Vol 5 | Issue 1 | 2015 | 18-23.

e-ISSN 2248 - 9169
Print ISSN 2248 - 9150

International Journal of
Experimental Pharmacology

www.ijepjournal.com

EVALUATION OF THE ANTICONVULSANT ACTIVITY OF OMEPRAZOLE IN COMPARISON WITH PHENYTOIN IN ALBINO RATS

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ABSTRACT

Study was conducted to evaluate the potential of omeprazole as an anti-epileptic drug and compare it with currently used drug such as phenytoin using Maximal Electroshock seizure (MES) model. Randomized, Prospective, controlled, open labelled study using Healthy Albino Sprague-Dawley rats. All animals were randomly divided into 4 subgroups, and each subgroup comprises 6 rats. Study Drugs & Dosage are for control**:** Distilled water, Study drug: Omeprazole - 0.5 mg/kg & 1 mg/kg, Standard drug: Phenytoin - 20 mg/kg. Both doses of omeprazole (0.5 mg/kg and 1 mg/kg) reduce all three parameters and appear to be effective in protecting the animal against seizure induced by Maximal Electroshock (MES) model. Have efficacy comparable to phenytoin. Omeprazole at the doses (0.5 mg/kg and 1 mg/kg) has an anticonvulsant activity in Maximal electroshock (MES) models.

Keywords: Omeprazole, Maximal Electroshock model (MES), Phenytoin.

INTRODUCTION

Epilepsy is a group of disorders of the central nervous system (CNS) characterized by paroxysmal cerebral dysrhythmia caused by abnormalities in the electrical activity of brain. About 50 million people worldwide have epilepsy and nearly 90% of epilepsy occurs in developing countries [1]. There are more than 10 million persons with epilepsy in India and its prevalence is about 1% of our population [2]. The annual cost per capita is between $27.51 to $47.73 for epileptic patient [3, 4].

The ideal Antiseizure drug would suppress all seizures without causing any unwanted effects. Unfortunately the drugs used currently have low therapeutic index, they not only fail to control seizure activity in some patients, but frequently cause unwanted effects that range in severity from minimal impairment of CNS to death, from aplastic anaemia or hepatic failure. Also patients of absence seizure respond to Valproate or ethosuximide but the seizures can be exacerbated by Phenytoin and carbamazepine [5]. With these drugs control of seizures can be achieved in about half of the patients, while another 20-30% can be improved significantly and rest remains resistant. These patients who do not respond to the classical anti-epileptic therapy end up suffering from refractory epilepsy, an entity that is associated with considerable medical, social and psychiatric morbidity, and enormous financial costs [5]. Thus a need for a new

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antiepileptic drug which control and totally prevent all seizure activity with minimal side effects is perpetual. Carbonic anhydrase (CA) enzyme is quite abundant in the brain, mainly as the cytosolic isoenzyme CA II, CA VII, and membrane bound isoform CA XIV. Inhibition of this enzyme significantly increases the latency of onset of seizures and alteration of intracellular and extracellular PH decreases the efficacy of synaptic transmission in several areas of brain, prevent seizures [6]. Carbonic anhydrase (CA) inhibitors like Acetazolamide show such activity but due to rapid tolerance limits its use. Omeprazole a proton pump inhibitor, one of the most widely prescribed drugs internationally and is available over the counter in some countries. Omeprazole suppresses gastric acid secretion by specific inhibition of the H^+-K^+-ATPase in the gastric parietal cell. It also inhibits Carbonic anhydrase (CA) I, II and IV. The average brain concentration of omeprazole was 53.2 ± 6.9 ng/g of brain tissue [7].

Maximal Electroshock seizure (MES) model was used to study anticonvulsant activity of Omeprazole.

Therefore this study was conducted to evaluate the potential of omeprazole as an anti-epileptic drug and compare it with currently used drugs such as phenytoin.

MATERIAL AND METHODS:

Randomized, Prospective, controlled, open labelled study was done in the Department of Pharmacology, tertiary care hospital, Mumbai after approval from the institutional animal ethics committee. The animals were procured from the animal house of Department of Pharmacology, tertiary care hospital, Mumbai.

