Original Article

A Comparative study of the Anti convulsant effect of Nimodipine and Ketamine combination with standard anticonvulsant drug in Rodents

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ABSTRACT

Aim of the study: To evaluate and compare the anticonvulsant property of nimodipine and ketamine combination with a standard drug like Sodium valproate in electrically and chemically induced seizures in mice.

Methods: The maximal seizure pattern was induced in mice by giving an alternating current of 50 mA for 0.2 sec, 50 Hz and 220 volts (maximal electroshock method), while tonic-clonic seizures were seen with 60mg/kg sc. dose of pentylenetetrazole (PTZ). The test drugs were administered 30min before electrical or chemical induction. The ability of the test drug to abolish or reduce tonic-clonic seizures is taken as antiepileptic criteria. The dose of the test drugs nimodipine was kept constant and combined with increasing dose of ketamine.

Results: In maximal electroshock (MES) method, nimodipine (5mg/kg) in combination with ketamine (50mg/kg) only at a higher dose abolished tonic-clonic seizures. In pentylenetetrazole (PTZ) induced method, nimodipine (5mg/kg) in combination with ketamine at doses of 30, 50 mg/kg abolished seizures.

Conclusion: The result suggests that nimodipine and ketamine combination was effective against maximal electroshock (MES) induced seizures in animals. This is the best validated model for evaluating drugs for the treatment of generalized tonic-clonic seizures (GTCS) in human. The combination is also effective against pentylenetetrazole (PTZ) induced seizure in animals and may be effective in absence seizure in human.

KEYWORDS: Nimodipine, ketamine, pentylenetetrazole (PTZ), maximal electroshock (MES)

INTRODUCTION

Epilepsy is one of the most common serious primary brain disorders, affecting 40 million people worldwide[(1)]. It is a heterogeneous symptom complex-a chronic disorder characterized by recurrent seizures. Seizures are finite episodes of brain dysfunction resulting from abnormal discharge of cerebral neurons.The term epilepsy refers to a group of chronic neurologic conditions characterized by recurrent epileptic seizures. Epileptic seizures are the clinical manifestations (signs and symptoms) of excessive and /or hypersynchronous, usually self-limited, abnormal activity of neurons in the brain[(2)]. Some epileptic seizures may be primarily generated at a subcortical level.

As calcium ion influx is involved in the origin of seizures, a calcium channel blocker like Nimodipine has vital role as an antiepileptic drug and the combination with Ketamine, a N-methyl-D-aspertate (NMDA) receptor antagonist may have effective action in blocking seizures since N-methyl-D-aspertate (NMDA) receptors activate the voltage sensitive calcium channels. Maximal electroshock (MES) method is the measure of the ability of an anticonvulsant drug to abolish or decrease in the duration of fore limb flexion or hind limb extension was taken as an index of protection induced my means of an Electroconvulsometer[(3)]. Maximal electroshock (MES) seizures and Pentylenetetrazole induced seizures are the widely used evaluation methods for substances with potential antiepileptic activity.

MATERIALS AND METHODS

The anticonvulsant profile of nimodipine and ketamine combination was evaluated in two conventional experimental methods of epilepsy; (i) Pentylenetetrazole

(PTZ) induced convulsions, (ii) Maximal electroshock (MES) induced convulsions.

Animals: Sixty male albino mice weighing between 24 to 40 grams were used. Mice were kept in groups of 3 to 4 per cage and fed regularly except during test period. The study was approved by Institutional Animal Ethics Committee (IAEC). Experiments were carried out at around the same time every day.

Drugs: Nimodipine (5mg/kg, ip)[5], Ketamine (10, 30 & 50 mg/kg, ip) were dissolved in distilled water and administered in combination, Pentylenetetrazole (PTZ) (60mg/kg, sc).

i) Pentylenetetrazole (PTZ) induced convulsions in mice: Thirty male mice were divided into 5 groups consisting of six mice in each group. Control group received Distilled water, 0.5ml/mice, Standard group received Sodium valproate, 150mg/kg., Test 1, 2 and 3 groups received Nimodipine 5mg/kg in combination with Ketamine 10mg/kg, 30mg/kg and 50mg/kg, intraperitoneally, respectively. Pentelenetetrazole (PTZ) 60mg/kg injected, subcutaneously, after 30 minutes of administration of control, standard and test drug. Each animal was placed in individual plastic cage and observed for 1 hour for time of onset, duration of convulsion, time to recover[4].

ii) Maximal electroshock (MES) induced convulsions: Thirty male mice were divided into 5 groups consisting of six mice in each group. Control group received Distilled water, 0.5ml/mice, Standard group received Sodium valproate, 150mg/kg., Test 1, 2 and 3 groups received Nimodipine 5mg/kg in combination with Ketamine 10mg/kg, 30mg/kg and 50mg/kg, intraperitoneally, respectively. After 30 minutes of administration of control, standard and test drug Maximal electroshock given to induce convulsions. An ear-clip electrode was used to deliver the stimuli with the current strength of 50 mA for 0.2 sec, 50 Hz and 220 volts[6]. The experimental animals were closely observed for 5 minutes and the following parameters were recorded[7].

