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ANTI-SEIZURE EFFICACY OF NIMODIPINE IN PENTYLENETETRAZOLE AND KAINIC ACID COMBINED SEIZURE MODELS IN MICE

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Abstract : The present study aimed at establishing two models of experimental seizures by combination treatment with subconvulsive doses of PTZ and kainic acid in adult male mice and evaluating the modulatory role of cerebroselective dihydropyridine calcium channel blocker, nimodipine. The CD50±SEM value for PTZ was found to be 20.00±0.92 mg/kg, ip in kainic acid (administered at *per se* subconvulsive dose of 1.00 mg/kg, ip) pretreated mice while CD50±SEM value for kainic acid was found to be 0.30±0.08 mg/kg, ip in PTZ (administered at per se subconvulsive dose of 30.00 mg/kg, ip) pretreated mice. Nimodipine (5.00 mg/kg, ip) significantly protected the mice from seizure in both of the combination *in vivo* seizure models. The results suggested synergistic interaction between PTZ and kainic acid at subconvulsive dose combination while the protective efficacy of nimodipine suggested the role of calcium ion as an important mediator for the genesis of seizures.

Key words : kainic acid pentylenetetrazole nimodipine seizure

INTRODUCTION

Kainic acid has been used for centuries as a naturally occurring anthelmintic agent (1). It was reported that kainic acid induced neuropathological damages mimicked those observed in human temporal lobe epilepsy (2). Kainic acid is reported to act on kainate receptors, which are widely distributed in central nervous system and thereby could overwhelm the cells Ca^{2+} homeostatic capabilities (3). Further, pentylenetetrazole (PTZ) has been studied as a chemoconvulsant agent and the clonic seizures evoked by PTZ in small animals respond to anticonvulsant drugs that are effective in absence seizures in human beings (4). However, there are no reported studies on the interaction between kainic acid and PTZ. Therefore, the present study was designed to develop seizure

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models based upon the interaction between the two chemoconvulsants in mice and further, to ascertain the effect of nimodipine, a cerebroselective dihydropyridine calcium channel blocker in such seizure models.

MATERIAL AND METHODS

Animals

Experiments were carried out on adult male, Swiss strain mice weighing between 25–28 g. The animals were kept in polystyrene cages under standard laboratory conditions i.e. at temperature of 25±1°C, relative humidity of 60±2% and were exposed to a 12 h photoperiod. They had free access to food (Hindustan Lever Chow) and tap water. In the present study, a group size of 10 mice was used for each experiment. The research protocol was approved by the institutional animal ethics committee.

Drugs and chemicals

Kainic acid and PTZ were sourced from Sigma Chemicals Co. (MO, USA). Pure powder of nimodipine was obtained from Cadila Pharmaceuticals Limited (Baroda, India). All other chemicals and reagents used in the study were of AR grade. All the drug solutions were prepared afresh and administered intraperitoneally (ip) in a volume not exceeding 10 ml/kg.

Preparation of drug solutions

Kainic acid was prepared in strength of 1.00 mg/ml in phosphate buffer (pH 6.9) solution. PTZ solution was prepared by dissolving 25.00 mg of PTZ in 10 ml of 0.9% normal saline. Nimodipine was dissolved in the vehicle solution containing ethanol and propylene glycol (50:50 v/v) in a strength of 0.8 mg/ml.

Kainic acid induced seizures in PTZ pretreated mice

Experiments were performed with graded, single, subconvulsive doses (0.12 to 1.00 mg/kg, ip, unpublished data) of kainic acid administered to five groups of mice. The mice were pretreated with the *per se* subconvulsive dose of PTZ (30.00 mg/kg, ip, unpublished data). The pretreatment time was 30 min. The mice were placed in perspex cages at an ambient temperature of 25±1°C in a quiet room between 10.00–12.00 h and observed for 1 h for the recording of seizure score; the seizure score was recorded according to the scale: 0–no response; 1–ear and facial twitching; 2–1 to 20 myoclonic body jerks in 10 min; 3–more than 20 body jerks in 10 min; 4–forelimb convulsions; 5–generalized clonic convulsions with episodes of rearing and failing down and 6–generalized epilepticus. The response to kainic acid was considered positive when the seizure score was ≥3 (5). From the dose-response study, the CD50 (the dose which showed positive seizure score in 50% of animals) and CD97 (the dose which showed positive seizure score in 97% of animals) doses of kainic acid were obtained in PTZ–pretreated mice.

