An experimental study of the anticonvulsant effect of amlodipine in mice

Sathyanarayana Rao K N, Subbalakshmi N K

ABSTRACT

Introduction: The need for the rational development of newer and adjuvant drugs to treat epilepsy has prompted this study of the potential anticonvulsant effect of amlodipine.

Methods: The acute effect was studied in mice in single doses of 1 mg/kg, 2 mg/kg and 4 mg/kg of amlodipine and the chronic effect was studied in doses of 1 mg/kg and 4 mg/kg (administered daily for 21 days) using the maximal electroshock seizure and pentylenetetrazole-induced seizure models of epilepsy. Sodium valproate and normal saline were used as the standard and control, respectively.

Results: For the acute study, in the maximal electroshock seizure model, the administration of 1 mg/kg of amlodipine resulted in the complete abolition of seizures in 33 percent of the mice, and this was increased to 67 percent with the administration of 4 mg/kg. In the pentylenetetrazole-induced seizure model, the administration of 1 mg/kg and 2 mg/kg amlodipine protected 33 percent of the animals from mortality, and 67 percent were protected with the administration of 4 mg/kg. For the chronic study, in the maximal electroshock seizure model, the administration of 1 mg/kg amlodipine resulted in the complete abolition of seizures in 40 percent of the mice and in 60 percent, with the administration of 4 mg/kg. In the pentylenetetrazole-induced seizure model, 50 percent of the mice were protected from mortality with 1 mg/kg amlodipine and 60 percent, with 4 mg/kg amlodipine.

Conclusion: These findings indicate that amlodipine may be a good candidate as an add-on therapy for epilepsy.

Keywords: abolition of seizure, add-on therapy, amlodipine, anticonvulsant, epilepsy, maximal electroshock, pentylenetetrazole
gradual onset of action and sustained effect that provides for continuous and consistent activity throughout a 24-hour period.\(^{(12)}\) In addition, free plasma concentrations of anti-epileptic drugs that are used concurrently are not altered by amlodipine.\(^{(13-15)}\) Since epilepsy is a chronic disease that requires long-term management, oral administration of the investigational drug daily over a period of time may be more appropriate in determining its efficacy than a single dose. Hence, unlike previous experiments that have been conducted, this study aimed to evaluate the anti-epileptic effect of amlodipine based on chronic (repeated doses) in addition to acute (single dose) administration.

**METHODS**

This study was conducted in the experimental pharmacology laboratory of Kasturba Medical College, Mangalore, India. The study was duly approved by the Institutional Ethics Committee.

The anticonvulsant profile of amlodipine (acute and chronic effects) was evaluated in two conventional experimental models of epilepsy: the maximal electroshock seizure (MES) test and the pentylenetetrazole (PTZ)-induced seizure test in mice.

Inbred albino mice (Swiss strain) of both genders weighing 20–30 g were used for the study. The mice were housed in groups of three to five in clean polypropylene cages in the laboratory environment at a temperature of 24°C–27°C, with cross-ventilation, a natural day/night cycle and free access to food and water. The mice were screened 24 hours prior to the study. Only the mice that showed all phases of convulsion with the maximal electroshock current were selected. In total, 30 mice were included in the acute experimental model. These were distributed into five groups of six mice each, of which three were males and three were females. Of the five groups, Group I served as a control, while Groups II, III and IV received the test drug and Group V received sodium valproate as a standard drug.

A total of 40 mice were included in the chronic experimental model. These were distributed into four groups, each containing ten mice, of which five were male and five were female. Among these, Group I served as the control group, while Groups II and III received the test drug and Group IV received sodium valproate as a standard drug. For the preliminary dose selection study, six mice were tested for each level of dosage.