- Duration of clonic flexion phase

- Duration of tonic extension phase

- Duration of post-ictal depression phase

- Time of recovery

RESULTS

i) Pentylenetetrazole (PTZ) Induced Convulsios in Mice

Control group (Distilled water 0.5ml/mice): All animals developed clonic tonic convulsion by PTZ. The mean time of onset of convulsion was 653.6 ± 179.04 seconds, mean duration of convulsion was 12.33 ± 4.3 seconds and mean time of recovery was 214.33 ± 108.38 seconds. All animals

Table - 1
Effect of Drugs on Pentylenetetrazole (PTZ) Induced Convulsios in Mice

Groups and Drugs with doses	CONVULSIONS			
	Mean time of onset of convulsion (sec)	Mean duration of convulsion (sec)	Mean time of recovery (sec)	Percentage of protection (%)
Control Distilled water 0.5ml/mice	653.6 ± 179.04	12.33 ± 4.3	214.33 ± 108.38	0 %
Standard Sodium valproate 150mg/kg	–	–	–	100 %
Test – 1 Nimodipine 5mg/kg + Ketamine 10mg/kg	2289.83 ± 408.49**	7.67 ± 1.967*	12.83 ± 3.060**	0 %**
Test – 2 Nimodipine 5mg/kg + Ketamine 30mg/kg	–	–	–	100 %
Test – 3 Nimodipine 5mg/kg + Ketamine 50mg/kg	–	–	–	100 %

- Mean values represented as Mean ± S.D.
- n = 6 in each group
- *P < 0.05 – Significant, **P < 0.001 – highly Significant

Table - 2
Effect of Drugs on Maximal Electroshock (MES) Induced Convulsions in Mice

Groups and Drugs with doses	CONVULSIONS				
	Mean duration of forelimb flexion (sec)	Mean duration of hind limb extension (sec)	Mean duration of Post-ictal depression (sec)	Mean time of recovery (sec)	Percentage of protection (%)
Control Distilled water 0.5ml/mice	4.167 ± 1.169	26.67 ± 7.340	75.67 ± 16.367	115.67 ± 36.098	0%
Standard Sodium valproate 150mg/kg	–	–	–	–	100%
Test – 1 Nimodipine 5mg/kg + Ketamine 10mg/kg	0.833 ± 0.983	13.33 ± 20.695**	4.33 ± 6.743**	23.67 ± 36.713**	50%
Test – 2 Nimodipine 5mg/kg + Ketamine 30mg/kg	0.667 ± 0.968	1.167 ± 1.835*	2.83 ± 4.401*	3.833 ± 5.947**	67%
Test – 3 Nimodipine 5mg/kg + Ketamine 50mg/kg	–	–	–	–	100%

- Mean values represented as Mean ± S.D
- n = 6 in each group
- *P < 0.05 – Significant, **P < 0.001 – highly Significant

experienced one episode of convulsion and no mice died.

Standard group (Sodium valproate 150mg/kg): On administration of standard drug, the mice remained normal and no animal developed convulsion in one hour observation after PTZ administration. One mouse showed minimal twitching for 4 seconds. It was inferred as 100% protection against convulsion.

Test - 1 group (Nimodipine 5mg/kg + Ketamine 10mg/kg): All animals developed convulsion after PTZ administration with a mean time of onset of 2289.83 ± 408.49 seconds, mean duration of convulsion of 7.67 ± 1.967 and mean duration of recovery of 12.83 ± 3.060 seconds which implies that all events occurred after a prolonged period when compared to control group.

Test - 2 Group (Nimodipine 5 mg/kg + Ketamine 30mg/kg): The mice were monitored for one hour after PTZ administration. 4 out of 6 mice had tail rising whereas 2 mice had a minimal twitch and no mice developed convulsion. In this group 100% protection was provided by test drug.

Test - 3 Group (Nimodipine 5 mg/kg + Ketamine 50mg/kg): All animals were protected (100%) from convulsions and no jerks or twitches were observed. The mice looked normal and active and the test dose was effective.

