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Research Article

## EFFECT OF AMLODIPINE AND INDOMETHACIN IN ELECTRICAL AND PICROTOXIN INDUCED CONVULSIONS IN MICE

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### ABSTRACT

**Background and Objectives:** Antiepileptic drugs (AEDs) are the drugs used in the treatment of epilepsy. Many AEDs have been developed, but the ideal AED which can not only prevent but also abolish seizures by correcting the underlying pathophysiology is still not in sight. Calcium channel blockers (CCBs) may form such a group, as the initiation of epileptogenic activity in the neuron is connected with a phenomenon known as “intrinsic burst firing” which is activated by inward calcium current. In this study, Amlodipine, a CCB of the dihydropyridine class was evaluated for its anticonvulsant activity in mice. It was compared with Phenytoin sodium, one of the oldest anti epileptic drugs. Amlodipine was also combined with Indomethacin, a conventional NSAID, to look for any potentiating effect of this prostaglandin-synthesis inhibitor. **Materials and Methods:** A total of 48 adult Swiss albino mice of either sex weighing 20-30 G were used for this study; 48 were divided into 8 groups, each group containing 6 mice. Group 1-4 MES (50 m Amp for 0.1 secs) induced convulsion method, Group 5-8 evaluated by using the chemo-convulsant, picrotoxin (0.7 mg / kg). Group 1, 5 are controls of MES, Picrotoxin (without treatment). Group 2 & 6 administered standard drug phenytoin (0.5mg/100mg i.p), Group 3 & 7: Amlodipine group (8 mg / kg i.p) and Group 4 & 8: Amlodipine (8 mg/kg) and Indomethacin group (20 mg / kg). In MES method Duration of tonic hind limb extension, Clonic convulsions, Recovery period were studied. In Picrotoxin method Latent period before onset of convulsions, severity of convulsions assessed. **Results:** In electrically induced seizures, the 3 parameters compared are duration of tonic hind limb extension, THLE, ( $P < 0.05$ ); duration of clonic seizures ( $P > 0.05$ ); duration of recovery phase ( $P < 0.0001$ ) and in picrotoxin-induced seizures, the 2 parameters are onset of seizures ( $P < 0.05$ ) and severity of seizures ( $P < 0.05$ ). **Conclusion:** The combination of Amlodipine and Indomethacin showed a superior anticonvulsant effect than the use of Amlodipine alone, in both electrically-induced seizures and picrotoxin-induced seizures in mice.

**Key words:** Anti epileptic drug,  $Ca^{+2}$  channel blocker, Maximal electroshock, Picrotoxin-induced seizures, Tonic hind-limb extension (THLE).

### INTRODUCTION

Antiepileptic drugs (AEDs) are the drugs used in the treatment of epilepsy. Many anti epileptic drugs have been developed, but the ideal AED is still not in sight. The ideal AED should not only prevent & abolish seizures, but also correct the aberrant pathophysiology of epileptogenesis, without interfering with the normal neural transmission.

Therapy is symptomatic in that available drugs inhibit seizures, but neither effective prophylaxis nor total cure is available. Compliance is a major problem because of the need for long term therapy together with the unwanted effects of many drugs. Overall drugs introduced after 1990 like gabapentin, topiramate, tiagabine, levetiracetam and zonisamide

present fewer problems with respect to drug interactions, but have insufficient evidence as monotherapy and are mainly useful as add on drugs<sup>1</sup>. As a general rule, complete control of seizures can be achieved in up to 50% of patients while another 25% can be improved significantly.<sup>1</sup> It has been observed that the presently available antiepileptic drugs are unable to control seizures effectively in as many as 25% of the patients.<sup>2</sup> The mounting number of drugs, the additional adverse effects, drug interactions and other limitations contribute to cause decreased patient compliance, especially if epilepsy is co-existent with other chronic diseases like hypertension.

