

EVALUATION OF ANTICONVULSANT ACTIVITY OF MAGNESIUM OXIDE ALONE AND ALONG WITH CARBAMAZEPINE

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ABSTRACT

Objectives: To evaluate the anticonvulsant effect of magnesium oxide alone and its activity alongside carbamazepine in albino rats.

Materials and methods: In MES (maximal electric shock) method, 36 male albino rats weighing between 100-200gm, were divided into 6 groups each containing 6 animals. All were treated with magnesium oxide (500, 1000mg/kg) for 10 days orally. On 11th day Carbamazepine (50, 30mg/Kg) was given orally an hour before MES induced seizures except control and only magnesium oxide groups. Abolition of tonic hind limb extension reflex and time taken to regain righting reflex were noted for each animal after MES induced seizures.

Results: Low dose oral administration (500mg/kg) for 10 days in healthy rat showed 33.33% protection to MES, while the group with high dose magnesium (1000mg/kg) showed no protection to MES. Low oral dose of magnesium (500mg/kg) along with low dose carbamazepine (30mg/kg) showed better results than groups with only carbamazepine and high dose carbamazepine (50mg/kg) along with low dose magnesium oxide (500mg/kg).

Conclusion: Low dose magnesium has anticonvulsant activity in MES seizure model, moreover combination of low dose magnesium oxide with low dose carbamazepine showed better results considering both the end points (THLE and TRR) in MES induced seizure model.

Key words: THLE (Total hind limb extension abolition), TRR (Time taken to regain righting reflex), MgO (Magnesium oxide)

INTRODUCTION

Traditional medicinal practices have remained as a component of health care system of many societies in spite of the availability of well established alternatives¹. Epilepsy is a chronic neurological disorder characterized by a person's predisposition to generate repeated seizures due to hypersynchronous neuronal activity in a brain. Epilepsy is one of the most common of the serious neurological disorders.² About 3% of people will be diagnosed with epilepsy at some time in their lives.³ The treatment aspect is constantly evolving starting from bromides which provided the first line of treatment to the most recent newer antiepileptics.⁴

Current trends in the treatment of epilepsy with the standard epileptic drugs is known to have been associated with dose related toxicities, drug interactions and refractoriness. The corollary is to achieve a high therapeutic plasma concentration with minimal toxicity. Hence, the hunt for a substance or a drug which enhances the efficacy and reduce the toxicity continues.

Carbamazepine is a iminostilbene derivative approved for the treatment of epilepsy since 1974. It causes dose dependent side effects like diplopia, dizziness, nausea, drowsiness, lethargy, idiosyncratic reactions, aplastic anemia and hyponatremia.⁵

Studies of neuroactive amino acids in surgically excised focally epileptic human brain tissue depict the involvement of these amino acids in epilepsy, in that concentrations of glutamate, aspartate and glycine are significantly increased in epileptogenic cerebral cortex⁶. Magnesium the endogenous antagonist of NMDA receptors, is necessary for normal function, and obviously "well tolerated". Mg²⁺ shows strong voltage-dependency and very low affinity which is associated with fast blocking kinetics. Mg²⁺ which normally acts as a filter or switch is too weak to serve this role and the NMDA receptor can no longer function as a coincidence detector. Magnesium ions and various salts have been used by different routes like intravenous, intracranial, intraperitoneal, subcutaneous, have known to exert a significant anticonvulsant effect in animal experimental models.⁷⁻¹⁰ It has been shown that magnesium is actively transported across the blood brain barrier¹¹, and even on supplementing magnesium oxide orally, significant serum magnesium levels can be achieved.⁷

Considering this concept, the current study was planned to notify if there was any effect with the use of magnesium in epilepsy and also its combination with carbamazepine in albino rats.

MATERIALS AND METHODS

Chemicals

Magnesium oxide was purchased at Davangere while carbamazepine was obtained from Sun Pharmaceuticals.

Animals

36 Healthy male albino rats weighing 100-200 grams were selected and procured from the Central Animal house of JJM Medical College, Davangere after screening. Animals were inbred under suitable conditions of housing, temperature, ventilation and nutrition with a 12hour light: 12hour dark cycle. The animals will have free access to standard pellet and water.

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.

