doses of ondansetron i.p., at 3mg/kg, 6mg/kg and 8mg/kg and control group received normal saline 0.1 ml i.p. for 3 days. On 3rd day, mice were subjected to MES, of different strength half an hour after ondansetron administration and findings were recorded. The minimum threshold current at which tonic hind limb extension occurred was recorded. Each animal was observed for incidence and duration of tonic hind limb extension and the strength of current was noted. In PTZ model, mice were subjected to subconvulsive dose of PTZ 45mg/kg and convulsive dose of PTZ 60mg/kg. The incidence and onset of convulsion at 45 and 60mg/kg dose of PTZ were recorded.
Results: Mice receiving ondansetron 3mg/kg, showed significant decrease in duration of tonic hind limb extension at convulsive current strength of 50mA ($p<0.001$). While group receiving 6mg/kg, showed decrease in seizure threshold. (40mA current strength) Mice receiving 3mg/kg, showed significant increase in onset of seizures ($p<0.001$) at convulsive 60mg/kg dose of PTZ. While mice receiving 6mg/kg showed decrease in seizure threshold at sub convulsive 45mg/kg dose of PTZ. Group receiving 8mg/kg ondansetron, showed 100% mortality due to convulsions caused by ondansetron.
Conclusions: Ondansetron at low therapeutic dose (3mg/kg) has an anticonvulsant action, while it has a proconvulsant action at a high therapeutic dose (6mg/kg). Ondansetron causes convulsions at toxic dose (8mg/kg). So, care should be taken while giving ondansettron in high doses to prevent chemotherapy induced emesis.

Keywords: Anticonvulsant, Ondansetron, Proconvulsant, Seizures

INTRODUCTION

The use of ondansetron, a selective serotonin 5-HT_3 receptor antagonist, is well established in patients with nausea and vomiting associated with cancer chemotherapy, radiotherapy or anesthesia and surgery.[1] The wide distribution of 5-HT_3 receptors in the body and the role of these receptors in various pathologies have provided the rationale for investigation of ondansetron for novel applications.

Ondansetron has also shown effectiveness in certain pain or CNS-related disorders [e.g. alcohol (ethanol) dependence, opiate withdrawal, vertigo, cerebellar tremor and Parkinson's disease treatment-related psychosis].[2] In contrast to conventional antiemetics, ondansetron is generally well tolerated with a lower incidence of sedation and only isolated case reports of extrapyramidal reactions.[3] 5HT is a CNS neurotransmitter which is probably involved in sleep, temp., mood regulation, behavior and cognitive functions. But recent evidence from experimental animal studies suggest that $5HT_3$ antagonist activity of ondansetron has controversial effect on seizure activity. Several lines of evidence suggest 5HT as a modulator of seizure activity with anticonvulsant effect.[4,5] However,

there are few case reports showing epileptic seizures can follow intravenous infusion of ondansetron.[6,7]

The important role of 5HT in modulation of seizure threshold raises the hypothesis that ondansetron may affect the seizure susceptibility through antagonist activity on 5 HT_3 receptors. Hence in the present study we intend to examine the possible effect of different doses of ondansetron on seizure susceptibility in experimental model of seizure. This research could provide new insight into the pharmacological role of the 5-HT_3 receptor antagonist in modulating seizures, which could in turn divulge this branch in antiepileptic drug studies.

Aims and objectives

- To study the proconvulsant and anticonvulsant action of ondansetron in experimental seizures models in mice.
- To evaluate whether these effects if observed are dose dependent or not.

METHODS

Animals

There were 48 Adult male Swiss Albino mice (Haffikine Institute, Mumbai), aged 6-8 weeks and weighing 20-25g were used. If needed the animals were reused in the study after giving adequate washout period of 15 days.

The animals were housed in a temperature-controlled (around 24ºC) colony room. Six animals were accommodated in polypropylene cages with grill on top. They were maintained in a 12-hrs on and 12-hrs off light/dark schedule with free access to food and water, except during experimental procedures. After one week period of acclimatization in animal laboratory at room temperature, the study was started. Animals' body weights were recorded on the first day of initiation of study. The experiments and procedure were approved by Institutional Animal Ethics Committee. All the procedures were carried out in accordance to standard guidelines of CPCSEA.

