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Research Article

INVIVO-ANTICONVULSANT ACTIVITY OF ACTIVE MOIETY OF GAP JUCTIONAL BLOCKER CARBENOXOLONE IN ALBINO RATS

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ABSTRACT

Epilepsy is a common disorder with an incidence of approximately 0.3-0.5% in different populations throughout the world and prevalence of 5-10% per 1000. The current drug discovery process for antiepileptic drugs should be reevaluated. Current evidence strongly suggests a role for gapjunctional communication in neuronal synchrony and seizure generation undernormal and pathological conditions. So that gapjunctional blockers likecarbenoxolone of mechanism of action was quite clear in this study. The carbenoxolone is an analogue of triterpinoid, derived from glycyrrhizinic acid a natural constituent of liquorice. This has been shown to have the property of inhibiting gapjunctional interneuron communication at the cellular level. The objective of this study was to screen the anticonvulsant activities by PTZ and MES methods. Methods: Carbenoxolone was tested for anticonvulsive effect in albino rats subjected to seizures by the PTZ and MES at three doses 100 m/kg, 200 m/kg, 300 m/kg. In the PTZ model parameters observed were seizure protection, seizure latency and seizure duration. In the MES model parameters observed were seizure protection and seizure duration.Results: In the PTZ model carbenoxolone produced a statistically significant increase in seizure latency, decrease in seizure duration and seizure protection. In the MES model carbenoxoloneproduced a statistically significant decrease in seizure duration (8.6Sec) at the dose of 300 mg/kg.Conclusion: Carbenoxolone has invivo anticonvulsive effect and could be useful in both petit mal and grand mal seizures. The protective effect of carbenoxolone could be due to blockade of GJ channels that mediate electrotonic coupling and thereby prevent the neural synchronization that is characteristic of seizures. The study also supports the view that gap junctions have a functional role in the electrophysiology of seizures and gapjunctional blockers have potential as a new class of antiepileptic drugs.

Key words: Carbenoxolone, gap junctions, anticonvulsant, PTZ, MES

INTRODUCTION

Epilepsy is a common heterogeneous disorder with an incidence of approximately 0.3-0.5% in different populations throughout the world and prevalence of 5-10% per 1000. The drug therapy of epilepsy is empirical therapy and doesn't address the underlying pathophysiology. Also, in spite of addition of large number of efficacious antiepileptic drugs provide relief in only up to 75% patients with absence of seizures [1]. The gap junctional communication has its important role in electrophysiology of CNS [2].

The gapjunctional communication enables high transmission speeds, an ability to transmit sub threshold signals in a reciprocal manner and synchronization of firing of neurons in a network [3]. Current evidence strongly suggests a role for gapjunctional communication in neuronal synchrony and seizure generation undernormal and pathological conditions [4]. Evidence from invitro studies show that inhibitors of GJ communication can inhibit seizures discharges [5].The generated seizure impulses were transmitted through GJ so that GJ blockers like carbenoxolone play a significant role in prevention of attack of seizures.

The progression from discoveries of promising agents made at cellular and molecular levels into the next stage of development requires screening in validated animal models. Thus carbenoxolone is being screened for anticonvulsive effects invivo to validate the findings made at the cellular level into the mechanisms in epilepsy and also for possible development of a new class of AEDs.

OBJECTIVES

The objectives of present investigations are:

- To study the anticonvulsant activity of carbenoxolone in experimentally induced seizures in Wister albino rats models.
- To probe the functional role of gap junctions in seizures.

METHODS

Experimental animals

Wistar albino rats for the study were obtained from the Central Animal House, JJM Medical College, Davangere. The animals were housed under standard conditions with free access to food and water. For the experiment healthy male rats between 150-200g showing normal behavior and activity were chosen. The rats were previously unused for any other experiment. The acute toxicity study was performed as per OECD guide lines. The study was conducted with the approval of the Institutional Ethical Committee, JJM, Medical College, Davangere and in accordance with their guidelines.

Drugs and Chemicals

Pentylenetetrazole, Diazepam and Carbenoxolone were procured through NICE chemicals, Bangalore (India).

Assessment of anticonvulsant activity by Pentylenetetrazole (PTZ) method

Thirty five albino adult rats of either sex weighing between150-200 were selected and divided into five groups consisting of 7 animals each to carry out another experiment by chemicals [6]. All the animals were fasted for 24 hrswith *ad libitum.*PC- control group received normal saline (i.p), PS- group received standard drug (diazepam) – 0.5mg/kg (i.p), P1- group received test drug (carbenoxolone) -100 mg/kg (i.p), P2- group received test drug (carbenoxolone) -200mg/kg (i.p) and P3- group received test drug (carbenoxolone) -300 mg/kg (i.p). The grouped rats were marked for identification and placed in separate labeled cages.

