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EVALUATION OF ANTICONVULSANT ACTIVITY OF MAGNESIUM OXIDE ALONE AND WITH PHENYTOIN AGAINST EXPERIMENTALLY INDUCED CONVULSIONS IN ALBINO RATS

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ABSTRACT

The objective of the study was to find the effect of oral magnesium oxide supplementation alone and with standard anticonvulsive doses of phenytoin in the animal models of maximal electroshock seizures (MES) induced seizures. Healthy male albino rats (n=42) were divided into 6 groups, each group including seven animals. Magnesium oxide (Mgo) supplementation is given orally in the dose (500 mg/kg/day) for 10 days for all groups except for group A(control) . On day 10, responses to MES (180 mA for 0.2 s) were tested 1hr after pre-administration of phenytoin orally in different doses(100,200,400 mg/kg). In Combination studies in group B,C and D the time taken for recovery was better in low dose magnesium and phenytoin combination (group B) . Compared to magnesium alone group (group F) all the combination groups (group B,C and D) were better in controlling THLE (Tonic Hind Limb Extension).Study also shows that Magnesium may help in faster recovery from seizure activity. Hence, we may reduce the standard dose of each drug and related adverse effects.

KEY WORDS

Magnesium Oxide, Phenytoin , Convulsions ,MES (Maximal Electroshock Seizures)

INTRODUCTION

Epilepsy refers to a clinical phenomenon rather than a single disease entity, since there are many forms and causes of epilepsy. Using the definition of epilepsy as two or more unprovoked seizures, the incidence of epilepsy is ~0.3- 0.5 % in different populations throughout the world , and the prevalence of epilepsy has been estimated at 5-10 persons per 1000[1]. Nearly one-third of the patients with epilepsy do not respond to treatment with a single antiepileptic drug, and it becomes necessary to try combination of drugs to control seizures. In most cases the initial combination therapy combines first-line drugs , i.e., carbamazapine, phenytoin, valproic acid, and lamotrigine. The principles concerning the monitoring of therapeutic response, toxicity, and serum levels for monotherapy ,can be applied for polytherapy and potential drug interaction need to be recognized. If there is a response for multidrug combination then the less effective or less well-tolerated of the two drugs should be gradually withdrawn[2].

MATERIALS AND METHODS

The animal experiment was carried out in the central animal house of department of pharmacology, JJM. Medical college, Davanagere inbred under suitable conditions of housing, temperature ventilation and nutrition with a 12 hour alternating light and dark cycle with adequate food and water supply. Healthy male albino rats (n=42) were divided into 6 groups each group including seven animals. Magnesium oxide (Mgo) supplementation is given orally in the dose (500 mg/kg/day) for 10 days for all groups except for group A(control) and . On day 10, response to MES (180 mA for 0.2 s) was tested 1hr after pre-administration of phenytoin orally.

***Inclusion criteria*:**

1. Animals weighing between 100-200g.
2. Age between 3-4months.
3. Healthy with normal behavior and activity.

Exclusion criteria:

1. Animals weighing <100 and >200g.
2. Age less than 3months or more than 4months.
3. Within 21days of use for other studies.
4. Pregnant rats

TABLE 1

A total 42 rats were divided into 7 groups each containing seven rats each. The drugs were given as in table 1

Group A	control group (normal saline)
Group B	500mg/kg of Mgo +100mg/kg of phenytoin
Group C	500mg/kg of Mgo +200mg/kg of phenytoin
Group D	500mg/kg of Mgo+ 400mg/kg of phenytoin
Group E	400mg/kg of phenytoin
Group F	500mg/kg of Mgo

Chemicals:
Magnesium oxide (Mgo) ,phenytoin

MES induced seizures
A stimulus of 180mA for 0.2s duration was given using the electro-convulsimeter and the responses were tested 1 hour after administration of phenytoin in all the groups.

RESULTS

The total animals analyzed finally after all the experiment were 6 in each group as in group B,C and D there were death of rats (one in each group).In groups A, and F there were death among rats after MES(one in each group).In E group one rat did not recover even after 3 hours(this animal was also not included for the analysis)

TABLE 2

All the animals were observed for the total hind limb extension(THLE) .If the animal does not show any show any hind limb extension then the animal is protected by prior administered drug .

Groups	THLE positive	THLE negative	Percentage of protection
Group A	6	0	0
Group B	0	6	100
Group C	0	6	100
Group D	0	6	100
Group E	0	6	100
Group F	3	3	50

All the animals in groups B,C,D and E were protected from MES induced seizures demonstrated by absence of tonic hind limb extension .The animals in group A(control) were not protected and hence all the animals were positive for THLE.

Table 3

The animals in group F(only Mgo group) were partially protected from MES as only three animals showed THLE positive results.

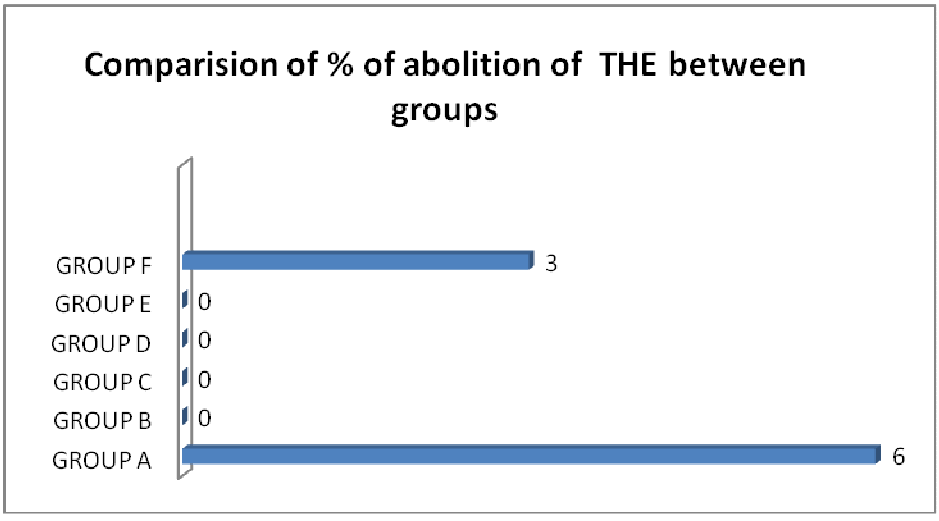

TABLE 4
Results of RRR time
(in seconds)