Experimental Animals: Healthy Albino Sprague-Dawley rats bred & reared in-house aged about 6-8 weeks with average weight around 150-200 gms. Sample size is 24 rats of either sex divided in six animals per group. Animals were not involved in any other study. These animals were housed under standard laboratory conditions in a well ventilated room & fed on standard pellet diet. The animals have free access to diet & water and ad libidum. Two rats each were placed in clean polypropylene cages with grill on top. The floor of the cages were stacked with grain husk which was replaced every second day. The animals were inspected frequently to rule out infections. Cages were identified by means of properly labelled cage tag, and in each cage the animals were identified by marking them with picric acid. An acclimatization period of one week in animal laboratory at room temperature was allowed for the rats before the experiment was started. The body weight of the animals was recorded on first day of initiation of study.

Equipments: Equipments used in the study are electroconvulsiometer with ear-clip electrodes to minimize animal suffering used to deliver the electric shock, Digital weighing scale to weight the drugs, Digital stop watch to record the time, Test tubes to prepare drug solution & storage, test tube racks to keep test tubes, Syringes: 2 cc, 1 cc to inject required amount of drugs, Sterile hypodermic needle: 25G for injecting drugs and hand gloves for cleanliness to avoid infection.

Study Drugs & Dosage: Study Drugs & Dosage for Maximal Electroshock model (MES) Model are for control**:** Distilled water, Study drug: Omeprazole - 0.5 mg/kg & 1 mg/kg, Standard drug: Phenytoin - 20 mg/kg.

Doses of Omeprazole (0.5 mg/kg & 1 mg/kg) were calculated using similar study where anticonvulsant activity of omeprazole in rats was evaluated [7]. A Body surface conversion chart was used to calculate doses. Dose of phenytoin 20 mg/kg was extrapolated from their average therapeutic dose in humans using the body surface conversion chart [8]. All drugs were weighed using a digital weighing scale and solutions prepared in Distilled water which was used as vehicle. All drugs were administered intraperitoneally in every animal in volumes of 1 ml.

Study Procedure: After an initial period of acclimatization of 7 days, all animals were randomly divided into 4 groups, and each group comprises 6 rats.

The Electroconvulsiometer (Bijou, Prasad Scientific) with ear-clip electrodes was used to deliver the electric shock. Ear clip electrodes were used instead of corneal electrodes to minimize animal suffering since blindness is a potential complication of corneal electrodes. The animals were acclimatized to the feel of the ear-clip electrodes so that they did not resist unduly at the time of administering the electric shock.

The maximal electroshock seizure pattern was induced in animals by using a convulsiometer [9]:

- Duration of current: 0.2 sec using digital stop watch.
- Voltage: 100 V
- Strength of current: 150 mA.

On the previous day of testing the pre-determined strength of current for above mentioned duration was given to each of the animals. On the day of experiment, the animals were brought to the experimental laboratory from the animal house. All the animals were checked to rule out any infection, injury or any other illness. The animals were weighed before the beginning of the experiment. The ears of the animals were cleaned with spirit to remove any oil film due to sebaceous gland secretions in the skin of the ear. Distilled water was used to moisten the ear for better electric contact. The animals were injected the control, test and standard drugs, intraperitoneally under all aseptic precautions, as per the study subgroups. 30 minutes later, the rats were subjected to an electric shock. The animal was restrained with the help of an assistant and the ear clip electrodes were applied. The electric current was immediately applied to the animal. The resultant

convulsions were timed using the digital stop clock. The occurrence of a tonic hind limb extension was taken as a positive response for MES; abolition of tonic hind limb extension was taken as protection against MES seizures.

The following parameters were noted:
1. Duration of Hind limb tonic extensor (HLTE) phase in sec.
2. Duration of entire convulsion in sec.
3. Duration of post-ictal phase in sec. i.e. time to resumption of normal activity following post-ictal Stunning.
At the end of the experiment the animals were inspected for any injury or residual damage. No mortality was observed in the entire procedure. All the animals used for the study were kept separately in the animal house and were not used for any other experiment.