The drugs were injected to rats prior to subjecting them to chemo convulsion by pentylenetetrazole (70 mg/kg.,s.c. into the scruff of the neck). The control group rats were injected normal saline 60 minutes before, the standard group were injected diazepam 30 minutes before and test groups were injected carbenoxolone 60 minutes before. Animals are observed

over a 30 minutes for the occurrence of seizures. The occurrence of tonic clonus for more than 5 seconds was taken as a positive seizure response and abolition of tonic clonus was considered as protection against PTZ seizures. The parameters noted were: i) occurrence of seizure, ii) seizure latency (time for onset of seizure) and iii) duration of tonic clones. Results are tabulated in Table-1.

Assessment of anticonvulsant activity by Maximal Electroshock (MES) method

Thirty five albino adult rats of either sex weighing between150-200 were selected and divided into five groups consisting of 7 animals and fasted for 24 hrs with *ad libitum.* MC- control group received normal saline, MS- standard drug (diazepam) group received -5mg/kg, M1- test drug group received -100mg/kg, M2- test drug group received -200mg/kg and M3- test drug group received -300mg/kg. The grouped rats were marked for identification and placed in separate labeled cages.

After half an hour of the treatment the convulsions were induced by delivering current of 150mA through corneal electrodes foe a period of 0.2sec using electroconvulsiometer [7]. The occurrence of a tonic hind limb extensor was taken as a positive response for MES. Abolition of tonic hind limb extensor was taken as protective against MES seizures results are tabulated in Table-2.

Table 1: Comparison between groups with PTZ-induced Seizures

Rat Group	Mean Seizure Latency Mean ± S.E	P Value	Mean Seizure Duration Mean ± S.E	P Value	Seizure Protection %
PC	127.3 ± 5.97	------	767 ± 15.76	-----	0%
PS	794.3±50.65 ***	<0.0001	80.3±38.17***	<0.0001	57.14%
P100	140.9 ± 3.7 ^	0.078	719.7±17.65 ^	0.069	0.0%
P200	339.1 ± 93.73 *	0.044	463.1 ± 80.13 *	0.0029	14.28%
P300	598 ±107.34 ***	0.0009	298.9±77.48 ***	<0.0001	28.56%

PC: (Normal Saline); PC: Diazepam 0.5 mg/kg); P100: Carbenoxolone 100 mg/Kg; P200: Carbenoxolone 200 mg/Kg; P300: Carbenoxolone 300 mg/Kg. Results are expressed as Mean ± S.E and analysed for significance by the Turkey-Kramer Testp>0.05 not significant, * p<0.05 significant, ** p<0.01 very significant, *** p<0.001 highly significant.

