Objective: To evaluate the anticonvulsant activity of nimodipine (NMD) alone and in combination with diazepam (DZP) against PTZ induced status epilepticus (SE).

Methods: The study was conducted in mice and SE was induced by administering i.p. 50 mg/kg of phenytoin followed 2 hour later by PTZ, 100 mg/kg, s.c. ED$_{50}$ of NMD and DZP was calculated using different doses of NMD and DZP. Effect of combination of a fixed dose of NMD (ED$_{25}$) with different doses of DZP was also seen and new ED$_{50}$ of DZP in the presence of fixed dose of NMD (83.3 mg/kg) was also calculated.

Results: NMD when administered alone produced dose dependent protection against PTZ induced SE in mice only at higher dose levels and ED$_{50}$ was found to be 119.8 mg/kg. The protective effect of DZP was enhanced by NMD and ED$_{50}$ of DZP was reduced to 0.35 mg/kg from 1.33 mg/kg.

Conclusion: NMD potentiated the anticonvulsant activity of diazepam, so it can be considered as useful add on therapy against SE.

SUMMARY

INTRODUCTION

Calcium ions play a central role in the control of neuronal excitability$^1$. Accumulating evidence shows that Ca$^{2+}$ channels modulated by dihydropyridines play a facilitatory role in experimental seizures$^{2,3}$. Intensive research has highlighted that calcium is an important factor involved in epileptogenesis and neurotoxicity during status epilepticus (SE)$^{4,5}$. Ca$^{2+}$ currents may contribute to epileptogenesis by a) undergoing bursting in pacemaker cells, b) enhancing postsynaptic excitatory responses in dendrites and somatic nerve cells, c) providing post-burst re-excitation$^6$. Ca$^{2+}$ is not only important in genesis and spread of seizures but is also involved in neuronal injury which is caused as a result of repeated seizures and SE$^7$.

Ca$^{2+}$ channel antagonists have been shown to block various aspects of epileptogenesis and are effective anticonvulsants in a number of in vivo models$^{8-10}$. Thus the present study was undertaken to investigate the anticonvulsant activity of nimodipine (NMD) per se and to evaluate the effects of NMD on the anticonvulsant activity of diazepam (DZP) against mouse model of PTZ induced SE.

MATERIALS AND METHODS

Animals: Swiss albino mice (ICRC) of either sex, weighing between 20-30 g were used in this study. They were housed under standard laboratory conditions for one week before experiments were started and were kept in groups of 3-4 per cage at controlled temperature (23°C) and humidity (50%) with light-dark cycle beginning at 7.00 AM. They received standard diet and water ad libitum. Each mouse was used for one seizure test only. All experiments were performed between 8 am and 12 pm to minimise circadian influences. The study was approved by the Institute Ethics Committee.
**Drugs:** Diazepam was dissolved in normal saline. NMD (a generous gift from Cipla Limited, Mumbai, India) was suspended in 0.5% solution of Tween 80 and sonicated. NMD is sensitive to light, so all syringes used for mixing and injecting were covered with aluminium foil. The drugs were injected intraperitoneally and SE was induced 30 minutes after vehicle/drug administration.

**Induction of SE:** SE was induced by the method of Raines et al. Phenytoin Sodium (50 mg/kg) dissolved in alkalinized saline was administered i.p. in a volume of 0.1 ml/10 g body weight to prevent the terminal tonic hind-limb extension produced by PTZ. PTZ was administered 2 hours later, in a dose of 100 mg/kg, s.c. The injection was made in the loose skin behind the neck, in a volume of 0.01 ml/10 g body weight.

The time needed for the development of unequivocal sustained clonic seizure activity involving the limbs (isolated myoclonic jerks or other preconvulsive chewing behaviour were not counted) was carefully noted. Seizure free state for a period of 1 hour was taken as protection. To determine the ED$_{50}$ of DZP, NMD and combination of fixed dose of NMD (ED$_{25}$) with DZP, 4-5 dosages of each drug were tried and six mice were used for each dose level.