A new group of drugs with antiepileptic activity, without sedative properties is an interesting prospect. The results from experimental animal models of epilepsy & theoretical considerations suggest that calcium ( $\text{Ca}^{2+}$ ) antagonists may form such a group. The initiation of epileptogenic activity in the neuron is connected with a phenomenon known as “intrinsic burst firing” which is activated by an inward  $\text{Ca}^{2+}$  current.<sup>3</sup>  $\text{Ca}^{2+}$  is described as the primary mediator of excitotoxic neuronal damage during seizure activity. There is a decrease in the extracellular calcium concentration prior to the onset of seizure activity followed by an increase in the intracellular calcium concentration.<sup>4</sup> Considering the crucial role played by calcium, Calcium Channel Blockers (CCBs) can be used in the treatment of epilepsy.

In this study, Amlodipine, a Calcium channel blocker of the dihydropyridine class is evaluated for its anticonvulsant property in mice. Amlodipine has unique pharmacokinetic and dynamic properties among all the CCBs. It has a prolonged half life varying between 36-50 hours. It has slow, sustained action and is suited for chronic therapy. Amlodipine is also an antagonist of the N and P/Q type of calcium channels unlike the other CCBs, Verapamil & Diltiazem which are mainly L-type calcium channel antagonists.<sup>5</sup> Experimental evidence indicates that N-type calcium channels are responsible for glutamate release in the cerebral cortex and hippocampus. Glutamate is the major excitatory neurotransmitter in the brain and is crucial for epileptogenesis. It is also noted that calcium current through the N-type calcium channel accounts for 20% of the total inward calcium current in isolated cortical neurons obtained

from epileptic patients.<sup>5</sup> Hence the effect of Amlodipine has been evaluated.

Studies have indicated that some prostaglandins especially  $\text{PGF}_2$  have pro-convulsant properties.<sup>6</sup> Subsequently prostaglandin synthesis inhibitors or Cyclooxygenase inhibitors like Aspirin, Indomethacin, Naproxen, Nimesulide and Rofecoxib have been tried and proven to have an adjuvant role in the treatment of epilepsy in animal models.<sup>7</sup> In this study, Indomethacin has been combined with Amlodipine to potentiate the latter's effect on experimentally induced seizures. The combination of Amlodipine with Indomethacin, two drugs with two different mechanisms of action could result in an additive or synergistic effect.

## MATERIALS AND METHODS

The present study was conducted in the Department of Pharmacology. The approval for the study was taken from the Institutional Animal Ethics Committee.

In the present study, anticonvulsant activity of Amlodipine and combined effect of Amlodipine and Indomethacin is evaluated using electrically induced and picrotoxin-induced convulsions in mice.

**Grouping:** The mice were divided into 8 groups, each group contained 6 mice. (N=48), Groups 1-4 were MES method and Group 5-8 were picrotoxin induced seizures

Group 1: MES Control Group (without any treatment, administered normal saline 0.1 ml. i.p.)

Group 2: Phenytoin Group (administered Phenytoin sodium 0.5mg/100mg i.p.)<sup>8,9</sup>

Group 3: Amlodipine Group (administered Amlodipine 8 mg / kg i.p.)<sup>10</sup>

Group 4: Amlodipine and Indomethacin (administered Amlodipine 8 mg/kg and Indomethacin 20 mg/kg, i.p.,<sup>11</sup>

Group 5: Picrotoxin Control Group (without any treatment, administered normal saline 0.1 ml. i.p)

Group 6: Phenytoin Group – administered Phenytoin Sodium 0.5mg/100g i.p

Group 7: Amlodipine Group – administered Amlodipine 8 mg/kg i.p.

Group 8: Amlodipine & Indomethacin (administered Amlodipine 8 mg/kg i.p. and Indomethacin 20 mg/kg i.p.)

**I. Supra maximal Electroshock or Maximal Electro Shock (MES test):** 24 mice were subjected

to maximal electroshock through ear electrodes with an intensity of 50 m Amp of alternating current for 0.1 secs 60 minutes after the intra peritoneal injections in mice.<sup>8,9</sup> using Techno Electroconvulsometer. This resulted in almost immediate onset of convulsions, preceded by tonic hind limb extension (THLE) and followed by post ictal depression and recovery. The following 3 parameters were recorded.