PTZ induced seizures in kainic acid pretreated mice

PTZ solution (0.25% in 0.9% NaCl) was injected ip in graded, single, subconvulsive doses (10 to 30 mg/kg, unpublished data) to five groups of mice. The mice were pretreated with the *per se* subconvulsive dose of kainic acid (1.00 mg/kg, ip,

unpublished data). The pretreatment time was 30 min. The mice were observed for 1 h for the recording of seizure score and percentage of positive responders as described above. The CD50 and CD97 doses of PTZ were obtained from the dose-response study in kainic acid pretreated mice.

Kainic acid induced seizures in PTZ and nimodipine pretreated mice

Mice were injected with 30.00 mg/kg, ip, dose of PTZ (*per se* subconvulsive dose). After 15 min of PTZ pretreatment, the mice were injected with nimodipine (5.00 mg/kg, ip) or 0.2 ml of vehicle. After 15 min of nimodipine or vehicle administration, the animals were injected with either CD50 (0.30 mg/kg) or CD97 (1.00 mg/kg) dose of kainic acid, ip. The mice were observed for 1 h for the recording of seizure score and percentage of positive responders as described above.

PTZ induced seizures in kainic acid and nimodipine pretreated mice

Mice were injected with 1.00 mg/kg, ip dose of kainic acid (*per se* subconvulsive dose). After 15 min of kainic acid pretreatment, the mice were injected with nimodipine (5.00 mg/kg, ip) or 0.2 ml of vehicle. After 15 min of nimodipine or vehicle administration, the animals were injected with either CD50 (20.00 mg/kg, ip) or CD97 (30.00 mg/kg, ip) dose of PTZ. The mice were observed for 1 h for the recording of seizure score and percentage of positive responders as described above.

Statistical analysis

Data for positive responders were compared by Fisher's exact probability test. The CD50 values for PTZ and kainic acid were determined by the graphic method. The logdose-probit lines were obtained by least-square regression analysis. The approximate standard error of mean (SEM) of CD50 values were calculated using the formula (log CD84–log CD16)/$2N^{-1/2}$ where N is the total number of animals employed in between 4 and 6 probits. The log values for CD84 and CD16 were obtained from the regression line corresponding to probits 6 and 4 respectively (6). Data on seizure score (expressed as Mean±SEM) for the different groups of mice were analyzed by nonparametric Mann-Whitney U test. A P-value of less than 0.05 was considered statistically significant.

RESULTS

Table I shows the dose-response study with kainic acid in five groups of PTZ pretreated mice. The data (Mean±SEM) are expressed in terms of seizure score and initiation time to seizure against graded doses of kainic acid ranging from 0.12 to 1.00 mg/kg. At 0.12 mg/kg, the mice showed a seizure score of 1.4±0.24 while at 1.00 mg/kg, the observed seizure score was 4.9±0.15.

TABLE I: Dose-response study with kainic acid in PTZ pretreated mice.

Dose of kainic acid (mg/kg)	*Seizure score (Mean±SEM)*	*Seizure initiation time (min) (Mean±SEM)*
0.12	1.4±0.24	50.0±1.45
0.25	2.4±0.16	45.6±1.02
0.50	3.6±0.16	42.4±1.09
0.75	4.2±0.13	34.5±0.94
1.00	4.9±0.15	32.5±0.89

n=10
Seizure score scale: 0–6
Per se subconvulsive dose of PTZ: 30 mg/kg, ip
pretreatment time: 15 min.

Seizure score data showed graded incremental response with increasing doses of kainic acid. Seizure initiation time showed graded decremental response to increasing doses of kainic acid. The animals became sluggish, less active, initially on kainic acid administration. After sometime, in a dose-dependent manner, the animals became restless, showed piloerection, clonic convulsions, rearing and falling down episodes. All the animals showing convulsions had increased salivation, urination and defecation.

Fig. 1 shows the graded log dose-probit curve derived from the percentage of positive responders (seizure score ≥ 3) against five graded doses of kainic acid ranging from 0.12 to 1.00 mg/kg in PTZ pretreated mice. The observed positive seizure response at 0.12 mg/kg was found to be 20% (probit = 4.16) while at 1.00 mg/kg, 100% (corrected value = 97.5%; probit = 6.96) of the mice had positive seizure response. There was linear increase in positive responders with increasing doses of kainic acid. The derived CD50±SEM value for kainic acid was found to be 0.30±0.08 mg/kg. Fig. 1 inset shows the doses of kainic acid against probit 4, 5 and 6 which were 0.10 mg/kg, 0.30 mg/kg and 0.90 mg/kg respectively.

Table II shows the dose-response study with PTZ in kainic acid pretreated five

TABLE II : Dose-response study with PTZ in kainic acid pretreated mice.

