

Evaluation of anticonvulsant activity of amlodipine in albino rats

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ABSTRACT

Background: The objective of the study was to evaluate the anticonvulsant activity of amlodipine in albino rats.

Methods: Anticonvulsant activity of amlodipine was done in three graded doses (1 mg/kg, 2 mg/kg, 4 mg/kg), and combination group with low dose of amlodipine (1 mg/kg) and standard drug (phenytoin) in maximal electroshock seizures (MES) experimental animal model.

Results: Amlodipine in dose of 2, 4 mg/kg showed dose dependent significant anticonvulsant effect and combination of low dose amlodipine and low dose of standard drug also showed significant anticonvulsant effect in MES model.

Conclusions: Amlodipine is having anticonvulsant activity and also potentiated the anticonvulsant effect of phenytoin in MES model.

Keywords: Amlodipine, Anticonvulsant, Maximal electroshock seizures, Phenytoin

INTRODUCTION

Epilepsy is a chronic neurological disease, which affects roughly 1% of the human population.¹ The initiation of seizure is associated with high frequency burst of action potential (AP), i.e. caused by long lasting depolarisation of the neuronal membrane triggered by a large influx of calcium ions into cells.^{2,3} Although, vast number of drugs were introduced for the treatment of epilepsy, still there is 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.⁴ Various new drugs with their own unique advantages have been introduced, but failed to provide satisfactory seizure control because of dose related side effects. So there is an ever increasing need for research and development of newer drugs for the treatment of epileptic seizures.

As there is role of calcium channels in the initiation of seizure potential, there may be a role of calcium channel blocker (CCBs) like amlodipine, nifedipine, nimodipine in the treatment of epilepsy.

Amlodipine belongs to the 1, 4-dihydropyridine (DHP) group of CCBs which are lipophilic in nature.⁵ Amlodipine has proven efficacy in cardiovascular diseases such as hypertension and angina pectoris. In contrast to other calcium channel blockers, amlodipine exhibits unique features that afford a smooth gradual onset of action and sustained effect that provides for continuous and consistent activity throughout a 24 hour period. It does not alter the plasma concentration of concurrently used other antiepileptics.^{6,7}

With these advantages of Amlodipine over other DHPs, the present study was conducted to evaluate the anticonvulsant activity of amlodipine on electroconvulsion model in albino rats.

METHODS

The study was carried out in Research Laboratory, Department of Pharmacology Kamineni Institute of Medical Sciences, Narketpally, Nalgonda, Telangana during the period of 2013 to 2014.

Choice and care of animals

Adult healthy wistar albino rats of either sex, weighing 150-200gm were used in this experiment. The animals were procured from National Institute of Nutrition (NIN), Hyderabad. All the animals were housed in an air cooled Central animal house of Kamineni Institute of Medical Sciences, Narketpally in accordance with the guidelines of CPCSEA. The animals were acclimatized to the laboratory conditions (12 hourly dark / light, 25 degree C).

Randomization and selection of rats

Selection was done randomly from the total rats available in the Central animal house, KIMS, Narketpally. Selection was done using random number table.

Design of experiment

Randomized control based study on laboratory animal (albino rats) models after approval from the Institutional Animal Ethics Committee (IAEC).

Maximal ElectroShock seizure (MES) model using the electroconvulsimeter

Rats were screened one day before the experiment by subjecting to maximal electroshock with an alternating current of 150 mA intensity for duration of 0.2 seconds through ear electrodes. Only those rats that showed characteristic course of convulsions were selected for the experiment. Animals were fasted overnight but water was given ad libitum. Amlodipine was procured from sigma aldrich, Hyderabad and Phenytoin injection from Vulcan laboratory PVT limited, Kolkata), distilled water were used. Seven groups of rats, each having 6 were taken for the study. Three groups of rats were administered Amlodipine in 3 graded doses (1, 2 and 4mg/kg). Among other groups, one group were administered distilled water (control) and 2 groups received Phenytoin (12.5mg/kg) and (25 mg/kg) taken as reference anticonvulsant drug and a group of combination of Amlodipine 1 mg/kg + Phenytoin 12.5 mg/kg (Table 1).

Table 1: Grouping of animals for Maximal Electro Shock Seizure model.