Statistical Analysis: All quantitative data were presented as mean & standard error of mean (SEM). Data of MES induced seizure in group of Omeprazole was analysed using unpaired't' test. Multiple group comparison was done by One-Way ANOVA followed by post-hoc Dunnet's test. For all tests, a 'p' value of < 0.05 was considered as significant.

RESULT

In the present study, the anticonvulsant activity was evaluated for Omeprazole using maximal electroshock seizure (MES) model in albino rats. Omeprazole was compared with Phenytoin in maximal electroshock seizure (MES) model.

1. Duration of Hind limb tonic extensor (HLTE) phase (in Sec):

For Distilled water 10.90±1.31 sec, Omeprazole (0.5 mg/kg): 3.82±0.33 sec, Omeprazole (1 mg/kg): 3.25±0.43 sec and Phenytoin 20 mg/kg: 2.84±0.3 sec.

Comparisons:

- **With control**: Omeprazole (0.5 mg/kg): 3.82± 0.33, Omeprazole (1 mg/kg): 3.25±0.43 and Phenytoin 20mg/kg: 2.84±0.3 significantly reduced the duration of Hind limb tonic extensor (HLTE) phase as compared to Distilled water 10.90 ± 1.31; p<0.001.

- **Between two subgroups of Omeprazole:** There was no significant difference in the duration of Hind limb tonic extensor (HLTE) phase between the two subgroups of Omeprazole (Omeprazole 0.5 mg/kg: 3.82±0.33 sec and Omeprazole 1 mg/kg: 3.25±0.43 sec: p > 0.05).

- **Two subgroups of Omeprazole with Phenytoin:** There was no statistical difference between Omeprazole 0.5 mg/kg: 3.82±0.33 sec compared with Phenytoin 20mg/kg: 2.84±0.3 and Omeprazole 1 mg/kg: 3.25±0.43 sec and Phenytoin 20mg/kg: 2.84±0.3 in duration of Hind limb tonic extensor (HLTE) phase. (p>0.05).

2. Duration of entire Convulsion (in sec):

Duration of entire convulsion for Distilled water 22.58± 1.06 sec, Omeprazole 0.5 mg/kg: 14.20±0.57 sec, Omeprazole 1 mg/kg: 13.12±0.49 sec and Phenytoin 20 mg/kg: 12.72±0.36 sec.

Comparisons:

- **With control:** Omeprazole (0.5 mg/kg): 14.20 ± 0.57, Omeprazole (1 mg/kg): 13.12 ± 0.49 and Phenytoin 20 mg/kg: 12.72±0.36 showed a significant decrease in duration of entire convulsion compare to Distilled water 22.58± 1.06; P<0.001.

- **Between two subgroups of Omeprazole:** There was no significant difference in the duration of entire convulsion between the two subgroups of Omeprazole (Omeprazole 0.5 mg/kg: 14.20±0.57 sec and Omeprazole 1 mg/kg: 13.12±0.49 sec: p > 0.05).

- **Two subgroups of Omeprazole with Phenytoin:** There was no statistical difference between Omeprazole 0.5 mg/kg: 14.20±0.57 compared with Phenytoin 20mg/kg: 12.72±0.36 and Omeprazole 1 mg/kg: 13.12±0.49 and Phenytoin 20mg/kg: 12.72±0.36 in duration of entire convulsion. (p>0.05).

3. Duration of post-ictal phase (in sec):

Duration of post-ictal phase for Distilled water 32.68± 0.74 sec, Omeprazole 0.5 mg/kg: 14.78 ± 0.20 sec, Omeprazole 1 mg/kg: 14.23±0.36 sec and Phenytoin 20 mg/kg: 13.93± 0.33 sec.

Comparisons:

- **With control:** Omeprazole (0.5 mg/kg): 14.78 ± 0.20, Omeprazole (1 mg/kg): 14.23 ± 0.36 and Phenytoin 20 mg/kg: 13.93± 0.33 showed a significant decrease in duration of post-ictal phase compare to Distilled water 32.68± 0.74; P<0.001.