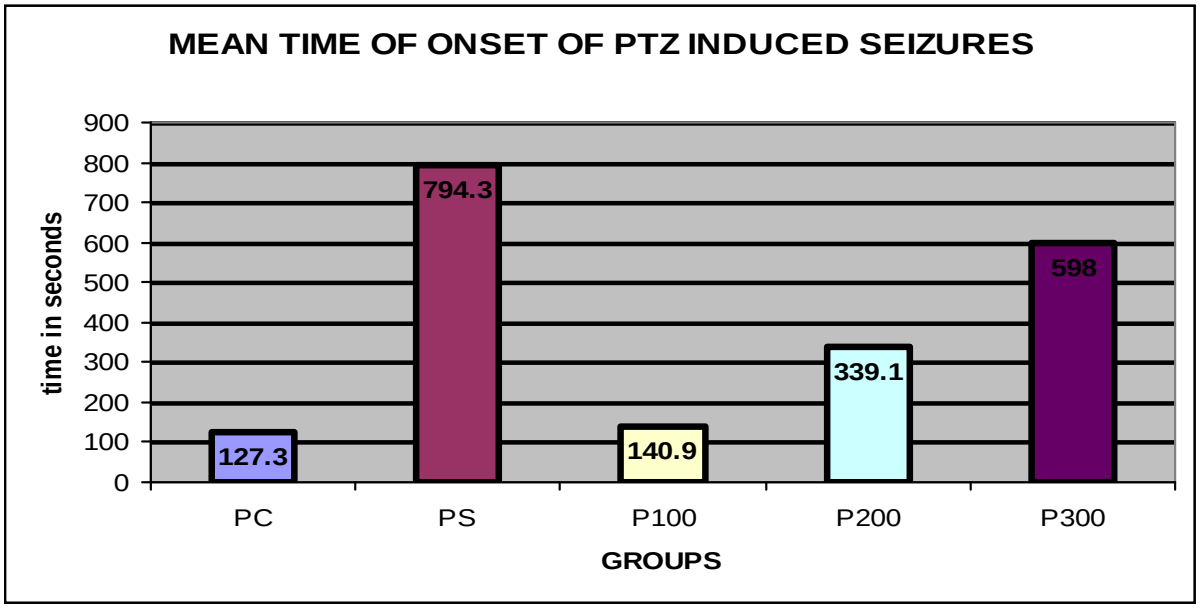

Fig 1: PC: Normal Saline; PC: Diazepam 0.5mg/kg; P100: Carbenoxolone 100mg/Kg; P200: Carbenoxolone 200mg/Kg; P300: Carbenoxolone 300mg/Kg

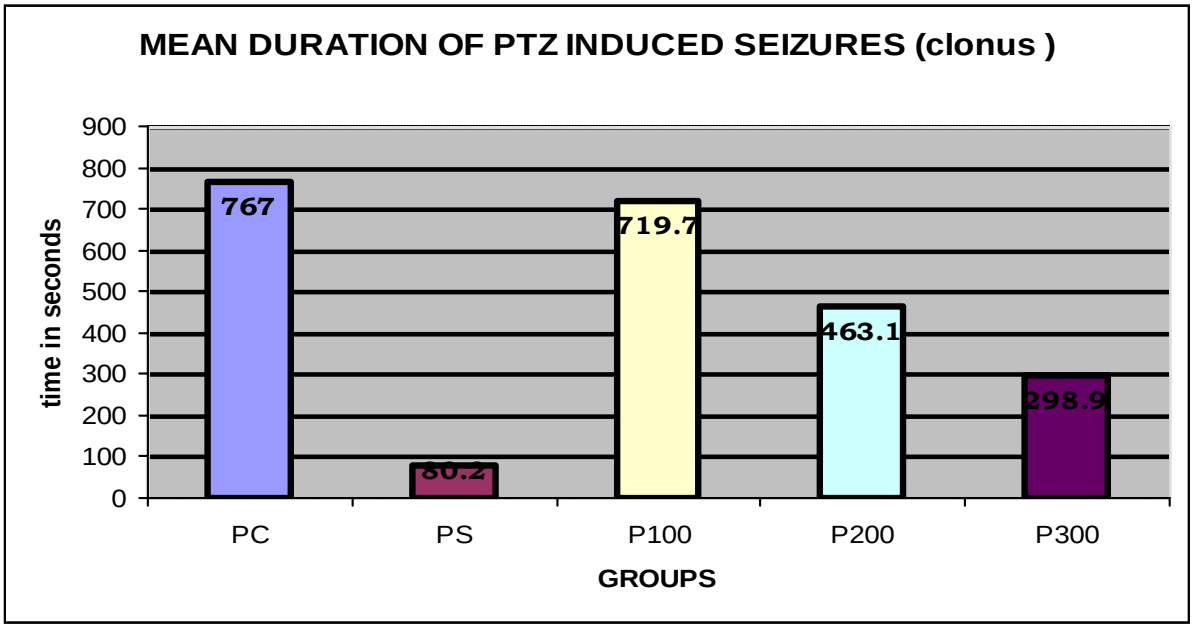

Fig 2:PC: Normal Saline); PC: Diazepam 0.5mg/kg); P100: Carbenoxolone 100 mg/Kg; P200: Carbenoxolone 200 mg/Kg; P300: Carbenoxolone 300mg/Kg

Table 2: Comparison of groups with MES induced Seizures

Rat Group	Treatments	Seizure %	Seizure duration Mean ± S.E	p value	Seizure Protection %
MC	Normal Saline	100%	10.6± 0.57	--------	0%
MS	Diazepam	42.85%	3.1 ± 1.52 ***	0.0006	57.14%
M100	Carbenoxolone 100 mg/Kg	100%	9.7 ± 0.71 ^	0.37	0%
M200	Carbenoxolone 200 mg/Kg	100%	9.1 ± 0.51 ^	0.086	0%
M300	Carbenoxolone 300 mg/Kg	100%	8.6 ± 0.48 *	0.02	0%

Results are expressed as Mean ± S.E and analysed for significance by the Tukey-Kramer Test*p<0.05 significant, ***p<0.001 highly significant, ^ P>0.05 not significant.