Groups	Drugs	Dose (mg/Kg) .	Route of administration
I	Distilled water (control)	5 ml/kg	Oral
II	Phenytoin	12.5	IP
III	Phenytoin	25	IP
IV	Amlodipine	1	Oral
V	Amlodipine	2	Oral
VI	Amlodipine	4	Oral
VII	Amlodipine + Phenytoin	1 +12.5	Oral + IP

Total animals taken for study are 42 and rats in each group are 6
IP - Intraperitoneal

Depending upon the group drugs were administered. All the groups were subjected to maximal electro shock at 150 mA intensity for 0.2 seconds in rats using ear electrodes 30 minutes after the intra peritoneal injection of the phenytoin drug groups and one hour after the oral administration of distilled water and amlodipine drug groups. In combination group amlodipine is administered one hour before followed by phenytoin 30 minutes before subjecting to maximal electro shock. Before putting ear electrodes normal saline was applied to prevent damage.

The following parameters are observed:

- Duration of Flexion in seconds
- Duration of Tonic hind limb extension (THLE) in seconds
- Duration of post-ictal sleep (PIS) which reflects loss of righting reflex in seconds.

The data is expressed in mean \pm standard error. Statistical analysis was done using one-way ANOVA and Post-hoc

test done by LSD method. SPSS version 19 was employed for statistical analysis

RESULTS

In MES model (Table 2) Amlodipine in doses of 2 and 4 mg/kg significantly decreased the duration of flexion (4.21 ± 0.16 and 3.21 ± 0.35 seconds $\alpha = 0.001$) comparison to control group (distilled water 5.4 ± 0.26 seconds). Combination of amlodipine 1 mg/kg and phenytoin sodium 12.5 mg/kg significantly decreased the duration of flexion (2.60 ± 0.22 seconds) in comparison to their individual drugs (amlodipine 1mg/kg 4.77 ± 0.23 and phenytoin 12.5 mg/kg 5.89 ± 0.26 seconds). The duration of tonic hind limb extension (THLE) was significantly decreased with 2 and 4 mg/kg doses of Amlodipine (7.17 ± 0.25 and 2.32 ± 0.08 seconds) in comparison to

distilled water (11.17 ± 0.35 seconds). The combination group significantly decreased the duration of THLE (1.48 ± 0.38 seconds) compared to their individual groups (amlodipine 1 mg/kg 10.95 ± 0.17 seconds and phenytoin 12.5 mg/kg 10.98 ± 0.14 seconds). Amlodipine 2 and 4 mg/kg significantly increased the duration of PIS (180.33 ± 3.49 and 205.00 ± 4.47 seconds respectively) in comparison to distilled water (132.17 ± 2.37 seconds). The combination group significantly increased the duration of PIS (230.67 ± 4.03 seconds) in comparison to their individual drugs (amlodipine 1mg/kg (141.33 ± 2.39) and phenytoin 12.5 mg/kg (133.83 ± 3.10 seconds) alone, while low dose amlodipine (1 mg/kg) and low dose phenytoin 12.5 mg/kg groups did not significantly affect the duration of flexion, THLE and PIS compared to distilled water.

Table 2: Effect of drugs in MES model.

Drug	Dose mg/Kg	Duration of flexion Mean \pm S.E.M (sec)	Duration of THLE Mean \pm S.E.M (sec)	Duration of PIS Mean \pm S.E.M(sec)
Distilled water (control)	5 ml/kg	5.4 ± 0.26	11.17 ± 0.35	132.17 ± 2.37
Phenytoin	12.5	5.83 ± 0.26	10.98 ± 0.14	133.83 ± 3.10
Phenytoin (standard)	25	$1.50 \pm 0.24^{**}$	$0.58 \pm 0.27^{**}$	$239.33 \pm 13.41^{**}$
Amlodipine	1	4.77 ± 0.23	10.95 ± 0.17	141.33 ± 2.39
Amlodipine	2	$4.21 \pm 0.16^{**}$	$7.16 \pm 0.25^{**}$	$180.33 \pm 3.49^{**}$
Amlodipine	4	$3.21 \pm 0.35^{**}$	$2.32 \pm 0.08^{**}$	$205.00 \pm 4.47^{**}$
Amlodipine + Phenytoin	1 + 12.5	$2.60 \pm 0.22^{**}$	$1.48 \pm 0.38^{**}$	$230.67 \pm 4.03^{**}$

Results are expressed in Mean \pm S.E.M. Test applied is ANOVA followed by Post hoc LSD test

