

Potential of anticonvulsant activity of phenytoin by calcium channel blockers (verapamil and amlodipine) against maximal electroshock seizures in rats

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ABSTRACT

Background: The objective of this work was to study the anticonvulsant activity of calcium channel blockers verapamil and amlodipine and their interaction with an established antiepileptic drug diphenylhydantoin (DPH) in experimental model of epilepsy in albino rats.

Methods: Maximal electroshock (MES) convulsions were induced by electroconvulsimeter and different phases of MES were noted in control and drug treated groups. Effect of different doses of verapamil (dose-5, 10 and 15 mg/kg), amlodipine (dose-2.5, 3 and 3.5 mg/kg) and DPH (dose-0.5 and 1 mg/100 g) on MES was studied. Finally, effect of combined treatment consisting of non-protective dose of DPH with different doses of verapamil and amlodipine were also studied on MES induced seizures.

Results: Combination of non-protective dose (0.5 mg/100 g) of DPH with all the three doses of verapamil and amlodipine offered significant protection against MES induced seizures.

Conclusions: From the present investigation, it may be concluded that the dose of DPH may be reduced in an antiepileptic individual who is on verapamil and amlodipine therapy.

Keywords: Epilepsy, Seizures, Diphenyl hydantoin, Maximal electric shock, Verapamil, Amlodipine

INTRODUCTION

Epilepsy comprises of sudden excessive depolarization of group of cerebral neurons, which may remain localized or which may spread to cause generalized seizures.

The action potential develops due to opening up to voltage dependent ion channels which provides rapid change in ion permeability.¹ This leads to rapid prolongation of signals along axon and cause excitation secretion coupling that release neurotransmitters from the presynaptic sites. Neurotransmitters are stored within the synaptic vesicles.² During the development of action potential ion channels upon leading to influx a calcium ion. This calcium ion causes the fusion of pre-synaptic vesicles to the pre-synaptic axonal membranes. The point to their fusion breaks open and there is release of neurotransmitters,

which interact with post-synaptic receptors to produces the response.^{3,4}

The release of neurotransmitters can directly be affected by calcium channel blockers. Calcium channel blockers have central effect after penetration through blood brain barrier, but against central form of neuronal activation when voltage operated channels activated relatively for long time as in epilepsy.⁵

Studies with diphenylhydantoin (DPH) and carbamazepine have suggested that neuronal calcium channel blocker may be important in preventing seizures propagation.⁶

Some investigators have reported that calcium channel blockers may prevent seizures induced by variety of physical and chemical stimuli.^{7,8} Flunarazin has been found

to be useful in reducing seizure activity in experimental epilepsy.

The present study has been undertaken to evaluate antiepileptic activity of verapamil and amlodipine in experimental epilepsy maximal electroshock (MES) in albino rats in different doses and to work out any additive effect with an established and commonly used antiepileptic drug, DPH.

METHODS

This study was conducted in the Pharmacology Department of Dr. Sampurnanand Medical College, Jodhpur, Rajasthan, India.

Animal used

Healthy albino rats weighing 150-200 g were used for throughout the study. All the animals were maintained in the department animal's house at the room temperature of 25°-30° with food and water available ad libitum.

Drug used

- Phenytoin-doses used were 0.5, and 1.0 mg/100 g intellectual property (IP) given 30 mins before the test.
- Amlodipine-doses used were 2.5, 3 and 3.5 mg/kg IP was administered 6 hrs before the expose to MES.
- Verapamil-doses used were 5, 10 and 15 mg/kg IP was administered 30 mins prior to the MES.

Electroshock seizures

In each animals, the ear was cleaned with cotton dipped in saline (saline facilitate the electric current). MES was induced by techno electroconvulsimeter (120 mA, 0.2 sec duration) through ear electrodes. Duration of tonic extensor phases of MES (the more important phase of seizure pattern) were noted with the help of stop watch. The abolition and reduction of tonic extensor phase were considered as an index for anti-epileptic activity of the drugs under study. The same procedure was done for all groups of animal. The animals were divided in the following group (n=6 for each group).

Group 1	Received saline (control) intraperitoneally
Groups 2 and 3	Two doses of DPH (0.5 mg/100 g and 1.0 mg/100 g) i.p. respectively.
Groups 4, 5 and 6	Three doses of verapamil (5 mg/kg, 10 mg/kg and 15 mg/kg) i.p. respectively.
Groups 7, 8 and 9	Three doses of amlodipine (2.5 mg/kg, 3.0 mg/kg and 3.5 mg/kg) i.p. respectively.
Group 10	DPH (0.5 mg/100 g)+verapamil (5 mg/kg) i.p.