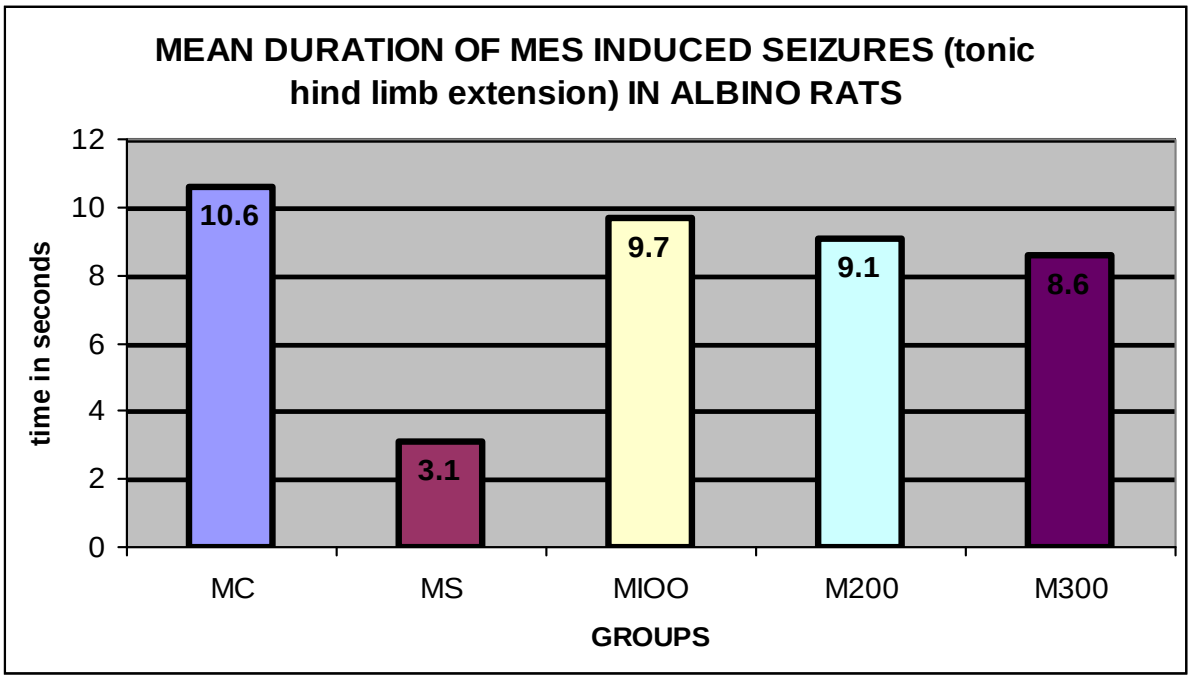

Fig 3:MC: control group; MS: standard group (diazepam); M100: test group (carbenoxolone 100mg/kg); M200: test group (carbenoxolone 200mg/kg); M300: test group (carbenoxolone 300mg/kg)

RESULTS AND DISCUSSION

In this study the protection provided by carbenoxolone seems to be stronger in the PTZ model than the MES model. In the PTZ model carbenoxolone provides seizure protection in addition to reducing the duration of seizures. In the MES model carbenoxolone only produced a

reduction in the seizure duration but did not provide seizure protection. The results of this study support similar studies conducted on mice by Hosseinzadehet.al., in which carbenoxolone showed anti convulsant activity in both PTZ and MES models[8].

The study also provides evidence for the possible role of gap junctions in seizure as blocking of the gap junctions was associated with a protective effect against seizures. Gapjunctions could therefore be promising targets in the search for new antiepileptic drugs.The test samples are effective in the PTZ model are usually thought to have activity against absence seizures(petit-mal seizures) and drugs showing activity in the MES model are presumed to have effect against tonic-clonic seizures(grand mal seizures)[9].

The exact mechanism of action of carbenoxolone is still speculative. Carbenoxolone is supposed to bind to the connexin molecules of the GJs between neurons, leading to a conformational change and this possibly leads to closure of the GJs [10]**.** This blockade could hinder the development of electrotonic coupling between neural populations and thereby prevent neuronal synchronization in the brain. Synchronization being an important feature in the electrophysiology of seizure spread, the blockade could be the reason for the anti-seizure activity of carbenoxolone.

CONCLUSION

Carbenoxolone has invivo anticonvulsant activity in both PTZ and MES induced seizures and may be useful in absence seizures and tonic-clonic seizures.

- The anticonvulsant action is better against PTZ induced seizures than MES seizures
- The study tends to support to the view that gap junctions have a role in pathophysiology of seizures
- Pharmacological agents that block gap junctions blockers could represent a potential new treatment for epilepsy.

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