Groups

MES -Induced Seizures: The various groups were as follows:

- Group A: Control Group: Received Normal Saline
- Group B: Low dose Magnesium Oxide (500mg/kg)
- Group C: High dose of Magnesium Oxide (1000mg/kg)
- Group D: Low dose Magnesium Oxide (500mg/kg) + High dose Carbamazepine (50mg/kg)
- Group E: Low dose Magnesium Oxide (500mg/kg) + low dose Carbamazepine (30mg/kg)

Group F: Carbamazepine (50mg/kg) only.

The study was approved by the Animal Ethics Committee, JJM Medical College, Davangere.

Albino rats of either sex weighing between 100-200gms maintained in well ventilated animal house will be used for the study. The animals were allowed access to water ad libitum and standard pellet diet. The animals were divided into six groups of six animals each. In MES method, 36 male albino rats weighing between 100-200gm, were divided into 6 groups each containing 6 animals were all treated with magnesium oxide(500,1000mg/kg) for 10 days orally. On 11th day Carbamazepine (50,30mg/Kg) was given orally an hour before MES induced seizures except control and only magnesium oxide groups. Abolition of tonic hind limb extension reflex and time taken to regain righting reflex were noted for each animal after MES induced seizures.

MES Induced Seizures

A stimulus of 180mA for 0.2 s duration was given using the electroconvulsimeter and the responses were tested 1 hour after administration of the concerned drug orally.

Parameters Observed: Abolition of tonic hind limb extension and time taken to regain righting reflex.

Statistical Analysis

The data was analysed ANOVA and Student t test. P value was significant < 0.05.

RESULTS

GROUP A: Control group treated with normal saline showed 0% protection for THLE and TRR(time taken to regain righting reflex), mean value being 105.8s with standard deviation being 19.71.

GROUP B: Low dose magnesium oxide 500mg/kg showed 33% protection for THLE and TRR (time taken to regain righting reflex), mean being 110.4s with standard deviation being 7.40.

GROUP C: High dose magnesium oxide 1000mg/kg showed 16.6% protection for THLE and TRR(time taken to regain righting reflex), mean being 97s with standard deviation being 18.49.

GROUP D: High dose Carbamazepine (50 mg/kg) + low dose Magnesium(500mg/kg) showed 100% protection for THLE and TRR (time taken to regain righting reflex) ,mean being 77.4 with standard deviation being 13.18.

GROUP E: Low dose Carbamazepine (30mg/kg) + low dose Magnesium oxide(500mg/kg) showed 100% protection for THLE and TRR(time taken to regain righting reflex), mean being 43.4 and standard deviation 8.8.

GROUP F: Carbamazepine only. Showed 100% protection for THLE and TRR(time taken to regain righting reflex), mean being 68.6 and standard deviation being 31.17.

Table 1: Response for THLE (total hind limb extension) abolition by MES method

Groups	Parameter		Protection %
	+	-	
A	6	0	0
B	4	2	33.33
C	5	1	16.6
D	0	6	100
E	0	6	100
F	0	6	100

