Original Article

Antiepileptic Effects of Amlodipine in Mice

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Abstract

Introduction: Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures with at least two unprovoked seizures occurring >24 hours apart. It is one of the most common neurological diseases globally. Earlier studies revealed that a potent calcium channel agonist induced convulsion and calcium channel antagonists produced antiepileptic activities. Hence, this study was carried out to assess antiepileptic effects of amlodipine since it holds a good safety profile among calcium channel blockers.

Methods: Inbred Swiss albino mice of both sexes weighing between 20-30 g were used. Antiepileptic effects were assessed using Maximal Electroshock Seizure (MES) test and Pentylene tetrazole (PTZ) induced seizure test. Mice were arranged into 5 groups, each containing 6 mice: Tween-80 (Negative control), Amlodipine at the doses of 1 mg/kg, 2 mg/kg, 4 mg/kg and Sod. valproate (Positive control). Comparison between the test and control was done using Mann-Whitney U test and dose-dependent effects by regression analysis. P value of less than 0.05 was taken as significant.

Results: In MES Test, Amlodipine in the dose of 2 mg/kg and 4 mg/kg significantly decreased the duration of tonic hind limb extension ($P<0.01$) with significant dose dependent effect ($r^2 = 0.96$).

In PTZ test, Amlodipine in the dose of 2 and 4 mg/kg significantly increased the latent period ($P<0.05$) with dose dependent increase in the latent period ($r^2 = 0.97$).

However, protection offered in both the seizure models are lower with amlodipine even in higher dose as compared to Sodium valproate.

Conclusion: Amlodipine is effective to control seizure in animal models of epilepsy especially in higher doses. Amlodipine can be a good add-on drug to sodium valproate rather than an alternative to it.

Keywords: Amlodipine, Antiepileptic, Electroshock, Pentylenetetrazole

Introduction

Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures with at least two unprovoked (or reflex) seizures occurring >24 hours apart.[1] An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. Approximately 50 million people worldwide have epilepsy, making it one of the most common neurological diseases globally and people with epilepsy respond to treatment approximately 70% of the time.[2] Though various new drugs have been introduced with their unique advantages, they fail to provide satisfactory seizure control in as many as 25% of the patients; their dose related neurotoxicities and other side effects become a major limitation in their clinical use.[3] Thus there is an ever increasing need for research into the pathophysiology of epilepsy and rational designing of newer and safer molecules for treating epileptic seizures.

The initiation of the seizure is associated with high frequency burst of action potential caused by relatively long lasting depolarization of neuronal membrane which may be triggered by a large influx of Ca^{2+} ions into the cells. [4] A potent calcium channel agonist Bay K-8644 has induced convulsion in experimental animals and its co-administration has decreased the effects of anti-epileptic drugs.[5,6]

Studies in animals have indicated the antiepileptic activities of calcium channel antagonists like Flunarizine and Nifedipine, Nimodipine and Nicardipine.[7-9]

Amlodipine belongs to 1,4 dihydropyridine group of calcium channel blockers. it shows gradual onset of action and produces continuous and consistent activity throughout 24-hour period.[10] Upon co-administration, it has not affected the blood concentration of antiepileptic drugs.[11]

Hence this study was carried out to assess the antiepileptic activity of amlodipine in two models of epilepsy in mice:

1) Maximal Electroshock Seizure (MES) Test: The animal model of tonic-clonic seizure
2) Pentylenetetrazole (PTZ) induced seizure Test: The animal model of absence seizure

Methods

Inbred albino mice (Swiss strain) of both sexes weighing between 20-30 g were used for the study. Mice were housed in a group of three to five in a clean polypropylene cage in the laboratory environment with temperature 24-27 ° C, cross ventilation and natural day/night cycle with free access to food & water. The study was approved by the Institutional Animal Ethics Committee.