A. Duration of THLE, B. Duration of clonic convulsions, C. Recovery period.

**II. Picrotoxin Induced Seizures :** 60 minutes after the above injections to induce convulsions (intraperitoneal injection of picrotoxin 0.7 mg / kg body weight)<sup>12,13</sup> and the resultant seizures with its various phases recorded.

The following parameters were considered with picrotoxin induced seizures.

1. Latent period before onset of convulsions. 2. Severity of convulsions-as assessed by a scoring system

The convulsions severity scoring system 1-7 is as follows:<sup>12</sup>

Hyper locomotion & Pilo erection=1, Catatonia, stunning = 2, Clonic body tremors =3, Prolonged Clonic tremors = 4

Tonic forelimb convulsions followed by clonus = 5, Repetitive fore limb convulsions followed by clonus = 6, Tonic extension of both fore limbs and hind limbs = 7, followed by clonus

## RESULTS

The onset of convulsions or their inhibition, nature of convulsions, duration of the tonic hind limb extension (THLE), a period of post ictal depression (when present) and recovery were observed and noted in all groups of animals and compared with the control group administered normal saline 0.1 ml. i.p. and Phenytoin group administered Phenytoin sodium 0.5mg/100mg i.p).<sup>8</sup>

Data were analysed and all descriptive statistics are expressed as Mean, Standard Deviation.. The results obtained from the study were analysed by ANOVA test and Student t test. P value <0.05\* was considered to be statistically significant.

Table 1: The duration of THLE is 20 seconds in the Control group mice. It is one second in the Phenytoin group. In the Amlodipine group it decreases to 12 seconds and with the addition of Indomethacin further decreased to 10 seconds.

The observed difference between the 4 groups as calculated by ANOVA is statistically significant at 95% confidence intervals P<0.001\*\*\*.

The mean duration of clonic phase in the control group is 60 seconds. It is shortened to 35 seconds in the Phenytoin group, 40 seconds in the Amlodipine group and to 35 seconds in the combined group. The observed difference between the 4 groups as calculated by ANOVA test is statistically highly significant P<0.00001\*\*\*

**Table 1: Duration of THLE, Clonic Phase, and recovery period (in seconds) by MES Method**

Group	Tonic hind limb extension		Clonic Phase		Recovery Period	
	Mean ± SD	P value <sup>§</sup>	Mean± SD	P value <sup>§</sup>	Mean ± SD	P value <sup>§</sup>
Group 1	20 ± 1.68		60±0.63		40±1.41	
Group 2	1±0.58	<0.0001***	35±0.63	<0.0001***	10±0.89	<0.0001***
Group 3	12±1.09	<0.0001***	40±0.89	<0.0001***	30±0.89	<0.0001***
Group 4	10±0.89	<0.0001***	35±0.63	<0.0001***	40±1.41	1 (ns)

\* Significant, \*\* Very Significant, \*\*\* Extremely significant Ns: Non significant

<sup>§</sup>P value comparison with Group 1

**Table 2: Onset of Seizures, Convulsion score by Picrotoxin method**

Group	Onset of Seizures		Convulsions Score <sup>12</sup>	
	Mean ± SD (in minutes)	P value	Mean± SD	P value
Group 6	12±1.41		7±0.89	<0.001***
Group 7	18±0.89	<0.0001***	5±0.63	<0.001***
Group 8	20±0.44	<0.0001***	4±1.41	<0.0001***
Group 9	30±0.89	<0.0001***	2±0.63	<0.001***

\* Significant, \*\* Very Significant, \*\*\* Extremely significant

On the convulsions severity scoring scale (1-7), control has 7, followed by phenytoin group with 5 and then Amlodipine group with 4. The combined use of Amlodipine with Indomethacin is highly effective, decreasing the severity to 2. The observed difference between the 4 groups as calculated by ANOVA test is statistically significant (P<0.05)\*(table 2)