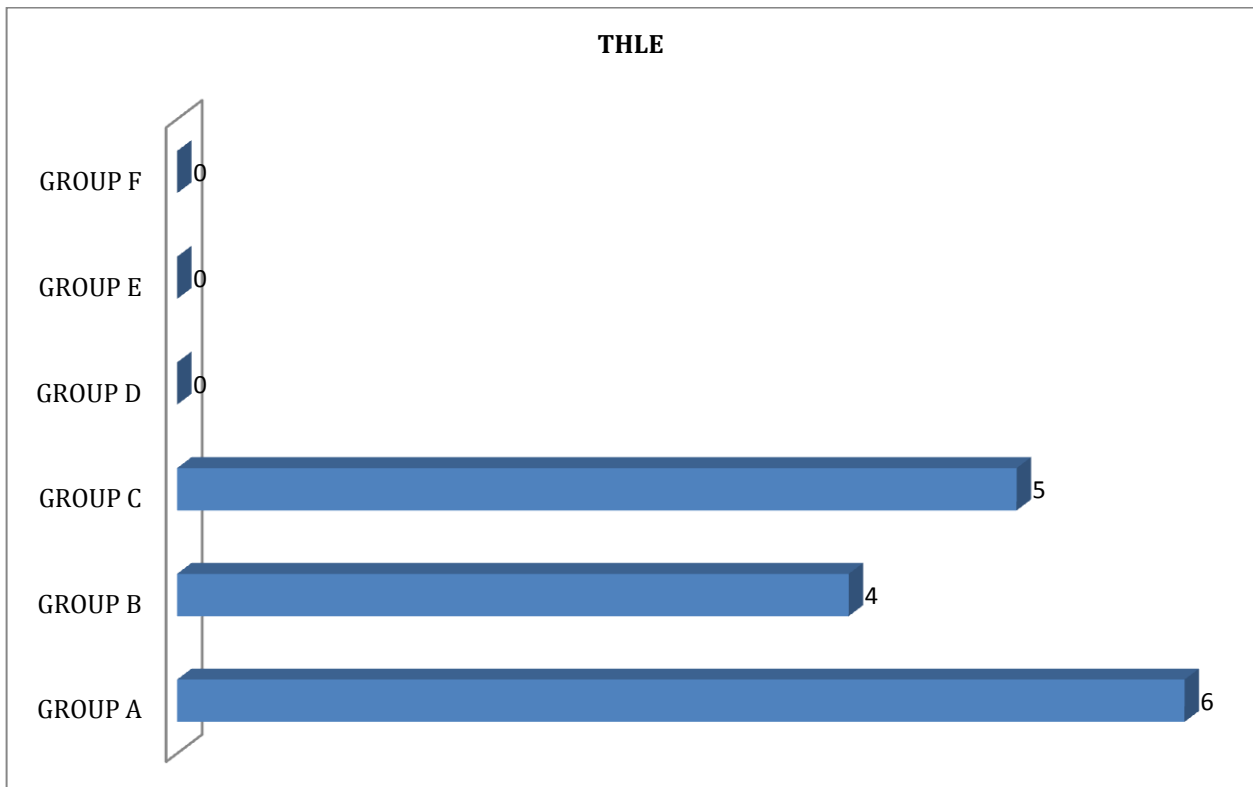


Fig1: Bar Chart

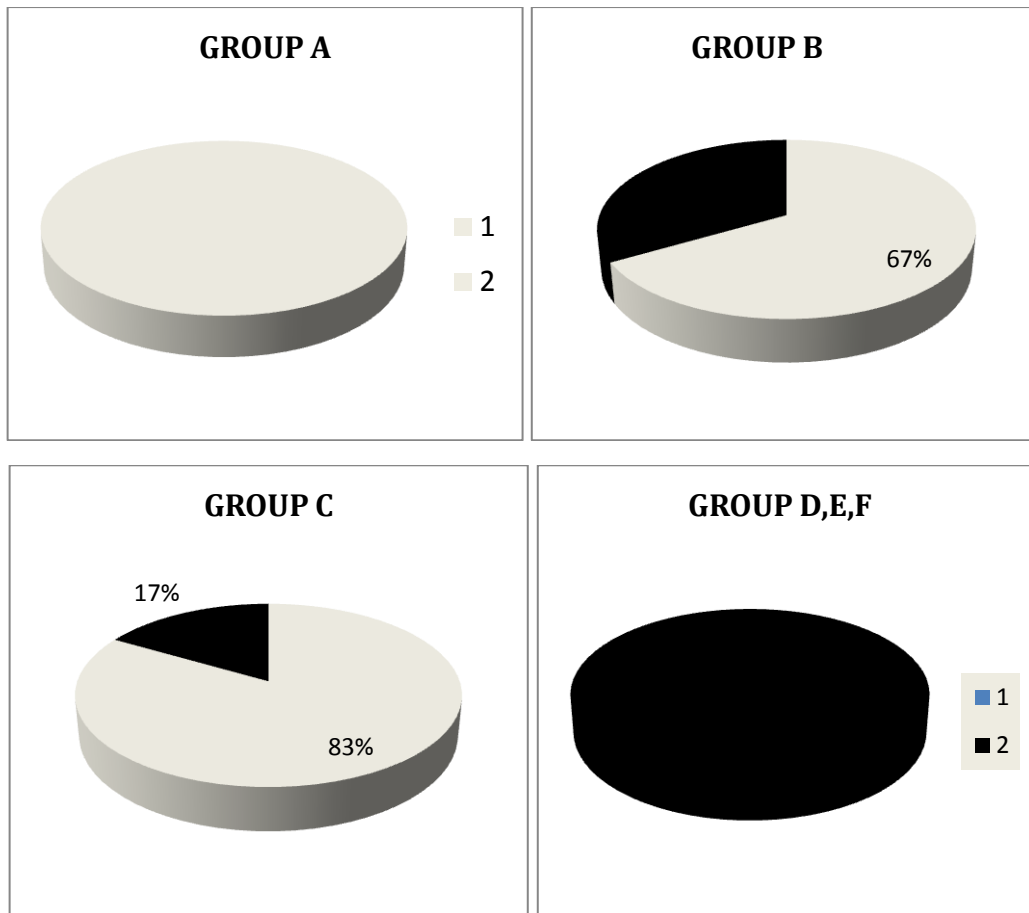


Fig 2: Pie Chart

Black colour denotes protection(%) for THLE, White colour denotes no protection.

Table 2: Time taken to regain righting reflex in seconds(TRR)

GROUPS	A	B	C	D	E	F
MEAN	105.8	110.4	97	77.4	43.4	68.6
SD	19.71	7.40	18.49	13.18	8.82	31.17

f = 11.724

p = 0.0001

Comparing with the control, the p value is highly significant, whilst when comparing between individual groups .

Group D vs Group E : t = 3.68

p = .0043 which is significant suggesting that thou the primary end point was same among the groups being 100% abolishment of THLE , when secondary end point was considered(TRR) group E (Low dose magnesium alongside low dose carbamazepine was much effective and statistically gave a significant p value. When secondary end points between other groups were calculated statistically the results were insignificant, but clearly low dose magnesium oxide along with low dose magnesium produced significant results. Thus, it is apparent from the present study that magnesium does have some beneficial effects in MES seizure model. Hence supplementation of low dose carbamazepine along with low dose magnesium could moreover play an important role in grandmal epilepsy.

DISCUSSION

Undertaken study demonstrates that magnesium oxide does have a protective role against MES induced seizure method. Hence, the above statement reveals its usage in protection against seizure, the dose used being critical , and needs to be decided. Already two decades ago a study with DBA/2 mice (audiogenic seizure model) showed that the NMDA-receptor is deeply involved in the initiation

or spread of epileptic neuronal hyperactivity. Studies of neuroactive amino acids in surgically excised focally epileptic human brain tissue depict the involvement of these amino acids in epilepsy, in that concentrations of glutamate, aspartate and glycine are significantly increased in epileptogenic cerebral cortex¹². The motto behind supplementing magnesium for a 10 day period is that, Magnesium helps in slowing the spread of electrical discharge from one area of the brain to the rest and therefore magnesium depletion can cause a