- **Between two subgroups of Omeprazole:** There was no significant difference in the duration of post-ictal phase between the two subgroups of Omeprazole (Omeprazole 0.5 mg/kg: 14.78± 0.20 sec and Omeprazole 1 mg/kg: 14.23± 0.36 sec: p > 0.05).

- **Two subgroups of Omeprazole with Phenytoin:** There was no statistical difference between Omeprazole 0.5 mg/kg: 14.78 ± 0.20 compared with Phenytoin 20mg/kg: 13.93± 0.33 and Omeprazole 1 mg/kg: 14.23± 0.36 and Phenytoin 20mg/kg: 13.93± 0.33 in duration of post-ictal phase. (p>0.05)

Table 1. Maximal Electroshock model (MES): Animal groups

Group	Description	Interventions	No. of Animals
1	Control	Distilled water	6
2	Test Group 1	Omeprazole 0.5 mg/kg i.p.	6
3	Test Group 2	Omeprazole 1 mg/kg i.p.	6
4	Standard	Phenytoin 20 mg/kg i.p.	6

Figure 1. Duration of Hind limb tonic extensor (HLTE) phase

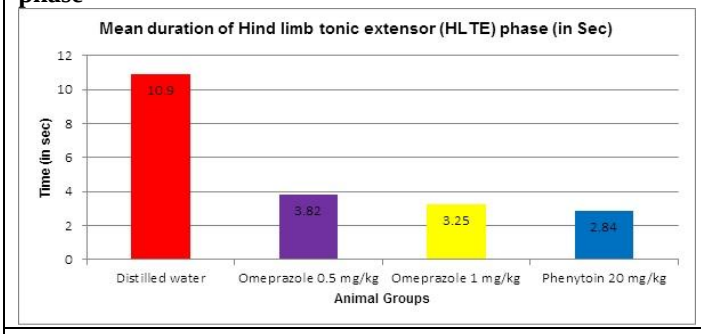


Figure 2. Duration of entire Convulsion

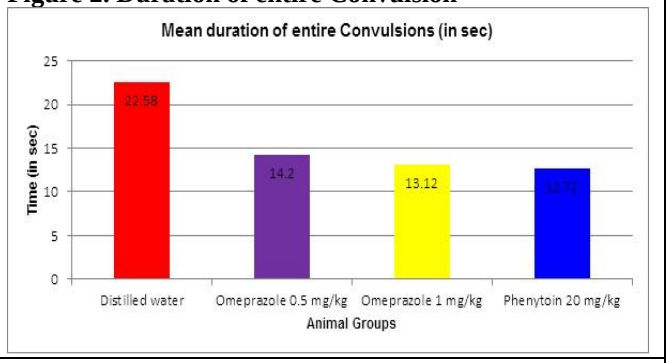
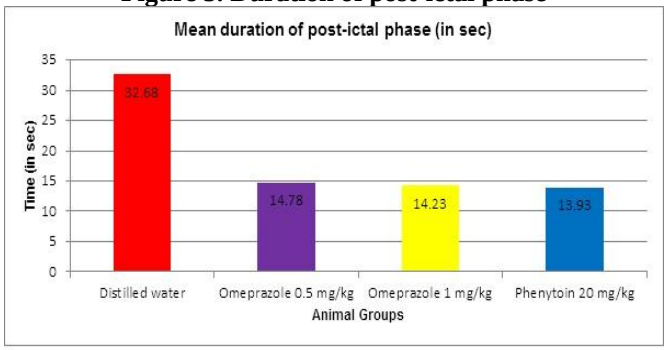


Figure 3. Duration of post-ictal phase



DISCUSSION

Carbonic anhydrase (CA) enzyme is quite abundant in the brain, mainly as the cytosolic isoenzyme CA II, CA VII, and membrane bound isoform CA XIV. Inhibition of this enzyme significantly increases the latency of onset of seizures and alteration of intracellular and extracellular PH decreases the efficacy of synaptic transmission in several areas of brain, prevent seizures [6]. Several carbonic anhydrase inhibitors like acetazolamide, methazolamide, topiramate and zonisamide are used as antiepileptic drugs. The anticonvulsant effects of these are probably due to CO_2 retention, secondary to inhibition of red cell and brain enzymes. However, there can be other mechanisms of activity such as blockade of sodium channels and kainite/ α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors as well as enhancement of GABA-ergic transmission [10].