Pure chemicals of amlodipine (Micronova Laboratory, Bangalore, Karnataka, India), sodium valproate (Micronova Laboratory, Bangalore, Karnataka, India) and pentylenetetrazole (Genuine Chemicals Co, Mumbai, Maharashtra, India) were used in this study. The preparation and administration of drugs were conducted in the following way. The test drug, amlodipine, was dissolved in 1% tween 80 (polyoxyethylene sorbitan mononucleate) solution. The standard drug, sodium valproate, was dissolved in distilled water. Three test doses of 1 mg/kg, 2 mg/kg and 4 mg/kg amlodipine were administered in the acute study and two test doses of 1 mg/kg and 4 mg/kg amlodipine were administered in the chronic study after the dose selection study was completed. This dose selection was done based on the findings of a preliminary study in which the anti-epileptic activity of amlodipine was observed in the MES test in increasing single doses of 1 mg/kg, 2 mg/kg, 4 mg/kg, 8 mg/kg and 16 mg/kg.

### Table I. Acute study: maximal electroshock seizure (MES) test (n = 6 in each group).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (ml/kg)</th>
<th>Mean ± SE (sec)</th>
<th>Abolition of seizure(^{1}) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (normal saline)</td>
<td>10</td>
<td>1.33 ± 0.17</td>
<td>16.33 ± 0.56</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>1</td>
<td>0.92 ± 0.30</td>
<td>9.67 ± 3.24</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.75 ± 0.33</td>
<td>6.67 ± 2.98(^{**})</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.50 ± 0.32</td>
<td>4.33 ± 2.75 (^{**\ast})</td>
</tr>
<tr>
<td>Sodium valproate</td>
<td>100</td>
<td>0.50 ± 0.32</td>
<td>4.17 ± 2.64</td>
</tr>
</tbody>
</table>

\(^{\ast}\)Significant at \(\alpha = 0.01\). \(^{**}\)Significant at \(\alpha = 0.005\) compared to the control group.

\(^{1}\)Denotes the abolition of the tonic hind limb extension and is considered as the end point of the test.

THLE: tonic hind limb extension; SE: standard error

### Table II. Acute study: pentylenetetrazole-induced test (n = 6 in each group).

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (ml/kg)</th>
<th>Mean latent period ± SE (sec)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (normal saline)</td>
<td>10</td>
<td>82.00 ± 7.36</td>
<td>83.33</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>1</td>
<td>100.67 ± 8.81</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>123.50 ± 8.43</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>142.50 ± 18.35*</td>
<td>33</td>
</tr>
<tr>
<td>Sodium valproate</td>
<td>100</td>
<td>132.50 ± 13.15</td>
<td>33</td>
</tr>
</tbody>
</table>

\(^{*}\)Significant at \(\alpha = 0.05\) compared to the control group.

SE: standard error
In the acute study, the drugs were administered orally in single doses at the maximum volume of 10 ml/kg one hour prior to the test procedure. In the chronic study, the drugs were administered orally at the maximum volume of 10 ml/kg once a day for 21 days. The mice were subjected to the test procedure one hour after the last dose on the 21st day.

MES was induced in the mice using an electroconvulsiometer (Techno India Ltd, Ambala, Haryana, India). MES stimuli, comprising 0.2 seconds of rectangular positive pulses (48 mA at 60 Hz; pulse width 0.4 ms) were delivered through ear clip electrodes. Each mouse was administered the drug or normal saline (control) 30 minutes prior to receiving an electroshock. The anticonvulsant activity of the drug was evaluated based on its ability to protect (in %) against MES and decrease the duration of tonic hind limb extension, flexion and clonus in unprotected animals (i.e. only in animals in which seizures were not abolished).

PTZ seizure was induced by administering a dose of 80 mg/kg PTZ intraperitoneally. Each mouse was administered the drug or normal saline (control) 60 minutes prior to the PTZ treatment. The mice were observed for the onset of clonus with a loss of the righting reflex, the onset of tonic hind limb extension and mortality. The anticonvulsant effect was evaluated based on the ability of the drug to prolong the duration of the latent period (i.e. the time taken for the onset of clonus with a loss of the righting reflex or tonic hind limb extension, whichever appeared first after the administration of PTZ) and its ability to decrease mortality.