marked irritability of the nervous system, eventually resulting in epileptic seizures^{13,14}. The magnesium depletion here refers to that in the CSF or in the brain, which is associated with the development of seizures¹⁵. CSF magnesium concentrations can readily be replenished following magnesium supplementation¹³, and this may explain the protective effect observed in the magnesium groups after a 10 day supplementation with oral MgO. The NMDA receptor is modulated by a number of endogenous and exogenous compounds, Mg²⁺ not only blocks the NMDA channel in a voltage-dependent manner but also potentiates NMDA-induced responses at positive membrane potentials, Channel is blocked by Mg²⁺ at resting membrane potential . Thus, NMDAR are silent until the activated AMPA receptors have sufficiently depolarized the neuronal membrane in order to relieve Mg²⁺-block, A positive change in transmembrane potential will make it more likely that the ion channel in the NMDA receptor will open by expelling the Mg²⁺ ion that blocks the channel from the outside. This property is fundamental to the role of the NMDA receptor in memory and learning, The normal function of the NMDA receptor complex depends on the dynamic equilibrium among its various parts. Loss of equilibrium may block the function of the entire system and result in the expression of excitotoxicity. The statement that magnesium is a physiological Ca²⁺ blocker¹⁶ suggests that magnesium competes with the Ca²⁺ uptake presynaptic reducing Ca²⁺ dependent neurotransmitter release which might exert magnesiums central anticonvulsant activity. The

other mechanism is that magnesium plugs the channel and does not allow access to other permeable ions (Ca^{2+}) in a voltage dependent manner.

Thus, it is apparent from the present study that magnesium does have some beneficial effects in MES seizure model. Hence supplementation of low dose carbamazepine along with low dose magnesium could moreover play an important role in grandmal epilepsy. The corollary to achieve high therapeutic concentration with minimal drug toxicity can be achieved by the above study and needs further application in clinical studies.

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