Drugs

Ondansetron infusion (Emset, 4ml) was purchased from local pharmacy. Each 1ml contains 2mg of ondansetron hydrochloride. The study was done with the intension to judge the clinical implication of ondansetron.

Therefore, the doses used for study was extrapolated from human dose and with the reference to previous study.[8,9] Ondansetron was used in 3 doses of 3, 6 and 8mg/kg.

The drug was administered by the intraperitoneal route (i.p.), for 3days and 30 minutes prior to the test on the third day. PTZ powder was obtained from Sigma chemicals. PTZ powder was dissolved in normal saline for i.p. injection.

Experimental seizure models[10,11]

Electroshock induced seizures

Electroconvulsiometer manufactured by Bhushan electronic was used for the study. The electroconvulsiometer was calibrated and its output checked. In this model, electrical stimulation was applied via ear electrodes with a stimulator that delivers constant current. Animals were screened twenty four hours before the study for convulsion. The ears were cleaned with spirit to remove any oil film due to sebaceous gland secretions on the skin of the ear and then with saline for electric contact.

Chemical induced seizures

In this PTZ is used for induction of convulsions. PTZ produces generalized clonic movements followed by tonic convulsions. In this model PTZ was administered by i.p route half hour after ondansetron administration. The study was carried out in three phases:

Establishment of model

Total 20 mice were used to find out the strength of current and the dose of PTZ required to produce convulsions in the mice. So, authors have started with a current of strength 30mA for 0.2 sec and then go on increasing to 40mA and 50mA till the mice got convulsion without mortality. Similarly, in PTZ induced convulsions the animals were challenged to different doses of PTZ.

Proconvulsant action[12,13]

MES model

In this model, total 24 mice were used. They were distributed in 4 groups of 6 animals each. The control group received normal saline 0.1ml i.p. for 3 days. Other groups received ondansetron 3mg/kg, 6mg/kg and 8mg/kg respectively for 3 days. On the third day, half hour after drug administration the animals were subjected to MES induced convulsions. The animals were challenged with subconvulsive current strength of 40mA and the findings were recorded. Each animal was observed for,

- Occurrence of convulsions (based on tonic hind limb extension).
- The parameters measured were
  - Duration of flexion
  - Duration of extension
  - Whether convulsion was followed by recovery or death.

PTZ model

In this model, total 24 mice distributed in 4 groups of 6 animals each were used. The control group received

normal saline 1ml i.p. for 3 days. Other groups received ondansetron 3mg/kg, 6mg/kg and 8mg/kg respectively for 3 days. On the third day, half hour after drug administration the animals were subjected to PTZ induced convulsions. The subconvulsive dose of PTZ i.e. 45mg/kg was used. Each animal was observed for:

- Occurrence of convulsions (based on clonic hind limb flexion).
- The parameters measured were
  - Onset of jerks,
  - Onset of seizures
  - Whether convulsion was followed by recovery or death.

Anticonvulsant action[10,11]

In this total 24 mice were used. The 12 animals received 3mg/kg ondansetron i.p. for 3 days and 12 animals were used as control group receiving normal saline 0.1ml i.p. On the third day, half hour after ondansetron administration the animals were subjected to convulsions induced by MES and PTZ methods.

6 animals receiving ondansetron 3mg/kg and 6 animals in control group receiving normal saline for 3 days were challenged to convulsive current strength of 50mA and the findings were recorded. The parameters measured were

- Duration of flexion
- Duration of extension
- Recovery or death occurred in experimental group.

There were 6 animals receiving 3mg/kg ondansetron and 6 mice in control group receiving normal saline for 3 days were subjected to convulsive dose of PTZ of 60mg/kg and the findings were recorded. The parameters measured were:

- Onset of jerks
- Onset of seizures
- Recovery or death.