## DISCUSSION

A large body of evidence supports the role of L-type calcium channels in epileptogenesis. Nifedepine was demonstrated to inhibit picrotoxin-induced seizure activity in adult Sprague-Dawley rats<sup>13</sup>. Intraperitoneal injection of Nifedepine at doses of 10-20 mg/kg body weight significantly decreased the severity of seizures after i.p injection of 4mg/kg picrotoxin in rats.<sup>13</sup> Other CCBs have been used for various experiments. Nifedepine 5mg/kg and Flunarizine 4mg/kg were found to have promising effects in both MES and audiogenic seizures<sup>9</sup>. Effect of Cinnarazine has been evaluated as a calcium channel blocker on antiepileptic activity of Maximal electroshock seizures in mice.<sup>2</sup>

In the experiment carried out by Kaminski et al, Amlodipine (up to 10mg/kg) reduced Pentylene tetrazole-induced clonic and tonic convulsions in mice.<sup>14</sup> Many other experiments have been carried out by combining amlodipine and other CCBs with antiepileptic drugs like carbamazepine, valproate. Lamotrigine and Topiramate.<sup>15</sup>

The mouse MES model has been universally accepted as the standard for generalized tonic-clonic seizures. MES and Pentylene tetrazole are the standard methods against GTCS and petitmal epilepsy. The aim of this study is to assess the anticonvulsant effect of Amlodipine alone and in combination with Indomethacin in experimentally induced seizure models in mice.. The above drugs are compared with both the Control (normal saline) and the standard (Phenytoin Sodium).

In electrically induced seizures, the 3 parameters compared are duration of tonic hind limb extension, THLE, ( $P < 0.05$ ); duration of clonic seizures ( $P > 0.05$ ); duration of recovery phase ( $P < 0.0001$ ) and in picrotoxin-induced seizures, the 2 parameters are onset of seizures ( $P < 0.05$ ) and severity of seizures ( $P < 0.05$ ).

The efficacy of CCBs to change the parameters in MES model correlates well with the ability to prevent partial and generalized tonic-clonic seizures and thus its capacity to prevent seizure spread..

“Role of prostaglandin synthesis inhibitors on chemically induced seizures” have been evaluated in albino mice.<sup>11</sup> Based on the findings that the levels of prostaglandins (PGs), the cyclooxygenase metabolites of arachidonic acid are increased in the brain during

experimentally-induced seizures in mice, a role for NSAIDs have been suggested. In our study efficacy of Amlodipine in combination with Indomethacin was evaluated and found to be comparable to Phenytoin in MES seizures and more than phenytoin in picrotoxin-induced seizures.

## CONCLUSION

The combination of Amlodipine and Indomethacin showed a superior anticonvulsant effect than the use of Amlodipine alone, in both electrically and chemically induced seizures with picrotoxin, in mice. In MES seizures, the combined anticonvulsant effect was comparable to that of the standard drug, phenytoin.

In picrotoxin induced seizures, the combined anticonvulsant effect was superior to that of phenytoin both in delaying the onset of seizures and decreasing the severity of seizures.

Hence the anticonvulsant potential of this combination is seen in both seizure models which are equivalent to generalized tonic clonic seizures and partial seizures. Further clinical investigation of these drugs is needed in the context of their being established drugs with no sedation, minor side effects and fewer drug interactions.

Epilepsy being a chronic disease may be coexistent with other chronic diseases like hypertension and osteoarthritis. In these clinical settings, the potentiating effect of calcium channel blockers like Amlodipine and Nonsteroidal anti-inflammatory drugs like Indomethacin may prove to be useful.

## Limitation of study

There is a definite limitation of this study as the number of animals, i.e. Mice studied are small groups (N=6). This preliminary study was to substantiate the mechanism of anti-epileptic action of both CCBs & NSAIDs. Further clinical studies are however needed to prove this action in humans.

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**Conflict of interest: None**

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