Dose of PTZ (mg/kg)	*Seizure score (Mean±SEM)*	*Seizure initiation time (min) (Mean±SEM)*
10	1.5±0.18	44.2±2.20
15	2.8±0.27	39.0±2.34
20	3.2±0.44	31.1±3.14
25	3.8±0.21	27.0±1.44
30	4.2±0.15	26.6±2.53

n=10
Seizure score scale : 0–6
Per se subconvulsive dose of kainic acid : 1 mg/kg, ip
pretreatment time : 15 min.

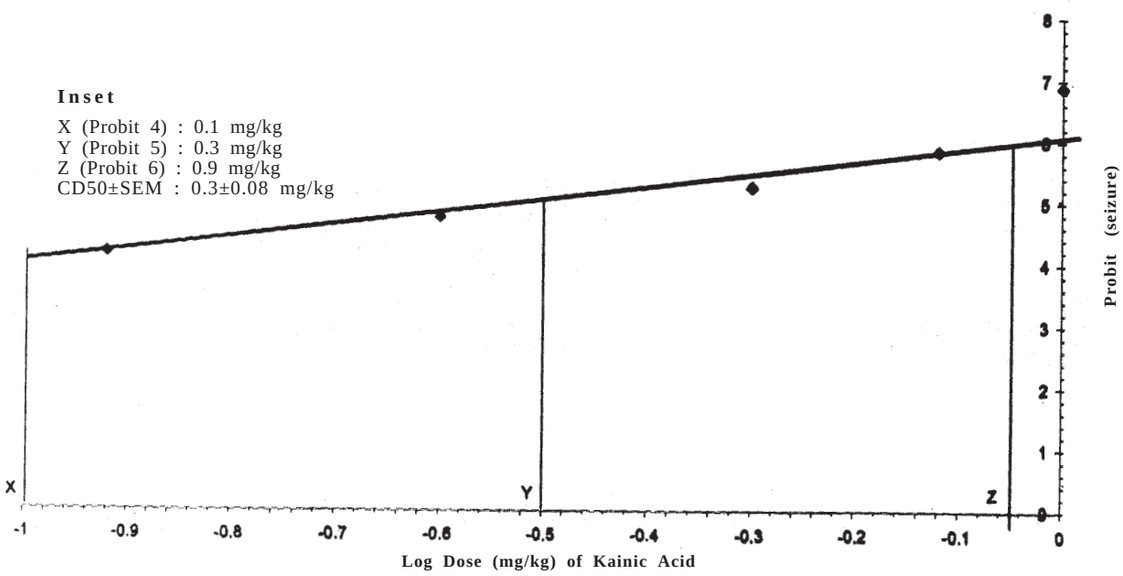

Fig. 1 : Log-dose probit curve depicting CD50 value of kainic acid in PTZ (30 mg/kg) pretreated mice.

groups of mice. The data (Mean±SEM) are expressed in terms of seizure score and initiation time to seizure against graded doses of PTZ ranging from 10.00 mg/kg to 30.00 mg/kg. At 10.00 mg/kg, the seizure score was 1.5±0.18 while at 30.00 mg/kg, it was 4.2±0.15. Seizure score data showed graded incremental response with increasing doses of PTZ. Initiation time to seizure showed decremental response with increasing doses of PTZ.

Fig. 2 shows the graded logdose-probit curve derived from the percentage of positive responders against five graded doses of PTZ ranging from 10.00 mg/kg to 30.00 mg/kg in kainic acid pretreated mice. The observed positive seizure response at 10.00 mg/kg was found to be zero (corrected value 2.5%; probit 3.03) while at 30.00 mg/kg, all the mice showed positive seizure response (corrected value 97.5%; probit-6.96). At intervening doses, there was graded response to positive responders. The derived CD50±SEM value for PTZ in kainic acid pretreated mice was found to be 20.00±0.92 mg/kg. Fig. 2 inset shows the doses of PTZ against probit 4, 5 and 6 which were 12.60 mg/kg, 20.00 mg/kg and 28.84 mg/kg respectively.

Table III shows the effect of nimodipine (5.00 mg/kg, pretreatment time: 15 min) on

TABLE III : Effect of nimodipine (5.00 mg/kg, ip) on CD50 (0.30 mg/kg, ip) and CD97 (1.00 mg/kg, ip) doses of kainic acid in PTZ (30.00 mg/kg, ip) pretreated mice (n=10).