Antiepileptic effects were assessed using two animal models of epilepsy. For each experimental model of epilepsy, mice were arranged into 5 groups, each containing 6 mice (three males and three females):

Table 1: Assignment of animals to various doses of test drugs and controls

Group	No. of Animals	Drug	Dosage (mg/kg B.W)
I	6	Tween 80	10 ml/kg
II	6	Amlodipine	1 mg/kg
III	6	Amlodipine	2 mg/kg
IV	6	Amlodipine	4 mg/kg
V	6	Sod. Valproate	100 mg/kg

Three graded doses of amlodipine were assessed along with sodium valproate as positive control and normal saline as negative control. Sodium valproate was dissolved in distilled water where as Amlodipine was dissolved in 1 % Tween 80 solution. A period of half an hour was allowed for proper dissolution before administration. Different doses were dispensed separately and drugs were given orally in the volume of 10 ml/kg one hour prior to the test procedures.

Maximal Electroshock Seizure (MES) Test:

Convulsion was induced by electro-convulsiometer (Techno India Ltd). The current of 48 mA was delivered for 0.2 seconds through ear clip electrode by attaching them to the pinna.

Parameters observed:

- Duration of flexion
- Duration of tonic hind limb extension
- Duration of clonus

Criteria for evaluation of anticonvulsant effect:

- Abolition or reduction of tonic hind limb extension
- Abolition or reduction of duration of clonus.

Pentylenetetrazole (PTZ) Induced Seizure Test:

PTZ was administered in a dose of 80 mg/kg intra-peritoneally 60 minutes after administration of the test drugs.

Parameters observed:

- Latent period
- Duration of Seizure
- Mortality

(Latent period was taken as a duration from administration of PTZ to the onset of clonus with loss of righting reflex or tonic hind-limb extension, whichever appears first.)

Criteria for evaluation of Anticonvulsant effect:

- Increase in duration of latent period
- Decrease in duration of seizure
- Decrease in mortality

Statistical analysis

Data were presented in the form of mean $\pm$ standard error of mean. Comparison between the effects of the control and test drugs was done using Mann-Whitney U test. The dose dependent effects were verified statistically

by regression analysis where r^2 value was taken as the parameter for dose dependent effect. P value of less than 0.05 was taken as significant

Results

Table 2: Maximal Electroshock Seizure (MES) Test

Treatment Group	Dose	No of Animals	Flexion	THLE	Clonus	Abolition of seizure
Control (Tween 80)	10 ml/kg	6	1.33 ± 0.17	16.33± 0.56	10 ± 0.26	0%
Amlodipine	1 mg/kg	6	0.92 ± 0.30	9.67± 3.24	6.5 ± 2.14	33%
	2 mg/kg	6	0.75 ± 0.33	6.67± 2.98 **	4.33 ± 1.98 *	50%
	4 mg/kg	6	0.50 ± 0.32	4.33± 2.75 **	1.33 ± 1.33 **	67%
Sodium Valproate	100 mg/kg	6	0.50 ± 0.32	4.17± 2.64 **	2 ± 1.29 **	67%

Durations of the above parameters are expressed in seconds.
THLE: Tonic Hindlimb extension.
* P< 0.05 ** P< 0.01

Amlodipine in the dose of 2 mg/kg and 4 mg/kg significantly decreased the duration of tonic hind limb extension (6.67 ± 2.98 and 4.33 ± 2. 75 as compared to 16.33 ± 0.56 seconds with control, P<0.01) as well as clonus (4.33 ± 1.98 and 1.33 ± 1.33 as compared to 10 ± 0.26 seconds with control, p<0.05 and P<0.01 respectively).

The decrease in THLE with 1 mg/kg of amlodipine was not statistically significant. However, there was dose dependent decrease in the duration of tonic hind limb extension ($r^2 = 0.96$).

33% of the animals showed complete abolition of seizure after administration of 1 mg/kg of amlodipine which increased upto 67% with 4 mg/kg of amlodipine. There was dose dependent abolition of seizures ($r^2 = 0.93$).

