The Antiepileptic Effect of Riluzole And In Combination With Phenytoin on Maximal Electroshock Induced Seizures In Albino Rats : An Experimental Study

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Abstract :

Despite the introduction of several new therapeutic options, a significant fraction of the patients with epilepsy continue to live with uncontrolled seizures. Although large no. of antiepileptic drugs are available in the market, most of these agents have a limited spectrum of antiepileptic activity and all have certain negative properties that limit their utility and complicate patient management, so 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 **Methodology :** Albino rats weighing 150-200g, 6-8 weeks old of either sex, were used in the study. Rats were randomly allocated in to 9 groups; each group consists of six. **Group 1: Control, II, III:** Riluzole 5, 10 mg/kg respectively, **IV,V,VI:** Phenytoin 25,50,100 mg/kg respectively, **VII,VIII,IX:** Riluzole 10 mg/kg in combination with Phenytoin 25,50,100 mg/kg respectively. Drugs were administered to all groups, orally by orogastric tube, as per the study subgroups. 30 minutes later, the rats were subjected to an electric shock by using an electro convulsiometer : Presence or absence of hind limb extensor, duration of Hind limb tonic extensor (HLTE) phase & duration of clonus in sec. **Result :** Riluzole at both dosages of 5 & 10mg/kg did not modify the MES induced convulsions. Phenytoin at a dose of 25mg/kg & 50mg/kg could not abolish the hind limb extension. However, phenytoin at 100mg/kg produced 100% abolition of Hind Limb extension. Phenytoin at 25mg/kg & 50mg/kg dosage insignificantly reduced the duration of Hind Limb Extensions without any effect on clonic phase. At the same time phenytoin at 100mg/kg significantly increased the duration of clonus from 9.8 to 18.3 seconds, but interestingly when Riluzole 10mg/kg was combined with Phenytoin 50mg/kg, the Hind Limb Extension was abolished in 50% animals. When the dose of Phenytoin was further increased to 100mg, it produced 100% protection by abolishing Hind Limb Extension. Similar effects were produced when Riluzole was combined with 100mg of Phenytoin. **Conclusion :** Riluzole alone was found to be ineffective against MES induced seizures, however it was found to enhance the antiepileptic effect of phentyoin against MES induced seizures.

Keywords : Maximal electroshock seizure, Riluzole, Phenytoin, Hind limb tonic extensor

Introduction : Epilepsy is one of the most common neurological disorders. The definition of epilepsy requires the occurrence of at least one epileptic seizure[1]. From a neurophysiologic point of view, an epileptic seizure has been defined as an alteration in CNS function resulting from spontaneous electrical discharge in a diseased neuronal population of cortical gray matter or the brain stem. Worldwide, more than 50 million people are suffering from epilepsy and the prevalence is estimated to be 0.5 – 1%, and there is a lifetime incidence of 1 – 3%.1 It is more common in young children and elderly people above 65 years[2]. The overall prevalence rate of epilepsy in India is 5.59 per 1,000 populations[3]. It has important medical, social and psychological consequences.

Despite the introduction of several new therapeutic options in the 1990s, a significant fraction of patients with epilepsy continue to live with uncontrolled seizures[4]. Although most people with epilepsy become seizure free with appropriate therapy, 30-40% of patients continue to have seizures despite the use of antiepileptic drugs either alone or in combination[5].

Glutamate and Aspartate are the major excitatory neurotransmitters found in the mammalian brain[6]. Focal application of glutamate to hippocampal slices induce a calcium ion current and depolarizes the neuron. Gamma-Aminobutyric Acid (GABA) has been shown to have inhibitory post-synaptic activity and is one of the principal inhibitory neurotransmitters in the mammalian brain[7].

The potential importance of Riluzole- a glutamate antagonist in modulating brain electrical activity has been described recently. Riluzole is known to act through a novel modulatory site on the Glutamate receptors, which mediate most excitatory neurotransmission in the mammalian brain. This has

been shown that riluzole enhances anti-seizure action of known anti-epileptic drugs[8].

Phenytoin is effective against all types of partial and tonic-clonic seizures but not absence seizures. Phenytoin exerts anti-seizure activity without causing general depression of the CNS. The most significant effect of Phenytoin is its ability to modify the pattern of maximal electroshock seizures. The characteristic tonic phase can be abolished completely, but the residual clonic seizure may be exaggerated and prolonged[9].

A standard antiepileptic drug includes barbiturates, hydantoins, iminostilbenes, aliphatic carboxylic acid, succinimides, and benzodiazepines. Most of these agents have a limited spectrum of antiepileptic activity and all have certain negative properties that limit their utility and complicate patient management, so 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 [10].

The present study was undertaken to find out the effect of riluzole alone and in combination with Phenytoin on MES in Swiss-Albino rats.