Statistical analysis

The primary endpoints, presence or absence of convulsion, is considered as a nominal data. Comparison of mean duration of various phases of seizures in seconds in various groups was done by one way analysis of variance (ANOVA) test followed by Tukey-Kramer post hoc test for multiple comparison. P values <0.05 were considered as significant.

RESULTS

Establishment of model

In this current strength of 30, 40 and 50mA were used and the findings were as described in Table 1.

Table 1: MES model results.

Current (mA)	30	40	50
N	6	6	6
No. of animals with convulsion	0	0	6
Duration of flexion (sec)	-	-	2.74±0.22
Duration of extension (sec)	-	-	16.47±0.23
Death	0	0	1

Based on these findings 50mA current strength was selected as convulsive strength and 40mA current was decided to use as sub convulsive strength in phase II and III.

In this PTZ dose of 45mg/kg and 60mg/kg were used and the findings were as mentioned in Table 2.

Table 2: PTZ Model results.

Dose of PTZ (mg/kg)	45	60
N	6	6
No. of animals with convulsions	0	6
Onset of jerks (sec)	-	144.97±1.21
Onset of seizures (sec)	-	318.89±2.01
Death	0	1

Based on these findings 60mg/kg of PTZ was selected as convulsive dose and 45mg/kg dose of PTZ was decided to use as sub convulsive dose in phase II and III.

Proconvulsant action

Authors have decided to carry out proconvulsant action in groups receiving ondansetron 3, 6 and 8mg/kg for three days. It was observed that in the pre-treatment of the animals with ondansetron for 3 days, the group receiving 8mg/kg showed 100% mortality due to convulsions caused by high dose of ondansetron itself on the 1st day of experiment.

Table 3: Convulsant behaviour of mice receiving ondansetron 8mg/kg (n=6).

	Onset of convulsions(mins)	Death (in mins)
Mean±SE	10.29±0.31	10.47±0.31

In MES model, to evaluate the proconvulsant potential, the mice were challenged to sub convulsive current strength of 40mA. The findings were as described in Table 4.

Also, the findings in the group receiving 6mg/kg and subconvulsant current were compared with the control group receiving normal saline and convulsant current. The results were as described in Table 5.

Table 4: Proconvulsant action of ondansetron using MES Model.

Groups	Control (NS)	Ondansetron 3mg/kg	Ondansetron 6mg/kg	Ondansetron 8mg/kg
N	6	6	6	6
Current strength (mA)	40	40	40	-
Observation period (days)	3	3	3	1
No. of animals with convulsion	0	0	6	6
Duration of flexion (sec)	-	-	3.42±0.25	-
Duration of extension (sec)	-	-	17.56±0.54	-
Death	0	0	3	6

Table 5: Results in MES Model.

Groups	Control group	Ondansetron 6mg/kg
N	6	6
Current strength (mA)	50	40
No. of animals with convulsion	6	6
Duration of flexion	2.74±0.22	3.42±0.25
Duration of extension	16.47±0.23	17.56±0.54
Death	1	3

P value is >0.05, not significant

In PTZ model method, the animals in the control group and the groups receiving ondansetron 3mg/kg and6mg/kg were subjected to sub convulsive dose of PTZ of 45mg/kg. The group receiving 8mg/kg of ondansetron was not included because of 100% mortality as observed in previous model. The readings were as described in Table 6.

Table 6: Proconvulsant action of ondansetron using PTZ Model.

Groups	Control (NS)	Ondansetron 3mg/kg	Ondansetron 6mg/kg
N	6	6	6
PTZ dose (mg/kg)	45	45	45
Observation period (days)	3	3	3
No. of animals with convulsions	0	0	6
Onset of jerks (sec)	-	-	144.59±1.17
Onset of seizures (sec)	-	-	321.18±2.37
Death	0	0	3

Also, the findings in the group receiving 6mg/kg and subconvulsant dose were compared with the control group receiving normal saline and convulsant dose. The results were as described in Table 7.

Table 7: Results in PTZ model.