The data is presented as mean ± standard error (SE). A comparison of the effect of the test drug with the control was done using one-way ANOVA followed by the post-hoc Bonferroni’s test. The level of significance was measured using a two-tailed test. The various analyses were performed using the Statistical Package for the Social Sciences version 11.0 (SPSS India, Bangalore, Karnataka, India), Microsoft Office Excel 2003 and Microsoft Windows XP Professional (Microsoft Corporation, Redmond, WA, USA). A p-value < 0.05 was considered to be significant.

RESULTS

For the acute study, in the MES model (Table I), complete abolition of seizures was observed in 33%, 50% and 67% of mice in the groups administered 1 mg/kg, 2 mg/kg and 4 mg/kg amlodipine, respectively. In unprotected animals, a significant decrease in the duration of tonic hind limb extension was observed in the dosages of 2 mg/kg and 4 mg/kg amlodipine (6.67 ± 2.98 seconds at $\alpha = 0.01$ and 4.33 ± 2.75 seconds at $\alpha = 0.005$, respectively, as compared to 16.33 ± 0.56 seconds in the control group). A significant decrease in clonus was observed in the dosage of 4 mg/kg (1.33 ± 1.33 seconds at $\alpha = 0.01$ as compared to 10 ± 0.26 seconds in the control group).

In the PTZ-induced seizure model (Table II), complete protection from PTZ-induced mortality was observed in 33% of the mice in the groups administered 1 mg/kg, 2 mg/kg and 4 mg/kg amlodipine, respectively. In unprotected animals, a significant decrease in the duration of tonic hind limb extension was observed in the dosage of 4 mg/kg (1.33 ± 1.33 seconds at $\alpha = 0.01$ as compared to 10 ± 0.26 seconds in the control group). There was no significant decrease in the duration of tonic hind limb extension in the dosage of 1 mg/kg amlodipine. There was no significant effect in the flexion at any level of dosage. The maximal effect observed with amlodipine at any of the above dosages did not exceed that of sodium valproate in any of the above parameters.

In the PTZ-induced seizure model (Table II), complete protection from PTZ-induced mortality was observed in 33% of the mice in the groups administered 1 mg/kg and 2 mg/kg amlodipine, and in 67% of the mice in the...
group administered 4 mg/kg amlodipine. In unprotected animals, a significant increase in the latent period was observed in the dosage of 4 mg/kg amlodipine (142.50 ± 18.35 as compared to 82.00 ± 7.36 seconds in the control group, at $\alpha = 0.05$). A significant increase in the duration of the latent period was not observed in the dosages of 1 mg/kg and 2 mg/kg amlodipine. The maximal effect did not exceed that of sodium valproate at any dosage in any of the above parameters.

For the chronic study, in the MES model (Table III), complete abolition of seizures was observed in 40% and 60% of mice in the groups administered 1 mg/kg and 4 mg/kg amlodipine, respectively. In unprotected animals, a significant decrease in the duration of tonic hind limb extension was observed in the dosages of 1 mg/kg and 4 mg/kg amlodipine (6.9 ± 1.89 seconds at $\alpha = 0.01$ and 4.8 ± 1.98 seconds at $\alpha = 0.005$, respectively, as compared to 16.0 ± 0.47 seconds in the control group). A significant decrease in clonus was observed in the dosages of 1 mg/kg and 4 mg/kg (4.3 ± 1.21 seconds at $\alpha = 0.01$ and 3.6 ± 1.61 seconds at $\alpha = 0.005$, respectively, as compared to 10.6 ± 0.37 seconds in the control group). The decrease in the duration of flexion was significant only at the dosage of 4 mg/kg amlodipine (0.60 ± 0.25 seconds as compared to 1.45 ± 0.05 seconds in the control group, at $\alpha = 0.05$). The maximal effect of amlodipine at any dosage did not exceed that of sodium valproate in any of the above parameters.