Methods :

Study design: It was an experimental animal based study.

Study location : The study was conducted in the Department of Pharmacology, PDVVPF'S Medical College and Hospital, Ahmednagar,

Ethical approval : The study was conducted only after approval of the Institutional Animal Ethics Committee (IAEC).

Sample size : Nine groups of each group n=6 (Total 54)

Inclusion criteria : Healthy Swiss Albino rats weighing 150-200 gm, 6-8 weeks old of either sex, were used in the study[11]. One week before the actual day of testing the pre-determined strength of current for mentioned duration (as per mentioned in methodology) was given to each of the animals. The occurrence of a tonic hind limb extension was taken as a positive response for MES. The rats which are producing convulsion are selected for present study.

Animal rearing : Animals were housed in plastic cages under standard conditions (ambient temperature of 22 ± 1°C, natural light-dark cycle, free access to chow pellets and tap water.) [8].

Drugs and chemicals:

- Riluzole was administered orally in the dose of 5mg/kg and 10 mg/kg[8] while Pheytoin was administered orally at 25 mg/kg, 50 mg/kg and 100 mg/kg dose[12].
- The control groups were administered distilled water was another drugs were prepared in water. The volume was fixed 2ml.

Animal Grouping : Rats were randomly allocated in to 9 groups; each group consists of 6 animals.

Table 1: shows subgroups and intervention done

Groups	Intervention
Group I	Distilled water (Control)
Group II	Riluzole 5 mg/kg
Group III	Riluzole 10 mg/kg
Group IV	Phenytoin 25 mg/kg
Group V	Phenytoin 50 mg/kg
Group VI	Phenytoin 100 mg/kg
Group VII	Riluzole 10 mg/kg + Phenytoin 25 mg/kg
Group VIII	Riluzole 10 mg/kg + Phenytoin 50 mg/kg
Group IX	Riluzole 10 mg/kg + Phenytoin 100 mg/kg

Independent variables: MES, Riluzole, Phenytoin

Dependent variables: Duration of Hind limb tonic extensor (HLTE) phase in sec., Duration of clonus in sec.

Methodology :

All experiments were done at the same period of the day (between 9.00 a.m. and 12.00 a.m.) to minimize circadian influences on seizure susceptibility [8].

After one week of acclimatization, on the day of the 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.

Drugs were administered orally by orogastric tube under all aseptic precautions, as per the study subgroups. 30 minutes later, the rats were subjected to an electric shock.

The maximal electroshock seizure pattern was induced in animals by using a electro convulsiometer:[13,14]

- Duration of current of: 0.2 Sec.
- Voltage: 100 V
- Strength of current: 150 mA

Ear-clip electrodes with cotton plugs were used to deliver the electric shock. Noraml saline was used to moisten the cotton plugs for better electrical contact.

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.

Parameters studied:[14]

1. Presence or absence of hind limb extension
2. Duration of Hind limb tonic extensor (HLTE) phase in sec.
3. Duration of clonus in sec.

Statistical Analysis : All quantitative data was presented as Mean ± Standard Error of Mean (SEM). Data was analyzed using the unpaired student t- test. For all tests, a 'p' value of < 0.05 was considered significant.

Fig1: Hind Limb Extension in Electroshock induced convulsions

Results :

Table 2 : Effect of Riluzole, Phynetoin and in combination on Electroshock Induced Convulsions:

	% With Hind Limb Extension	Duration of Hind Limb Extensions (in Sec)	Duration of Clonus (in Sec)
Control	100 %	14 ± 1.	9.8 ±0.8
Riluzole 5mg/kg	100 %	12.7± 0.7	9.2± 0.3
Riluzole 10mg/kg	100 %	11.5± 0.7	7.8 ± 0.6
Phenytoin of 25 mg/kg	100%	13.7± 1.2	9.2± 0.4
Phenytoin of 50 mg/kg	100 %	11.3±0.7	12.2± 0.6
Phenytoin of 100mg/kg	0 %	0.0± 0.0***	18.3± 1.1***
Riluzole 10mg/kg + Phenytoin 25mg/kg	100%	11.5± 0.6	8.2±0.5
Riluzole 10mg/kg + Phenytoin 50mg/kg	50%	0.8± 0.4***	10.2±0.3**
Riluzole 10mg/kg + Phenytoin 100mg/kg	0%	0±0***	13.5± 0.4***

Data was expressed as Mean ± SEM, P- Values as * (<0.05), ** (<0.01), & * (<0.001).**

In this study, Riluzole was used at 5 &10mg/kg to observe any effect on Electroshock induced convulsions in rats. Riluzole at both dosages of 5 & 10mg/kg did not modify the electroshock induced convulsions. All animals exhibited Hind limb Extensions & no change in duration of tonic & clonic phase of convulsion.