Table 3. Pentylenetetrazole (PTZ) Induced Seizure Test

Treatment Group	Dose	No of Animals	Latent Period	Duration of Seizure	Mortality
Control (Tween 80)	10 ml/kg	6	82.00 ± 7.36	40.33 ± 3.28	83.33%
Amlodipine	1 mg/kg	6	100.67 ± 8.81	34.75 ± 6.57	67%
	2 mg/kg	6	123.50 ± 8.43 *	26.33 ± 1.23 *	67%
	4 mg/kg	6	142.50± 18.35 *	24.50 ± 3.88 **	33%
Sodium Valproate	100 mg/kg	6	132.50± 13.15	22.00 ± 2.54	50%

Durations of the above parameters are expressed in seconds.
* P< 0.05 ** P< 0.01

Amlodipine in the dose of 2 and 4 mg/kg significantly increased the latent period (123.5 ± 8.43 and 142.5 ± 18.35 as compared to 82 ± 7.36 seconds with control, P<0.05) and significantly decreased the duration of seizure (26.33 ± 1.23, P< 0.05 and 24.5 ± 3.88, P<0.01 as compared to 40.33 ± 3.28 seconds with control). The effect in these parameters with 1 mg/kg of amlodipine was not statistically significant.

There was dose dependent increase in the latent period ($r^2 = 0.97$). 33% of the animals were protected from the mortality due to PTZ induced seizure after administration of 1 mg/kg and 2 mg/kg of amlodipine which increased upto 67% with 4 mg/kg of amlodipine.

However, there was no dose dependent abolition of seizures ($r^2 = 0.89$).

Discussion

The study was conducted to assess the antiepileptic effects of amlodipine using Maximal Electroshock Seizure (MES) test and Pentylenetetrazole (PTZ) induced seizure test in mice.

Amlodipine decreased the tonic hind limb extension and clonus in a dose dependent manner. The lowest dose (1 mg/kg), however, did not show significant alteration in these parameters. Kaminski and colleagues showed that 5 mg/kg of amlodipine significantly potentiated the protective activity of carbamazepine, phenytoin, phenobarbitone and sodium valproate, [12] where as in this study, 4 mg/kg of amlodipine showed significant protection in MES test.

Similar results were obtained during PTZ induced seizure test where significant prolongation of latent period and decrease in duration of seizure were observed after pre-treatment with Amlodipine. A previous study revealed that 2.5 mg/kg of amlodipine enhanced the protective effect of ethosuximide, phenobarbitone and sodium valproate against PTZ induced seizures.[13]

The effectiveness of Amlodipine in both the epileptic models suggests the role of Ca^{2+} ion modulation in antiepileptic mechanisms. A potent calcium channel agonist Bay K-8644 has induced convulsion in experimental animals and its co-administration had diminished the effects of anti-epileptic drugs.[5,6] Another study reveals that voltage-dependent calcium channel antagonists augment anticonvulsant effects of lithium chloride on pentylenetetrazole-induced clonic seizures.[14] This suggests the role of calcium channel blocker for potential antiepileptic effects.

This is further supported by the previous studies which demonstrated the inhibition of calcium influx by anticonvulsant drugs like Carbamazepine, Phenobarbitone and Sodium valproate.[15,16,17] However, the decrease in tonic hind limb extension in MES test and duration of seizure in PTZ could not be achieved at the extent of sodium valproate even with the highest dose of amlodipine studied. Even in a study done by Borowicz KK and colleagues found that amlodipine 20 mg/kg did not significantly affect the threshold for maximal electro-convulsions in mice but it significantly enhanced the anticonvulsant activity of lamotrigine.[18] This suggests that amlodipine may have a role of an adjuvant instead of an alternative to sodium valproate.

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Conclusion

Epilepsy is one of the most common disorders of central nervous system. Amlodipine is effective to control seizure in animal models of tonic-clonic as well as absence seizure especially in higher doses. It can be a good add-on drug to sodium valproate rather than an alternative to sodium valproate.

Conflict of interest: None declared

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