Groups	Control (NS)	Ondansetron 6mg/kg
N	6	6
PTZ dose (mg/kg)	60	45
No. of animals with convulsions	6	6
Onset of jerks (sec)	144.97±1.21	144.59±1.17
Onset of seizures (sec)	318.89±2.01	321.18±2.37
Death	1	3

P value is >0.05, not significant

Anticonvulsant action

In MES model the control group and the animals receiving ondansetron 3mg/kg were challenged to convulsive current strength of 50mA. The findings were as described in Table 8.

Table 8: Anticonvulsant action of ondansetron using MES Model.

Groups	Control	Ondansetron 3mg/kg
N	6	6
Current (mA)	50	50
Observation period (days)	3	3
No. of animals with convulsions	6	6
Duration of flexion (sec)	2.74±0.22	1.69±0.15
Duration of extension (sec)	16.47±0.23	10.77±0.50*
Death	1	0

*indicates p value<0.001

In PTZ model the control group and the animals receiving ondansetron 3mg/kg were challenged to convulsive dose

of PTZ i.e.60mg/kg and the findings were as described in Table 9.

Table 9: Anticonvulsant action of ondansetron using PTZ Model.

Groups	Control (NS)	Ondansetron 3mg/kg
N	6	6
PTZ dose (mg/kg)	60	60
Observation period (days)	3	3
No. of animals with convulsions	6	6
Onset of jerks (sec)	144.97±1.21	255.4±2.36*
Onset of seizures (sec)	318.30±2.02	676.51±2.56*
Death	1	0

*indicates p value<0.001

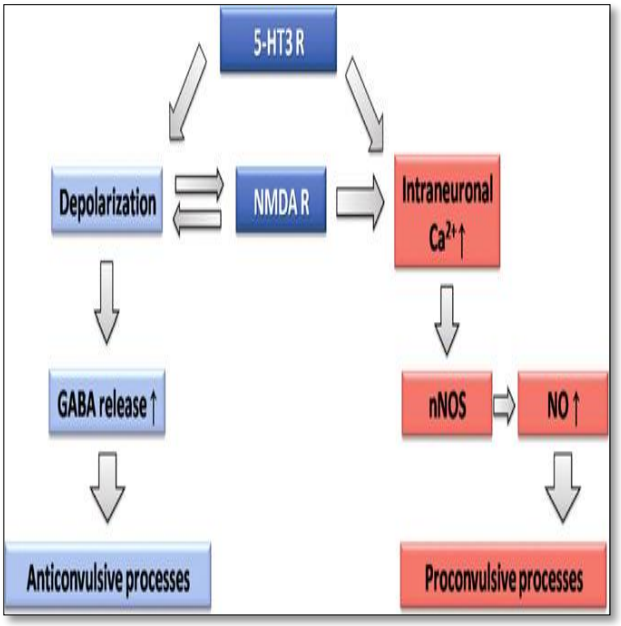

Figure 1: Dual action of 5HT3 receptor. Blocking this receptor ondansetron also has both anticonvulsant and proconvulsant action.[16]

DISCUSSION

Ondansetron is commonly used antiemetic in paediatric as well as in general population. The use of ondansetron, a selective serotonin 5-HT3 receptor antagonist, is well established in patients with nausea and vomiting associated with radiotherapy or anaesthesia[1]. In cancer chemotherapy especially caused by highly emetogenic drugs such as cisplatin it is considered a gold standard.[14]

Commonly seen side effects include constipation or diarrhoea, headache and dizziness.[1] Regarding ondansetron and seizure association the animal data is not adequate. There are some animal studies which claimed the anticonvulsant behaviour of ondansetron, while case reports are available which are in favour of proconvulsant action of ondansetron.[4-7]

Ill today no certain answer is available for ondansetron and seizure association. So, authors have decided to carry out the study which can throw light on this aspect. Our study aimed to clarify and reconfirm the influence of ondansetron on seizure threshold.

