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RESEARCH ARTICLE

EVALUATION OF ANTICONVULSANT ACTION OF CEREBRO-SELECTIVE CALCIUM CHANNEL BLOCKER NIMODIPINE AN EXPERIMENTAL STUDY

Dr. Sangita Totade, *Dr. Mahesh Kumar Jain and Dr. Swanand S Pathak

Department of Pharmacology, DMIMS (DU), Jawahar Lal Nehru Medical College, Sawangi (M), Wardha India

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ABSTRACT

Calcium (Ca++) a divalent cation, required to perform numerous physiological function within the body like cardiac and smooth muscle contraction. Increased excitatory and reduced inhibitory neurotransmission is responsible for excessive neuronal activity in epilepsy. Some of the CCBs induce reduction in current through Ca2+ channels and thus reducing the pacemaker current that underlies the thalamic rhythm in spikes and waves seen in generalized seizures. The dose of nimodipine was given at doses of 30 and 60 mg QID. This dose was given PO for the improvement of neurological outcome and is chaemic deficit in SAH patients. Either of the dose of nimodipine (30 and 60 mg QID) was not effective in maximum electroshock induced convulsions in experimental rats. When this dose was combined with standard anticonvulsant phenytoin at sub therapeutic dose in reducing tonic HLE was also not found statistically significant in our study.

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INTRODUCTION

Among the neurological disorders, epilepsy is the second most common disease of central nervous system after Stoke (http://www.ajpcr.com/Vol6Suppl2/1695.pdf; http://www.pharmascitech.in/admin/php/uploads/36_pdf.pdf). Epileptic seizures is defined by abnormal, excessive or synchronous neuronal activity in the brain, leading to transient occurrence of clinical features. Epilepsy affecting 01% of world human inhabitants. Synonyms of epilepsy are seizures, fits, and epileptic seizures. Consequences of epileptic seizures are numerous may be social, psychological, cognitive etc and hampering day to day activity (Fisher *et al*., 2005). Clinical definition of epilepsy was defined by International League Against Epilepsy (ILAE) was two unprovoked epileptic seizures occurrence which should be 24 hours apart. A transient factor provoking epileptic seizures in an otherwise normal brain by lowering the seizure threshold does not considered as a case of epilepsy (Fisher *et al*., 2014). Calcium (Ca^{++}) a divalent cation, required to perform numerous physiological function within the body like cardiac and smooth muscle contraction. The underlying mechanism of epileptic convulsion is an imbalance in the excitatory and inhibitory activity within the brain. Increased excitatory and reduced inhibitory neurotransmission is responsible for excessive neuronal activity (De Deyn and Macdonald, 1990; De Deyn *et al*., 1990; Louvel and Pumain, 1992). Calcium channel blocker(s) (CCBs) by blocking entry of Ca^{++} ions through L type calcium channels prevents numerous aspects of epileptic foci and was shown to be a very effective antiepileptic in various in vivo studies. For ex; in a hippocampal slice preparation, verapamil and methoxy verapamil (D-600) reduces Ca^{++} spikesamplitude generated by iontophoretic pulses of NMDA (N-Methyl D Aspartate) type glutamate receptor (Paczynski *et al*., 1990). Certain calcium channel blocking drugs like valproic acid, lamotrigene etc is used in epilepsy they act, either by reducing neurotransmitter release (N and P type) or by attenuating slow depolarisation (T-type) and spike wave discharges (Brunton *et al*., 12th edition). Some of the CCBs induce reduction in current through Ca^{2+} channels and thus reducing the pacemaker current that underlies the thalamic rhythm in spikes and waves seen in generalized seizures. Nimodipine a member of the dihydropyridine group of CCBs and having very high affinity for cerebral blood vessels and used frequently to reduce morbidity after SAH (Sub Arachnoid Haemorrhage). Some evidence also suggested that CCBs may also reduce cerebral damage after thromboembolic stroke (https://www.accessdata. fda.gov/drugsatfda_docs/label/2006/018869s014lbl.pdf; Katzung 11th ed.). This opens the door for research and development of novel and safer antiepileptic. Hence this study was planned to evaluate the anticonvulsant effect of cerebroselective calcium channel blocker Nimodipine by using experimental methods in adult male wistar rats.

***Corresponding author:* Dr. Mahesh Kumar Jain,**
Department of Pharmacology, DMIMS (DU), Jawahar Lal Nehru Medical College, Sawangi (M), Wardha India.

MATERIALS AND METHODS

This study was carried out in the animal house of Jawaharlal Nehru Medical College, after obtaining permission from institutional animal ethics committee vide letter no. DMIMS (DU)/IAEC/2015-16/01. To evaluate the anticonvulsant effect of nimodipine following material and methods were employed;

Animal Used

Arrive and Gold Standard guidelines were followed for maintaining the experimental conditions and care for wistar albino male rats weighing 150-300 gms. They have free access to water and food except during the experimentation. Timing selected for the experiment was in between 1000-1300 hrs at room temperature in noise proof and well-lit environment.