From the findings there was 100% protection from convulsions in standard group and also in Test - 2 and Test - 3 groups. On comparison of Test groups with control group, Test – 1, Test – 2 and Test - 3 were statistically highly significant in duration of onset and time of recovery i.e., P< 0.001.

ii) Maximal Electroshock (MES) Induced Convulsions in Mice

Control group (Distilled water 0.5ml/mice): All animals developed single episode of convulsion after MES with mean duration of forelimb flexion of 4.167 ± 1.169 seconds, mean duration of hind limb extension of 26.67 ± 7.340 seconds, mean duration of post-ictal phase of 75.67 ± 16.367 seconds and mean duration of recovery of 115.67 ± 36.098 seconds.

Standard group (Sodium valproate 150mg/kg): On administration of standard drug, no mice developed convulsion after MES. Three mice showed a minimal twitching and a jerky movement. It implies that 100% protection was offered against convulsion.

Test - 1 group (Nimodipine 5mg/kg + Ketamine 10mg/kg): Only three mice developed clonic convulsion of which one mice did not show tonic convulsion with mean duration of forelimb flexion of 0.833 ± 0.983 seconds, mean duration of hind limb extension of 13.33 ± 20.695 seconds, mean duration of post-ictal phase of 4.33 ± 6.743 and mean time of recovery of 23.67 ± 36.713 seconds.

Test - 2 Group (Nimodipine 5mg/kg + Ketamine 30mg/kg): It was observed that 2 out of 6 mice developed convulsion after MES with mean duration of forelimb flexion of 0.667 ± 0.968 seconds, mean duration of hind limb extension of 1.167 ± 1.835 seconds, mean duration of post-ictal phase of 2.83 ± 4.401 seconds and mean duration of recovery of 3.833 ± 5.947

seconds which implies that all events occurred after a prolonged period when compared to control group.

Test - 3 Group (Nimodipine 5mg/kg + Ketamine 50mg/kg): no mice developed convulsion. Only 1 out of 6 mice was observed with minimal twitches. In this group 100% protection was offered by test drug.

From the values recorded, the control group had 0% and standard group had 100% protection from MES induced convulsions. On comparison of test groups with control group, Test - 1 was statistically highly significant (P<0.001) in duration of hind limb extension. Test – 1 was highly significant and Test - 2 was significant (P < 0.05) in post-ictal phase. All test groups were highly significant (P < 0.001) in relation to the time of recovery. The combination of Nimodipine with Ketamine at a higher dose (50mg/kg) was effective against both PTZ and MES induced convulsions in experimental mice.

DISCUSSION

The result of this study indicated that the dihydropyridine calcium channel blocker Nimodipine has shown to be effective against Pentylenetetrazole (PTZ) and Maximal Electro shock (MES) induced convulsions when given in combination with Ketamine, a non-competi-tive antagonist of NMDA receptors in mice. NMDA receptors have been proposed to be involved in the generation and maintenance of epileptiform activity. Earlier studies have indicated that NMDA receptor antagonists more effectively block or antagonize the Maximal Electroshock (MES) induced seizures and they are either ineffective or weak against Pentylenetetrazole (PTZ) or Picrotoxin induced seizures in animals.

The present study is based on the combined effect of Nimodipine and Ketamine when compared to the standard antiepileptic drug against Pentylenetetrazole (PTZ) and Maximal ElectroShock (MES) induced convulsions in experimental mice. Nimodipine 5 mg/kg bodyweight is given in combination with Ketamine in doses of 10mg/kg, 30mg/kg and 50mg/kg bodyweight. It has shown that this combination reduced the duration of flexor and extensor phases as well as incidence of convulsions in both PTZ and MES induced convulsions. The study showed that Ketamine produced dose related anticonvulsant effect when given with Nimodipine 5mg/kg bodyweight. The anticonvulsant effects were significant (P <0.01) with Nimodipine 5mg/kg + Ketamine 30mg/kg. It was highly significant (P <0.001) with Nimodipine 5mg/kg + Ketamine 50mg/kg which blocked the flexor and extensor phases. This is in agreement with the findings of Irfuno et al[8] who observed that NMDA induced hyperactivity was antagonized by Ketamine in a dose-dependent manner. In a study done by Meyer et al, had stated that Nimodipine when administered in a dose of 5mg/kg/day orally for five days, increased the seizure - threshold by 50-60% in rabbits.

The efficacy of calcium channel blockers (CCBs) to reduce the duration of tonic hind limb extensor phase in the MES has been shown to correlate with the ability to prevent partial and generalized tonic clonic convulsions in man[9]. Theoretical consideration suggests that CCBs have anticonvulsant activity and this work correlates with the earlier studies[10]. These drugs have important potentials as adjuvants and as non-sedative antiepileptic agents[11].