In the PTZ-induced seizure model (Table IV), complete protection from PTZ-induced mortality was observed in 50% and 60% of the mice in the groups administered 1 mg/kg and 4 mg/kg amlodipine, respectively. In unprotected animals, a significant increase in the latent period was observed at the dosage of 1 mg/kg and 4 mg/kg amlodipine (116.3 ± 9.60 seconds at $\alpha = 0.01$ and 150.3 ± 14.02 seconds at $\alpha = 0.005$, as compared to 60.0 ± 3.82 seconds in the control group). The decrease in the duration of flexion was significant only at the dosage of 4 mg/kg amlodipine (0.60 ± 0.25 seconds as compared to 1.45 ± 0.05 seconds in the control group, at $\alpha = 0.05$). The maximal effect of amlodipine at any dosage did not exceed that of sodium valproate in any of the above parameters.

**DISCUSSION**

The aim of this study was to evaluate the anticonvulsant effects of amlodipine using the MES and PTZ-induced seizure models.

In the MES model, complete abolition of seizure (protection) was observed in 67% of animals that were administered the highest dose (4 mg/kg) in the acute study, which was equal to that of sodium valproate. 60% of the animals were protected in the chronic study compared to 70% for sodium valproate. In unprotected animals, amlodipine decreased the duration of tonic hind limb extension and clonus in a dose-dependent manner in the acute study. However, the lowest dosage (1 mg/kg) on a single-dose administration did not show statistically significant effects with these parameters, whereas the data was significant after continuous administration of the same dosage for 21 days in the chronic study. This finding suggests that the efficacy of this drug improves with long-term administration. Kaminski et al have shown that 5 mg/kg amlodipine significantly potentiated the protective activity of carbamazepine, phenobarbitone and sodium valproate; whereas in our study, 4 mg/kg amlodipine showed significant protection in the MES test.

In the acute study of the PTZ-induced seizure model, amlodipine increased the latent period in a dose-dependent manner. The lowest dosage of 1 mg/kg did not show significant effects in this parameter. However, the data was significant after continuous administration of the same dosage for 21 days in the chronic study. Despite this, the protection from mortality with the highest dose remained at 60%, as in the case of sodium valproate. A recent study has revealed that 2.5 mg/kg of amlodipine enhanced the protective effect of ethosuximide, phenobarbitone and sodium valproate against PTZ-induced seizures. Luszczki et al have recently evaluated the anticonvulsant action of second-generation anti-epileptic drugs in combination with amlodipine. The doses of amlodipine used by the authors were up to 20 mg/kg, which is significantly higher than the maximum dose of 4 mg/kg utilised in the present study. The doses of amlodipine used by Luszczki et al pertain to the effect of enhancing the anticonvulsant action of topiramate and lamotrigine. The use of higher doses indicates a completely different experimental setting compared to this study. The striking similarity is that there is a dose-dependent increase in the protective effect of amlodipine in both studies. Lower doses of amlodipine have also been shown to be effective in some previous studies. There is merit in evaluating the anticonvulsant effect of amlodipine using low doses (up to 4 mg/kg) as opposed to very high doses of up to 20 mg/kg in Luszczki et al’s study. As amlodipine is a potent vasodilator, lower doses carry a favourable risk-benefit ratio when it is used clinically as an add-on drug rather than in high doses. Demonstration of the anticonvulsant effect of amlodipine in lower doses experimentally, as in this study, is in our view, a very significant finding.

The effectiveness of amlodipine in the above epileptic models suggests the role of the blockade of Ca$^{2+}$ ion channels in mediating anti-epileptic mechanisms.
This is further shown by previous studies that have demonstrated the role of the inhibition of calcium influx by anticonvulsant drugs such as carbamazepine,(13) phenobarbitone(14) and sodium valproate.(17) Prior administration of amlodipine has resulted in significant protection against MES as well as PTZ-induced seizures in both the acute and chronic studies. However, the maximal effect did not exceed that of sodium valproate at any level of dosage. Hence, amlodipine can only be considered as an adjuvant drug rather than as an alternative to sodium valproate.

This study has supported and substantiated the hypothesis of the possible therapeutic role played by calcium channel blockers in epilepsy. Further experimental and clinical studies are required on the administration of amlodipine concurrently with other standard anti-epileptic drugs in order to assess its efficacy as an adjuvant drug.

REFERENCES