Omeprazole is a proton pump inhibitor used in the treatment of dyspepsia, peptic ulcer disease (PUD), gastroesophageal reflux disease (GERD), laryngopharyngeal reflux (LPR) and Zollinger–Ellison syndrome (ZES). Omeprazole is one of the most widely prescribed drugs internationally and is available over the counter in some countries. Omeprazole suppresses gastric acid secretion by specific inhibition of the H^+-K^+-ATPase in the gastric parietal cell. It also inhibits Carbonic anhydrase (CA) I, II and IV. The average brain concentration of omeprazole was 53.2 ± 6.9 ng/g of brain tissue [7]. In this study, role of Omeprazole as an anticonvulsant drug has been explored by its Carbonic anhydrase (CA) inhibition activity by evaluating it in most widely used epilepsy model such as Maximal electroshock (MES) model for generalized tonic-clonic seizures (GTCS) and also compared with established antiepileptic drugs like phenytoin.

Maximal Electroshock seizure (MES) model was used to study anticonvulsant activity of Omeprazole. This model was chosen because it is widely used and well-established model for generalized tonic-clonic seizures (GTCS). Phenytoin was taken for Maximal Electroshock seizure (MES) model because this is the first line drug used in GTCS and routinely prescribed for above condition.

In the present study, in this model Omeprazole was tested and compared with Phenytoin in rats. The rats were divided into four groups as mentioned in methods and materials. Three parameters were recorded, namely

Duration of tonic extensor phase (in sec), Duration of entire convulsion (in sec) and Duration of post-ictal phase (in sec). Distilled water, Omeprazole (0.5 mg/kg), Omeprazole (1 mg/kg) and Phenytoin (20 mg/kg) i.p. were tested for anticonvulsant activity.

There was significant reduction in duration of Hind limb tonic extensor (HLTE) phase when omeprazole 0.5 mg/kg (3.82 ± 0.33 sec, <0.001), omeprazole 1 mg/kg (3.25 ± 0.43 sec, <0.001) and phenytoin 20 mg/kg (2.84 ± 0.3 sec, <0.001) compared with distilled water (10.90 ± 1.31 sec). There was no significant difference in the duration of Hind limb tonic extensor (HLTE) phase between the two groups of Omeprazole ($p > 0.05$) and when the two groups of Omeprazole compared with Phenytoin ($p > 0.05$).

There was a significant reduction in duration of entire convulsion when Omeprazole 0.5 mg/kg (14.20 ± 0.57 sec, <0.001), Omeprazole 1 mg/kg (13.12 ± 0.49sec, <0.001) and Phenytoin 20mg/kg (12.72 ± 0.36sec, <0.001) compared with Distilled water (22.58 ± 1.06 sec). There was no significant difference in the duration of entire convulsion between the two groups of Omeprazole ($p > 0.05$) and when the two group of omeprazole compared with Phenytoin ($p > 0.05$).

There was significant reduction in duration of post-ictal phase when omeprazole 0.5 mg/kg (14.78 ± 0.20 sec, <0.001), Omeprazole 1 mg/kg (14.23 ± 0.36 sec, <0.001) and Phenytoin 20mg/kg (13.93 ± 0.33 sec, <0.001) compared with Distilled water (32.68 ± 0.74 sec). There was no significant difference in the duration of post-ictal phase between the two groups of Omeprazole ($p > 0.05$) and when the two group of omeprazole compared with Phenytoin ($p > 0.05$). Thus both doses of omeprazole (0.5 mg/kg and 1 mg/kg) reduce all three parameters and appear to be effective in protecting the animal against seizure induced by Maximal Electroshock (MES) model. Have efficacy comparable to phenytoin.