Contd...

Group 11	DPH (0.5 mg/100 g)+verapamil (10 mg/kg) i.p.
Group 12	DPH (0.5 mg/100 g)+verapamil (15 mg/kg) i.p.
Group 13	DPH (0.5 mg/100 g)+amlodipine (2.5 mg/kg) i.p.
Group 14	DPH (0.5 mg/100 g)+amlodipine (3.0 mg/kg) i.p.
Group 15	DPH (0.5 mg/100 g)+amlodipine (3.5 mg/kg) i.p.

RESULTS

MES convulsion induced by electro convulsimeter and different phases of MES were noted in control and drug treated groups.

Effect of different doses of verapamil (dose-5, 10 and 15 mg/kg), amlodipine (dose-2.5, 3 and 3.5 mg/kg) and DPH (dose-0.5 and 1 mg/100 g) on MES was studied. Finally, effect of combined treatment consisting of non-protective dose of DPH with different doses of verapamil and amlodipine were also studied on MES induced seizures.

DPH produced a dose dependent protection against MES induced seizures; however, all the three doses of both the change control boards (CCBs) under study could not protect the animals form MES induced seizures.

Combination of non-protective dose of DPH with all the three above mentioned doses of verapamil and amlodipine offered significant protection against MES induced seizures (Tables 1 and 2).

From the present investigation, it may be conclude that the dose of DPH may be reduced in an antiepileptic individual who is on verapamil and amlodipine therapy for other clinical conditions.

DISCUSSION

Epilepsy is a chronic disorder of central nervous system with a prevalence varying between 3 and 6/1000 of population it is a collective term for a group of chronic convulsive disorders.

The results of the present investigation suggest CCBs verapamil and amlodipine do not possess significant protective effect against MES seizures in rats. Combination of verapamil and amlodipine with DPH, enhance the anticonvulsant activity of phenytoin in experimental convulsions. A study was carried out by Kaminski et al. (2001) found that amlodipine does not seem a good candidate for a combination therapy in epileptic patients because of its adverse potential.⁹

Phenytoin is one of the most widely used anticonvulsant drug the major mechanism of anticonvulsant activity of phenytoin in excitable tissues involves a decrease in sodium influx in the neurons. It is likely that anticonvulsant action

Table 1: Effect of different doses of combination of DPH and verapamil on different phases of MES.

DPH (mg/100 g)+ verapamil (mg/kg) treatment	Phase of MES (in sec)					
	Flexor state		Extensor spasm		Clonus	
	% reduction		% reduction		% reduction	
Normal saline	1.8		9.0		5.3	
0.5+5	1.8	0	4.6	48.88	5.1	3.77
0.5+10	1.7	5.55	3.0	66.66	5.0	5.66
0.5+15	1.8	0	2.8	68.66	5.0	5.66

DPH: Diphenylhydantoin, MES: Maximal electroshock

Table 2: Effect of different doses of combination of DPH and amlodipine on different phases of MES.

DPH (mg/100 g)+ amlodipine (mg/kg) treatment	Phase of MES (in sec)					
	Flexor state		Extensor spasm		Clonus	
	% reduction		% reduction		% reduction	
Normal saline	1.8		9.0		5.3	
0.5+2.5	2	11.11	3.0	66.66	5.1	3.77
0.5+3.0	1.9	5.55	2.5	72.22	5.1	3.77
0.5+3.5	2.0	11.11	2.3	74.44	5.0	5.66

DPH: Diphenylhydantoin, MES: Maximal electroshock

of DPH may be in part due to inhibition of calcium influx under depolarization condition.

Though the exact mechanism of antiepileptic effect of CCBs is not clear, from the present investigation it may be speculated that they potentiate the antiepileptic activity of phenytoin possibly by blocked of voltage dependent calcium channel and thus exert a synergistic effect with a low dose of phenytoin, which inhibits sodium influx to a certain extent but not the calcium influx during depolarization.

Therefore, it is likely that enhancement of anticonvulsant action of phenytoin by amlodipine and verapamil could be due to either.

1. Blocked of voltage operated calcium channels in brain or
2. Pharmacokinetic interaction as reported by Kaminski et al. (1999) or both.

CONCLUSION

From the present investigation, it may be concluded that the dose of DPH may conjectured that the dose of DPH may be reduced in an antiepileptic individual who is on verapamil and amlodipine therapy.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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