In this study, the dosages of ondansetron that authors have selected are extrapolated from human dosage. As authors want to study the clinical implication of ondansetron, authors chosen the ondansetron dosages which are in clinical use. authors have also selected the higher dose, as we want to study the CNS effect produced by ondansetron poisoning. In this study, we used two experimental seizure models.[10,11]

- Electroshock induced seizures
- Chemical induced seizures: PTZ induced

Authors have carried out the experiment in 3 phases. The phase I part is for establishment of model for both proconvulsant and anticonvulsant action in this experimental set up. In this, we found the convulsive current strength in case of MES induced seizure model is 50mA while in case of PTZ induced seizure model the convulsive dose is 60mg/kg of PTZ. Therefore, 40mA and 45mg/kg were decided to be used as subconvulsive strength in MES and PTZ induced seizure model in phase II and III respectively (Table 1and 2).

In phase II, authors have pre-treated the animals with ondansetron in doses of 3, 6and8mg/kg for 3 days. On the first day of ondansetron treatment it was observed that all the 6 animals in 8mg/kg ondansetron group died because of convulsion induced by high dose of ondansetron itself. This indicates the convulsive potential of ondansetron in high doses (Table 3).

On the third day of ondansetron treatment we studied the proconvulsant effect produced by combination of ondansetron and sub convulsive strength in both MES and PTZ induced seizures.[12,13]

In this context, authors have found that 6mg/kg ondansetron significantly increases the duration of flexion and duration of tonic hind limb extension in MES induced convulsion. Also, in PTZ induced seizure ondansetron 6mg/kg significantly decreases the seizures threshold (Table 4 and 6).

The seizure caused by ondansetron 6mg/kg with combination with sub convulsive strength was similar to that of convulsion produced by convulsive strength (Table 5 and 7, p value >0.05).

This proconvulsant action of ondansetron can be explained by previous studies.[7,15,16]

- 5-HT3 receptor causes an early depolarization effect in postsynaptic membranes by directly conducting Na^+, K^+ and Ca^{2+} ions. Ondansetron competitively suppresses the chloride currents activated by GABA or glycine in patch clamped hypothalamic and hippocampal neurons of rats by preventing the influx of chloride ions (Cl^-) and inhibiting subsequent hyperpolarization.
- Ondansetron also competitively inhibits glycine induced hyperpolarization in mature neurons of rats
- Cortical and hippocampal interneurons expressing this 5-HT3 receptor are mainly inhibitory in nature, acting on excitatory neurons. Thus, inhibitions of these interneurons decrease the seizure threshold.

However, in phase III of our study, we found significant antiepileptic action of ondansetron at 3mg/kg (Table 8 and 9). This finding is supported by the study conducted by Balakrishnan et al, who reported that Ondansetron at lower doses is effective against maximal electroshock induced seizures in rats, with potentiation of the effect of phenytoin.

This antiepileptic action of ondansetron at low dose can be explained with the help of previous studies as follow:[4,5]

- At low dose ondansetron causes alteration in the ionic flux of cation resulting in inhibition of neuronal depolarisation. Ondansetron also competitively inhibits glycine induced depolarization in neonatal neurons.
- Nitric oxide synthase (NOS) enzyme has been shown to have close connection with other Ca^{2+} conducting neuronal channels, especially the glutamate NMDA receptor/channel, and to also take part in NMDA modulation of seizure paradigms. In general, NOS is considered as an enzyme highly dependent to Ca^{2+} and calcium binding proteins (especially calmodulin) in terms of activity and even destruction. NOS overactivity is shown to be proconvulsive. 5-HT3 receptor activation could directly affect NOS by changing cytosolic Ca^{2+} levels. By inhibiting 5HT3 receptor ondansetron possesses antiepileptic action.

Both anticonvulsive and proconvulsive activity of ondansetron can be explained as.[16] 5-HT3 activation may have two opposite effects on the inhibitory interneuron; one toward increased firing and subsequent GABA release, which is anticonvulsive, and another towards increased NOS activity and proconvulsive consequences.

So, by blocking 5HT3 receptors ondansetron also has both anticonvulsive and proconvulsive action (Figure 1).

CONCLUSION

Thus, it can be concluded that Ondansetron possesses both anticonvulsant and proconvulsant activities. These findings suggest that precaution has to be taken in using Ondansetron in general population receiving ondansetron in high doses (like in cancer chemotherapy) and in the patients having history of epilepsy. Clinical trials should be undertaken to address this important factor.

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