Phenytoin at a dose of 25mg/kg & 50mg/kg on oral administration could not abolish the hind limb extension. However, phenytoin at 100mg/kg produces 100% abolition of Hind Limb extension. This dose was found to be effective to produce the anti-convulsant effect in the electroshock induced convulsions model.

However, phenytoin at 25mg/kg & 50mg/kg dosage insignificantly reduce the duration of Hind Limb Extensions & no much effect on clonic phase. At the same time phenytoin at 100mg/kg significantly increased the duration of clonus from 9.8 to 18.3 seconds (table2).

Riluzole 10mg/kg & Phenytoin 25mg/kg had no effects, also against Electroshock induced convulsions. When both drugs are combined at similar doses did not produce any effect on Electroshock induced convulsions. Phenytoin 50mg/kg also could not give any protection against Electroshock induced convulsions, but interestingly when Riluzole 10mg/kg was combined with Phenytoin 50mg/kg, the Hind Limb Extension was abolished in 50% animals when the dose of Phenytoin was increased to 100mg, it gave 100% protection by abolishing Hind Limb Extension. Similar effects were produced when Riluzole was combined with 100mg of Phenytoin (Table No.2).

Discussion : Riluzole is a neuroprotective drug having anti-convulsant, anxiolytic and sedative properties [15,16]. The drug is primarily used to treat amyotropic lateral sclerosis due to its action on glutaminergic neurotransmission in the brain. This drug is reported to have an inhibitory effect on G-protein dependent release of glutamate to the synaptic cleft, reducing the release of glycine resulting in reduction of NMDA channel activity, diminishing sensitivity of AMPA-receptor, preventing calcium mobilization by activating G-proteins & blocking post-synaptic responses of glutaminergic receptors[8].

Blockade of glutaminergic transmission is believed to be followed by dose dependent inhibition of reuptake of several neurotransmitters like GABA[17], dopamine & acetylcholine[18], which may be responsible for the beneficial effect of Riluzole in the treatment of amyotropic lateral sclerosis and Huntington's disease[19].

Drugs inhibiting gluataminergic inhibition are known to be effective in the treatment of different types of epilepsy. Felbamate which inhibits NMDA receptor & potentiate GABA receptor was introduced in the treatment of partial & generalized seizures. However, the drug was withdrawn by US-FDA, due to its side effect producing aplastic anemia[9]. Riluzole being a glutamate antagonist was also taken for this study to explore the possibility of its protective effect in experimentally induced epileptic models in albino rats.

In the present study, the result shows Riluzole at 5mg & 10mg, body weight doses given orally could not produce any significant anti-epileptic effect against Maximal Electroshock Seizures.

Phenytoin at a dose of 100mg/kg protected 100% animals (0% Hind Limb Extensions) against electroshock induced convulsions, but when Riluzole was added to Phenytoin at 50mg/kg produce anti-convulsant effect in 50% rats in electroshock induced convulsions. This result indicates Riluzole has some synergistic effect on anti-convulsant effect of phenytoin.

Phenytoin is believed to produce its anti-epileptic effect by blocking Sodium channels & enhancement of responses to GABA in CNS[9]. It is therefore possible that Riluzole a glutamate antagonist when combined with a GABA mimetic agent can produce a synergistic effect in epilepsy .

Since riluzole does affect plasma concentrations of antiepileptic drugs, this positive interaction of riluzole with Phenytoin does not seem to be pharmacokinetic type[8].

Enhancing effect of riluzole may be due to its inhibitory action on excitatory neurotransmitters because in electrophysiological studies, riluzole prevented both NMDA and veratridine induced excitotoxicity in rat hippocampal slices[20]. Also in many experimental studies, riluzole was found to attenuate convulsions evoked by both glutamate and kainate in mice[21]. Moreover, the inhibitory effect on neurotransmission mediated by both the glycine/NMDA and AMPA/kainate receptor complex could be the reason of efficacy of riluzole in genetic model of seizure prone DBA/2 mice[22]. Riluzole may behave as a competitive[14] or as a noncompetitive[23] antagonist at NMDA receptor complex.

Observations of many studies related with NMDA

receptor antagonists have shown that these drugs enhance the anticonvulsant effect of many conventional antiepileptic drugs, allowing for their significant dose reduction[24].

So, riluzole could be used as add on drug along with phentyoin in the management of absence seizure in case of phentyoin is ineffective as monotherapy. Also we may reduce the dose of phentyoin by using riluzole as an adjuvant.

Limitations of the study : This is an experimental study so it is difficult to extrapolate results of animal study directly on human. So to confirm above results in humans, more clinical studies of riluzole in epileptic patients will be required.

Conclusion : Riluzole alone found ineffective against MES induced seizures, however it was found to enhance the antiepileptic effect of phentyoin against MES induced seizures.

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