The combined anticonvulsant activity may be due to the L-type Ca2+ channel blockade by Nimodipine and non-competitive antagonism of NMDA receptor by Ketamine. Ketamine has also been reported to inhibit voltage dependent Ca2+ channels[12]. In nerve cells, it has been shown that stimulation of NMDA receptor, activates voltage operated Ca2+ channels[13] and Nimodipine has been reported to counteract the increased Ca2+ influx evoked by NMDA receptors[14]. Furthermore, Nimodipine has also been shown to decrease Ca2+ dependent release of excitatory neurotransmitter glutamate.

In conclusion, all the observations and the findings in this experiment suggest that the anticonvulsant effect of the combination of Nimodipine and Ketamine is comparable to that of the standard drug, Sodium valproate. This indicates that, Nimodipine and Ketamine combination was effective against MES induced seizures in animals. This is the best validated model for evaluating drug for the treatment of GTCS in human. The combination is also effective against PTZ induced seizure in animals and may be effective in Absence seizure in human.

CONCLUSION

On the basis of present study, it can be stated that Nimodipine 5mg/kg in combination with Ketamine 10mg/kg was not effective but with Ketamine 30mg/kg and 50mg/kg had 100% protection which is similar to that of sodium valproate 150mg/kg in controlling seizures induced by Pentylenetetrazole. Nimodipine 5mg/kg with Ketamine at the doses of 10mg/kg, 30mg/kg and 50mg/kg had 50%, 67% and 100% protection against Maximal Electroshock induced convulsion in mice. On comparison with Sodium valproate, Nimodipine 5mg/kg + Ketamine 10mg/kg and 30mg/kg were comparatively less effective, whereas Nimodipine 5mg/kg + Ketamine 50mg/kg was equally effective, in controlling seizures induced by Maximal electroshock in mice. It may be concluded that Nimodipine and ketamine combination has anticonvulsant effect; an increase in anticonvulsant effect was observed when the dose of Ketamine was increased in combination with Nimodipine.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Pharmacology and the management of Chalmeda AnandRao Institute of Medical Sciences, Bommakal, Karimnagar, AP, for providing research facilities.

REFERENCES

1. Martin J Brodie, et al. Antiepileptic Drugs. N. Engl J. Med.1996; 334: 168 – 175.
2. Simon D Shorvon. Handbook of Epilepsy Treatment. 2nd edn; 2005.
3. Turner RA. Screening procedures in Pharmacology, Vol.2; 1972: 164 – 172.
4. Gupta YK, et al. Methods and considerations for experimental evaluation of antiepileptic drugs. Indian J Physiol Pharmacol. 1999; 43 (1): 25 – 43.
5. Shitak R, et al. Anti-seizure efficacy of Nimodipine in PTZ and Kainic acid combined seizure models in mice. Indian J. Physio Pharmacol 2006; 50(3): 265 – 272.
6. Vogle H Gerhard. Drug discovery and Evaluation: Pharmacological Assays. 2008; 3rd edn: 613, 692.
7. Gupta SK. Drug screening methods. 2004; 1st edition: 92 - 95.
8. Irfuno M, et al. N-Methyl-D-aspartate (NMDA) receptors in non-competitive NMDA receptor antagonist-induced hyperlocomotion in mice, Pharmacol Biochem Behav . 1995; 51: 291 - 296.
9. Meritt H and Putnam TJ, Sodium Diphenylhydantoin in treatment of convulsive disorders. JAMA 1938; 111: 1068 - 1073.
10. Sahadevan P, Rema MN. A comparative experimental study of anticonvulsant effect of three calcium channel blockers in albino mice. Indian J. Pharm. 2002; 34: 52 - 55.
11. Sharma AK, Khosla R, Mehta VL, Kela AK. Antiepileptic agents: Newer generation. Indian J Pharmcol. 1996; 28: 1-10.
12. Singh J, Khosla P, Srivastava RK. Nimodipine potentiates Anaesthesic effect of Ethanol, Pentobarbitone and Ketamine in Rats. Indian J. Pharmacol.2000; 32: 206 - 209.
13. Pocock JM, et al. The calcium channel coupled to the exocytosis of L-glutamate from cerebellar granule cells is inhibited by the spider toxin Aga-GI. Neuropharmacol. 1993; 32:1185 - 94.
14. Hasmin MA, et al. Modulation of solitarial deglutitive NMDA receptors by Dihydropyridines. Neuropharmacol. 1989; 28:923 - 9.