* < 0.05, ** < 0.001

DISCUSSION

The initiation of epileptogenic activity in the neuron is due to "intrinsic burst firing" caused by large influx of calcium ions.² So, there may be a role of calcium channel blockers (CCBs) in the treatment of epilepsy. Amlodipine exhibits unique features like smooth and gradual onset of action compared to other CCBs. The side effects like palpitation, flushing, hypotension, headache, drowsiness and nausea produced by other CCBs like Nifedipine, verapamil and diltiazem are not seen with amlodipine. Other dihydropyridines are short acting while amlodipine is long acting. Amlodipine shows sustained effect which provides for continuous and consistent activity throughout a 24-hour period. Amlodipine does not alter the plasma concentrations of anti-epileptic drugs that are used concurrently.⁶

The present study is done to evaluate the anticonvulsant activity of amlodipine in albino rats in the experimental animal model that included MES model. In MES model, amlodipine in doses of 2 and 4 mg/kg and phenytoin 25 mg/kg produced significant decrease in duration of flexion, tonic hind limb extension and significant increase

in duration of PIS in comparison to distilled water. But the maximal effect observed with amlodipine doses did not exceed the phenytoin 25 mg/kg suggesting that phenytoin 25 mg/kg is more effective than amlodipine. Low dose of amlodipine (1 mg/kg) and phenytoin (12.5 mg/kg) alone did not produced any significant change compared to control where as combination of amlodipine 1 mg/kg and phenytoin sodium 12.5 mg/kg significantly decreased the durations of flexion and THLE and significantly increased the duration of PIS indicating potentiating effect of amlodipine when used with phenytoin.

Satyanarayana et al, also reported decrease in duration of THLE in MES model with amlodipine pretreatment in graded doses (1 mg/kg, 2 mg/kg, 4 mg/kg).² Hassan et al, showed anticonvulsant effect of amlodipine when used in combination with lamotrigine, topiramate and gabapentin. Individual doses of amlodipine used in their study were 5, 10 and 20 mg/kg which did not show any anticonvulsant activity in mice. The main difference is experimental set up, that is current used to produced convulsions with tonic hind limb extension was 13 mA

and drug is given intraperitoneal 120 min before electroconvulsion.⁸

Kaminski et al, have shown that 5 mg/kg amlodipine significantly potentiated the anticonvulsant activity of carbamazepine, phenobarbitone and sodium valproate whereas in the present study, 4 mg/kg amlodipine showed significant anticonvulsant activity in the MES test when used alone and 1 mg/kg amlodipine had showed significant potentiation when used along with phenytoin.⁶

Hence, amlodipine have anticonvulsant activity in 2 and 4 mg/kg but phenytoin was more effective than amlodipine. When amlodipine was used in combination with phenytoin in subconvulsive doses, amlodipine had potentiated the anticonvulsant action of phenytoin in MES.

The anticonvulsant and potentiating effect of amlodipine can be explained as follows. Calcium influx occurs by L type voltage dependent calcium channels which cause long lasting depolarisation of the neuronal membrane. These L type calcium channels are present in CNS such as cortex, hippocampus, cerebellum and spinal cord.^{9,10} In addition to above mechanism, calcium enters the presynaptic terminal also through L type, N type and P/Q type channels and binds to calmodulin. This calcium-calmodulin complex regulates neurotransmitter release.¹¹

Amlodipine act as anticonvulsant by blocking these L-type calcium channels, the excitation/deploration of the neurons is inhibited.¹² In addition amlodipine also have high affinity to N- and P/Q-type calcium channels and block them.⁸ This presynaptic blockade results in inhibition of glutamate release from neurons which is a excitatory neurotransmitter.

The potentiation effect of amlodipine with phenytoin is due to pharmacodynamic based interaction i.e. phenytoin show anticonvulsant action by blocking these voltage dependent calcium channels.¹³ Synergism can also occur due to multiple mechanism of action of individual drugs like phenytoin prolongs the inactivated state of sodium channels. Studies showed that there were no pharmacokinetic interactions of amlodipine with phenytoin or other antiepileptic drugs.²

Epilepsy being a chronic disease may coexistent with other chronic diseases like hypertension. In these clinical settings, the potentiating effect of calcium channel blockers like amlodipine and antiepileptic drugs may prove to be useful. Moreover the doses of the standard antiepileptic drugs can be reduced when they are concurrently used with amlodipine so that the adverse effects of standard antiepileptic drugs may be reduced.

CONCLUSION

The present study suggests that amlodipine has an anticonvulsant effect in maximal electroshock induced

seizures in albino rats. Amlodipine potentiated anticonvulsant effect of phenytoin. However, further clinical studies are still required to establish the therapeutic role of calcium channel blockers as effective anticonvulsants either alone or in combination with existing antiepileptic drugs.

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