**Statistics:** Anticonvulsant ED$_{50}$ values were calculated by the method of Miller and Tainter. The significance of difference in percentage protection between vehicle and drug treated mice, between mice treated with diazepam alone and in combination with nimodipine was calculated by Fisher’s Exact Test. P <0.05 were considered significant.

**RESULTS**

All the ten animals tested in the vehicle treated group exhibited status at the doses of PTZ used in the study. The onset of seizures was found to be 3.6±0.34 min. and the mean seizure duration was 34.6±4.9 min.

NMD was found to be effective in preventing PTZ induced SE in mice. Protection produced against SE was dose dependent. All the five doses (40, 80, 100, 120 and 150 mg/kg) used to calculate ED$_{50}$ produced protection and the ED$_{50}$ was determined to be 119.8 mg/kg (Table 1).

**Table 1.** ED$_{50}$ value of nimodipine (NMD) against PTZ induced SE in mice.

<table>
<thead>
<tr>
<th>No.</th>
<th>Dose (mg/kg)</th>
<th>Latency (min)</th>
<th>Protection (%)</th>
<th>Duration of seizure (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>05.32±01.14</td>
<td>0</td>
<td>16.75±2.06</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>14.65±09.08</td>
<td>16.6</td>
<td>10.89±2.31</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>16.43±08.79</td>
<td>16.6</td>
<td>10.33±2.33</td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>33.05±12.06</td>
<td>50.0</td>
<td>06.61±3.32</td>
</tr>
<tr>
<td>5</td>
<td>150</td>
<td>52.60±07.34</td>
<td>83.3</td>
<td>02.33±2.33</td>
</tr>
</tbody>
</table>

Calculated ED$_{50}$ of nimodipine=119.8 mg/kg; ED$_{25}$ = 83.3 mg/kg n=6 for each dose level. Values are mean ± SE.

To calculate ED$_{50}$ of DZP, five doses used were 0.5, 0.75, 1.0, 2.0 and 4.0 mg/kg and the ED$_{50}$ was found to be 1.33 mg/kg (Table 2).

**Table 2.** ED$_{50}$ value of diazepam (DZP) against PTZ induced SE in mice.

<table>
<thead>
<tr>
<th>No.</th>
<th>Dose (mg/kg)</th>
<th>Latency (min)</th>
<th>Protection (%)</th>
<th>Duration of seizure (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.50</td>
<td>07.13±02.38</td>
<td>0</td>
<td>20.43±2.87</td>
</tr>
<tr>
<td>2</td>
<td>0.75</td>
<td>15.49±09.12</td>
<td>16.6</td>
<td>10.88±2.34</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>36.30±10.70</td>
<td>50.0</td>
<td>15.00±6.83</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>45.11±09.42</td>
<td>66.6</td>
<td>09.87±6.34</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>60.00±00.00</td>
<td>100</td>
<td>00.00±0.00</td>
</tr>
</tbody>
</table>

Calculated ED$_{50}$ of diazepam=1.33 mg/kg; ED$_{25}$ = 0.87 mg/kg n=6 for each dose level. Values are mean ± SE.

To evaluate the effect of NMD on anticonvulsant activity of DZP, a fixed dose of NMD 83.3 mg/kg (ED$_{25}$) was administered in combination with different doses of DZP. NMD potentiated the anticonvulsant activity of DZP. The ED$_{50}$ of DZP in presence of NMD was reduced from 1.33 mg/kg to 0.35 mg/kg. The protection afforded by diazepam in combination with nimodipine was higher at all the four doses tested. There was also an increase in latency and decrease in duration of seizure as compared to same doses of diazepam alone (Table 3).
The potentiation of anticonvulsant activity could be related to central blockade of calcium ion entry. L-channel is modulated by dihydropyridine agonists and antagonists\(^ {17}\). Therefore the anticonvulsant effects may be due to antagonism of Ca\(^ {2+}\) influx through dihydropyridine L-channel. It is currently accepted that GABA neurons are inhibitory through modulation of the L-channel, in addition to enhancing an inward Cl\(^{-}\) conductance and also provide a site at which the endogenous Ca\(^ {2+}\) antagonist could exert its anticonvulsant effects\(^ {18}\).