In one of the study, conducted by Demir Y *et al.* the effects of omeprazole, famotidine and ranitidine on bovine stomach carbonic anhydrase (EC 4.2.1.1.) isoenzymes have been investigated in vitro. Bovine stomach carbonic anhydrase (CA) was purified from four different cell localisations of bovine stomach using affinity chromatography by Sepharose 4B-L-tyrosine sulphanilamide. The inhibition or activation effects of three different medical drugs on CA isoenzymes were determined using esterase activity and the CO (2)-hydratase method by plotting activity % vs. The K(i) values for omeprazole, famotidine and ranitidine were determined in all localization CA, respectively. The I (50) values of the drugs exhibiting an inhibition effect were found by means of graphs. It was observed that omeprazole, famotidine and ranitidine showed inhibition of bovine stomach CA activity. In addition, in vivo studies were performed for these medical drugs in Sprague-Dawley rats. It was demonstrated that CA in erythrocytes was significantly inhibited by these drugs to 3 h [11]. Similar results were found in the study conducted by Puscus *et al*. who studied omeprazole and found that omeprazole has a dual mechanism of action: it inhibits both H^+-K^+ ATPase and gastric mucosa carbonic anhydrase enzyme in humans (in vitro and in vivo experiments) [12].

Study conducted by Balakrishnan *et al*. shows omeprazole to be an effective anticonvulsant, but rapidly develops tolerance to its anticonvulsant action. In this study CC 50, i.e. the threshold current inducing tonic hind limb extension in 50% of the rats was established using a technoconvulsometer which delivers currents of varying intensity via ear clip electrodes. The CC 50 was established 30 min after injection of omeprazole. In another group of rats, omeprazole 2 mg/kg was given for 6 days and the CC 50 determined on days 0, 1, 3 and 6. Also the concentration of omeprazole in the brain was determined using high performance liquid chromatography. The CC 50 in vehicle-treated rats was 98 mA, which increased to 126, 135 and 162 mA with 0.5, 1 and 2 mg/kg of omeprazole, respectively [7].

Thus in our animal study we got promising results for anticonvulsant action of omeprazole but this has to be confirmed by other experimental & human studies. We got very few references mentioning the effect of omeprazole on CA activity & lack of modern facilities to establish it; it creates a limitation to our study. Another limitation of the study was, the sample size which was taken for the study was less than required because of ethical constraints, hence there can be possibility of some deviation in the results which we got. By keeping all the limitations in mind, one should plan for a study or give a thought about potential use of omeprazole or like drugs which inhibit CA activity in brain on large sample size with all modern facilities available. In our study two different doses of Omeprazole have shown efficacy in GTCS seizures in MES model. There is hope that Omeprazole could be effective in patients who are refractory to presently available standard antiepileptic medication. Omeprazole could be of value for acute treatment of status epilepticus, perhaps in conjunction with conventional agents. Further studies need to be carried out to explore the potential of this drug. The need of the hour is to understand why epilepsy occurs and how best to control, if not cure it. Modulating ionic movements and their consequences, the release and action of neurotransmitters and modulators seems to be the best bet. Gene therapy and immune based therapies are alternative futuristic strategies. In this study Omeprazole was found to have antiepileptic activity in animal models, but this is to be confirmed in humans with controlled randomized clinical trials.

SUMMARY

In the present study anticonvulsant activity of Omeprazole was evaluated by using well-established model namely, Maximal electroshock (MES) model in albino rats.

Omeprazole (two doses: 0.5 mg/kg and 1 mg/kg) compared with Phenytoin in Maximal electroshock (MES) model used for generalized tonic clonic seizures by recording three parameters, namely Duration of tonic extensor phase (in sec), Duration of entire convulsion (in sec) and Duration of post-ictal phase (in sec). Omeprazole appears to be effective in protecting the animal against GTCS seizures induced by Maximal Electroshock (MES) model. Have efficacy comparable to phenytoin. These results indicate that Omeprazole at the doses tested above has an anticonvulsant activity in Maximal electroshock (MES) model.

CONCLUSION

- Omeprazole shows anticonvulsant efficacy in GTCS model of albino rats by using the Maximal Electroshock (MES) method.
- Anticonvulsant effect of Omeprazole in MES model of albino rats is comparable with the standard drug Phenytoin.

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