Grouping of Animals

Forty Two in number Wistar albino male rats, were divided into seven groups of six rats in each group by the method of random allocation (Block randomization open study) (Wang and Bakhai, 2006).

Group	Treatment	
1.	Control	Treated with Normal Saline (0.5 ml)
2.	Standard 1	Treated with Phenytoin (04 mg/Kg)
3.	Standard 2	Treated with Phenytoin (02 mg/Kg)
4.	Test 1	Treated with Nimodipine (120 mg/day)
5.	Test 2	Treated with Nimodipine (240 mg/day)
6.	Test 3	Treated with Nimodipine (120 mg/day) and Phenytoin (01mg/kg)
7.	Test 4	Treated with Nimodipine (240 mg/day) and Phenytoin (01mg/kg)

The animals in each group received corresponding drugs 30 minutes before the application of electroshock. Each animal after proper handling, current of 150 mA was passed for 0.2 sec transaurically as a single train of pulse using electroconvulsiometer. The reduction in tonic Hind Limb Extension (HLE) was considered as a protective action and was recorded for all the animals. The mean duration of tonic HLE was determined for each group.

Drug used

S. No.	Drug	Dose strength	Manufacturer	Country
1.	Tab Nimodip® (Nimodipine)	30 mg	USV Limited	India
2.	Inj Eptoin® Phenytoin	50 mg/ml	Akums Drugs and Pharmaceuticals ltd	India
3.	Normal Saline	-	-	India

Drug Preparation

Tab Nimodip® was grinded up to fine powder form and the appropriate weight of the powder was dissolved in double distilled water (DDW) and was given per oral (PO) using rat oral feeding needle because rapidly absorbed after oral administration. Phenytoin wasalso dissolved in DDW and administered intraperitoneally (i.p). The dose of nimodipine was given at doses of 30 and 60 mg QID. This dose was given PO for the improvement of neurological outcome and ischaemic deficit in SAH patients, so dose of 30mg QID and double of this dose was utilised. This human dose was converted to animal dose by applying the conversion factor based on body surface area and was given accordingly to the weight of the experimental rats (Medhi and Prakash, 2010). Study suggested deaths and serious life threatening adverse events was reported by the parenteral use of Nimodip (https://www. accessdata.fda.gov/drugsatfda_docs/label/2006/018869s014lbl.pdf). Phenytoin was used as standard anticonvulsant and was injected at a dose of 02 and 04 mg/kg, i.p. Control group treated with normal saline (0.5 ml) and given by i.p route. All aseptic precautions were taken into consideration while administering the drugs to the experimental rats.

Statistical Analysis

Results were entered in Microsoft office Excel sheet of Microsoft Office 2013 version and mean ± Standard deviation was calculated. Statistical analysis of data was performed by using Students paired **'t'** test and one way analysis of variance (ANOVA) and multiple comparison with Post Hoc Tukey test was done.

RESULTS

The mean duration of HLE in control group without drug was 11.18±1.93 and with drug it was 10.41±0.99 by using student paired t test statistically no significant difference was found in the mean duration of HLE without and with drug in control group (t=0.913, p=0.403). The mean duration of HLE in standard (std) 1 and std 2, without drug was 10.57±1.76, 10.41±0.99 respectively and with drug was 0±0, 1.55±2.42 respectively. This difference was found statistically significant by using students paired t test, for the mean duration of HLE in std 1 as well as std 2 group (std 1; t=14.639, p=0.0001 and for std 2; t=8.180, p=0.0001). Similarly the mean duration of HLE in test group 01 to 04, without drug was 10.18±0.77, 8.80±0.80, 10.31±1.52 and 9.06±0.99 respectively and with drug was 10.45±0.70, 10.09±1.08, 9.27±0.59 and 7.51±1.33 respectively. By using student paired t test statistically no significant difference was found in the mean duration of HLE without and with drug in all the test groups (Test 1; t=0.650, p=0.544. Test 2; t=2.001, p=.102. Test 3; t=1.562, p=0.179. Test 4; t=2.164, p=0.083)

Table 1. Comparison of duration of HLE in seven groups without and with drug

Student's Paired t test

Group	Without Drug	With Drug	t-value	p-value
Control	11.18±1.93	10.41±0.99	0.913	0.403,**
Std 1	10.57±1.76	0.00±0.00	14.639	0.0001,*
Std 2	10.41±0.99	1.55±2.42	8.180	0.0001,*
Test 1	10.18±0.77	10.45±0.70	0.650	0.544,**
Test 2	8.80±0.80	10.09±1.08	2.001	0.102,**
Test 3	10.31±1.52	9.27±0.59	1.562	0.179,**
Test 4	9.06±0.99	7.51±1.33	2.164	0.083,**

(*Significant, ** Not Significant)

The mean duration of HLE with drug in control group was 10.41±0.99, in std 1 and std 2 was 0.00±0.00 (complete protection) and 1.55±2.42 respectively, whereas in test group 1 to 4 it was 10.45±0.70, 10.09±1.08, 9.27±0.59 and 7.51±1.33 respectively, by using one way ANOVA statistically significant variation was found in the mean duration of HLE among seven groups (F=76.72, p=0.0001). On comparing mean duration of HLE in seven groups by using Post Hoc Tukey Test statistically significant difference was found in all the groups except control versus (vs) test 1, test 2 and test 3, Std 1 vs std 2, test 1 vs test 2 and test 3, test 2 vs test 3 and test 3 vs test 4 shows no significant differences.