In conclusion, this study has demonstrated that NMD has anticonvulsant action against PTZ induced SE and combination with DZP produced a synergistic and potent action as compared to DZP alone. The broader implication of this report suggests a role for calcium channel blockers as adjunctive therapy in SE, as their degree of anticonvulsant activity may not render them useful as anticonvulsants per se. NMD may be used as an add on therapy with DZP and combination of both drugs may provide a greater clinical effectiveness against SE.

**REFERENCES**


### Table 3.

<table>
<thead>
<tr>
<th>No.</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Latency (min) ± SD</th>
<th>Protection (%)</th>
<th>Duration of seizure (min) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DZP+ NMD</td>
<td>0.25</td>
<td>30.57 ± 09.43</td>
<td>33.3</td>
<td>13.30 ± 5.00</td>
</tr>
<tr>
<td>2</td>
<td>DZP</td>
<td>0.5</td>
<td>07.13 ± 02.38</td>
<td>0</td>
<td>20.43 ± 2.87</td>
</tr>
<tr>
<td></td>
<td>DZP+ NMD</td>
<td>0.5</td>
<td>50.30 ± 07.52</td>
<td>66.6*</td>
<td>04.00 ± 2.58</td>
</tr>
<tr>
<td>3</td>
<td>DZP</td>
<td>0.75</td>
<td>15.49 ± 09.12</td>
<td>16.6</td>
<td>10.88 ± 2.34</td>
</tr>
<tr>
<td></td>
<td>DZP+ NMD</td>
<td>0.75</td>
<td>55.50 ± 09.50</td>
<td>83.3*</td>
<td>03.67 ± 3.66</td>
</tr>
<tr>
<td>4</td>
<td>DZP</td>
<td>1.00</td>
<td>36.30 ± 10.70</td>
<td>50</td>
<td>15.00 ± 6.83</td>
</tr>
<tr>
<td></td>
<td>DZP+ NMD</td>
<td>1.00</td>
<td>60.00 ± 00.00</td>
<td>100</td>
<td>00.00 ± 0.00</td>
</tr>
</tbody>
</table>

Calculated ED\(_{50}\) of diazepam in combination with nimodipine = 0.35 mg/kg. ED\(_{25}\) = 0.23 mg/kg *p<0.05 Vs diazepam alone n=6 for each dose level. Values are mean ± SE.

**DISCUSSION**

Nimodipine has been found to be effective against SE induced by high doses of pilocarpine\(^ {19}\) and kainic acid\(^ {14}\) but not against lithium-pilocarpine model\(^ {13}\). There are very few studies which have evaluated the effect of NMD against PTZ induced SE. Meyer et al.\(^ {15}\) showed that NMD when administered in a dose of 5 mg/kg/day orally for five days, increased the seizure-threshold by 50-60% in rabbits. However, in our study NMD produced anticonvulsant activity only at higher doses and ED\(_{90}\) was determined to be 119.8 mg/kg. Various factors like age, sex, species, diet, water, day/night cycle, temperature, preparation, dose and injection of PTZ, route of administration are known to affect the response of the animal to PTZ induced seizures\(^ {16}\). These factors may have come into play to influence the ED\(_{90}\) in this study. When fixed dose of NMD (ED\(_{25}\)) was combined with DZP at various doses, it produced synergistic anticonvulsant activity against PTZ induced SE. To the best of our knowledge, there are no studies using combinations of DZP and NMD.


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