Groups	Mean	Standard deviation
Group A	104.5	17.91926
Group B	135.6667	15.73107
Group C	120.8333	21.43284
Group D	156	25.72936
Group E	140.3333	21.00159
Group F	96.16667	16.14208

Comparison between group B and F

Group B(500mg/kg of Mgo +100mg/kg of phenytoin) rats were completely protected as none of the rats showed tonic hind limb extension after MES and group F(500mg/kg of Mgo) rats were partially protected from the drug as only three of the rats were able to resist themselves from tonic hind limb extension .

Table 5
P value was significant with respect to the F group compared to B group as the recovery time was better in only magnesium group.

	THLE Positive	Mean	Standard deviation	P value
Group B	0	135.6667	15.73107	**0.001579**
Group F	3	96.16667	16.14208	

Comparison between group E and F

Group E(400mg/kg of phenytoin) rats were completely protected from the drug as none of them showed THLE positive results whereas the group F(500mg/kg of Mgo) showed partial protection as only half of them were protected and threw total hind limb extension.

Table 6
P value was significant with respect to the F group compared to B group as the recovery time was better in only magnesium group.

	THLE Positive	Mean	Standard deviation	P value
Group E	0	140.3333	21.00159	**0.001579**
Group F	3	96.16667	16.14208	

Comparison between group B and D

Both the groups B(500mg/kg of Mgo +100mg/kg of phenytoin) and D(500mg/kg of Mgo+ 400mg/kg of phenytoin) showed significant protection against MES induced seizures. There was no significant P value with respect to recovery period as both of groups recovered well .

Table 7

The low dose phenytoin group(group B) was better than high dose phenytoin combination(group D)

	THLE Positive	Mean	Standard deviation	P value
Group B	0	135.6667	15.73107	**0.1296**
Group D		156	25.72936	

DISCUSSION

Magnesium is an important cofactor for enzymatic reactions and plays an important role in neurochemical transmission and muscular excitability. Predominant deficiency effects are neurological , e.g., muscle irritability ,clonic twitching and tremors. Hypocalcemia and hypokalemia often follow low serum levels of magnesium. The large stores of magnesium present intracellularly and in bones is not mobilized sufficiently to maintain plasma levels[3]. Magnesium has been shown to have a central anticonvulsant effect by blocking N-methyl-D-aspartate (NMDA) receptors in neurons[4,5]. This property is useful in the prevention and management of seizures in pre-eclampsia and eclampsia and control of seizures in epilepsy, hypothyroidism and glomerulonephritis. Magnesium ions and various magnesium salts have been used by different routes like subcutaneous , intraperitonial intravenous and oral routes have shown to exert a significant anticonvulsant effect in experimental models . Studies have shown that combined use of conventional anti-epileptics with NMDA receptor blockade may have a synergistic effect[6,7]. 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. Phenytoin limits the repetitive firing of action potentials by a slowing of the rate of recovery of voltage-activated Na^+ channels from inactivation, an action that is both voltage-(greater effect if membrane is depolarized) and use-dependent. The toxic effects of phenytoin depend on the route of administration, the duration of exposure, and the dosage. Toxic effects associated with chronic treatment also are primarily dose-related cerebellar-vestibular effects but also include other CNS effects, behavioral changes, increased frequency of seizures, GI symptoms, gingival hyperplasia, osteomalacia, and megaloblastic anemia and hirsutism .Serious adverse effects, including those on the skin, bone marrow, and liver, probably are manifestations of drug allergy. Although rare, they necessitate withdrawal of the drug[8]. In order to retain the beneficial effects of phenytoin and decrease the adverse effects,(by decreasing the individual dose) we need to look for combination of drugs[9]. Our study is a sincere attempt in this direction by combining different doses of supplementation with standard dose of phenytoin.

Conclusion

1) Phenytoin being standard established drug in the treatment of seizures showed complete protection from MES induced seizures. In combination studies in group B,C and D the effect was better in low dose magnesium and phenytoin combination (group B) though the P value was not that significant. Compared to magnesium alone group, (group F) all the combination groups (group B,C and D) were better in controlling THLE. By supplementing magnesium oxide along with phenytoin we can reduce the standard dose of each drug and hence reduce the dose related adverse effects.

2) The P value was significant with respect to the F group compared to B group as the recovery time was better in only magnesium group. Group E(400mg/kg of phenytoin) rats were completely protected from the drug as none of them showed THLE positive results whereas the group F(500mg/kg of Mgo) showed partial protection as only half of them were protected and threw total hind limb extension. P value was significant with respect to the F group compared to B group as the recovery time was better in only magnesium group. This shows that Magnesium may help in faster recovery from seizure activity which has to be further studied.

3) Both the groups B(500mg/kg of Mgo +100mg/kg of phenytoin) and D(500mg/kg of Mgo+ 400mg/kg of phenytoin) showed significant protection against MES induced seizures.But there was no significant P value with respect to recovery period as both of groups recovered and there no significant difference as compared to magnesium alone group.

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