Table 2. Comparison of duration of HLE in seven groups

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		F-value	p-value
					Lower Bound	Upper Bound		
Control	6	10.41	0.99	0.40	9.37	11.46	76.72	0.0001,*
Std 1	6	0.00	0.00	0.00	0.00	0.00		
Std 2	6	1.55	2.42	0.98	-0.98	4.09		
Test 1	6	10.45	0.70	0.28	9.71	11.19		
Test 2	6	10.09	1.08	0.44	8.94	11.23		
Test 3	6	9.27	0.59	0.24	8.64	9.89		
Test 4	6	8.51	1.33	0.54	6.10	8.91		

(*Significant, ** Not Significant)

Multiple Comparison: Post Hoc Tukey Test

Groups		Mean Difference (I-J)	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Std 1	10.41	0.71	0.0001,*	8.18	12.64
	Std 2	8.86	0.71	0.0001,*	6.62	11.09
	Test 1	-0.03	0.71	1.00,**	-2.26	2.19
	Test 2	0.32	0.71	0.99,**	-1.90	2.55
	Test 3	1.14	0.71	0.67,**	-1.08	3.37
	Test 4	3.90	0.052	0.004,**	0.67	5.13
Std 1	Std 2	-1.55	0.71	0.33,**	-3.78	0.67
	Test 1	-10.45	0.71	0.0001,*	-12.68	-8.22
	Test 2	-10.09	0.71	0.0001,*	-12.32	-7.85
	Test 3	-9.27	0.71	0.0001,*	-11.50	-7.03
	Test 4	-8.51	0.71	0.0001,*	-9.74	-5.27
Std 2	Test 1	-8.89	0.71	0.0001,*	-11.12	-6.66
	Test 2	-8.53	0.71	0.0001,*	-10.76	-6.30
	Test 3	-7.71	0.71	0.0001,*	-9.94	-5.48
	Test 4	-6.95	0.71	0.0001,*	-8.18	-3.72
Test 1	Test 2	0.36	0.71	0.99,**	-1.86	2.59
	Test 3	1.18	0.71	0.64,**	-1.04	3.41
	Test 4	3.94	0.052	0.004,**	0.71	5.17
Test 2	Test 3	0.82	0.71	0.90,**	-1.41	3.05
	Test 4	2.58	0.052	0.015,**	0.34	4.81
Test 3	Test 4	2.76	0.052	0.20,**	-0.47	3.99

(*Significant, ** Not Significant)

DISCUSSION

It has been observed in this study that either of the dose of nimodipine (30 and 60 mg QID) was not effective in maximum electroshock induced convulsions in experimental rats. This is in contrast with the previous studies demonstrated that the dihydropyridine group of CCBs like nimodipine had a good effect in reducing convulsion induced by N_2O (nitrous oxide), PTZ (Phenylenetetrazole), MES (Maximum electroshock seizure), alcohol withdrawl (De Sarro *et al*., 1988; Meyer *et al*., 1986; Meyer *et al*., 1987; Meyer *et al*., 1986). In kainic acid induced convulsion Paczynski *et al* suggested that role of nimodipine was minimal when co mpared to diazepam, this is in favour of our findings. The exact reason for defending MES induced convulsion in rats was not clear but the possible reason for non-effectiveness of CCB nimodipine as an anticonvulsant in MES induced seizure could be that Nimodipine is highly selective blocker for L type voltage sensitive Ca^{++} channels within the CNS, either no voltage dependant/voltage independent Ca^{++} channels may be operative for the depolarisation in MES model of convulsions or intracellular cations ($Na^{+,}$ K^{+}) i.e., other than Ca^{++} could beresponsible for reducing seizure threshold. Phenytoin in the dose of 04 and 02 mg/ kg was found to be effective in reducing tonic HLE in rats. Combination of standard anticonvulsant phenytoin with test drug nimodipine was also analysed in this study; Combination 1-Phenytoin 01mg/kg + nimodipine 30 mg QID Combination 2-Phenytoin 01mg/Kg + nimodipine 60mg QID Both the combination was not effective in reducing tonic HLE, and not found statistically significant as compared to control. This was not effective in combination as well as individually. Hence it has been concluded that nimodipine is not at all effective in epilepsy alone as well as in combination with phenytoin in our study.

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