Sr. no.	*Groups*	*% of +ve responders*	*Seizure score (Mean±SEM)*
1.	Vehicle + PTZ + Kainic acid (0.30 mg/kg)	60	3.1±0.46
2.	Nimodipine + PTZ + Kainic acid (0.30 mg/kg)	20*	1.8±0.26*
3.	Vehicle + PTZ + Kainic acid (1.00 mg/kg)	100	4.6±0.15
4.	Nimodipine + PTZ + Kainic acid (1.00 mg/kg)	50#	2.8±0.35#

*P<0.05 versus serial no. 1.
#P<0.05 versus serial no. 3.

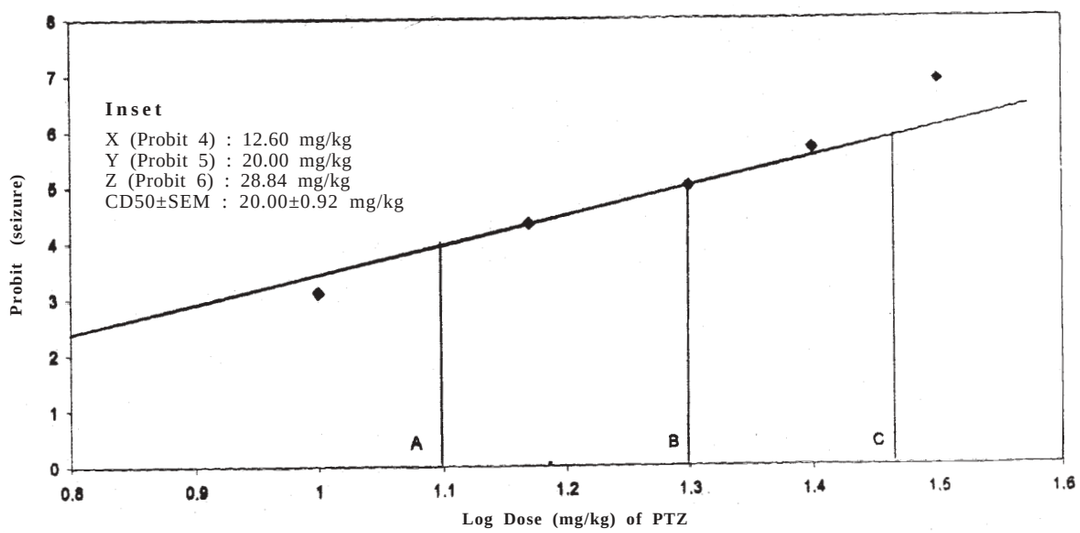

Fig. 2 : Log-dose probit curve depicting CD50 value of PTZ in kainic acid (1.00 mg/kg, ip) pretreated mice.

CD50 (0.30 mg/kg, ip, derived from logdose-probit line, Fig. 1) and CD97 (1.00 mg/kg, ip, obtained from actual observational study) doses of kainic acid in PTZ (administered at subconvulsive dose) pretreated mice. As compared to the vehicle treated control group, nimodipine significantly reduced the percentage of positive responders from 60% to 20% (against CD50 dose) and from 100% to 50% (against CD97 dose of kainic acid). Nimodipine also significantly reduced the seizure score from 3.1±0.46 to 1.8±0.26 (against CD50 dose) and from 4.6±0.15 to 2.8±0.35 (against CD97 dose of kainic acid).

Table IV shows the effect of nimodipine (5.00 mg/kg, ip, pretreatment time: 15 min) on CD50 (20.00 mg/kg, ip, derived from logdose-probit line, Fig. 2) and CD97 (30.00 mg/kg, ip, obtained from actual observational study) doses of PTZ in kainic acid (administered at subconvulsive dose) pretreated mice. As compared to the vehicle treated control group, nimodipine significantly reduced the percentage of positive responders as well as seizure score against both CD50 and CD97 doses of PTZ.

TABLE IV: Effect of nimodipine (5.00 mg/kg, ip) on CD50 (20.00 mg/kg, ip) and CD97 (30.00 mg/kg, ip) doses of PTZ in kainic acid (1.00 mg/kg, ip) pretreated mice (n=10).

Sr. no.	*Groups*	*% of +ve responders*	*Seizure score (Mean±SEM)*
1.	Vehicle + kainic acid + PTZ (20.00 mg/kg)	60	3.2±0.26
2.	Nimodipine + kainic acid + PTZ (20.00 mg/kg)	20*	1.7±0.32*
3.	Vehicle + kainic acid + PTZ (30.00 mg/kg)	100	4.8±0.40
4.	Nimodipine + kainic acid + PTZ (30.00 mg/kg)	40#	2.6±0.82#

*P<0.05 versus serial no. 1.
#P<0.05 versus serial no. 3.

DISCUSSION

In the present study, it was observed that PTZ at the subconvulsive dose synergised with the subconvulsive dose of kainic acid to produce frank seizures in mice. Similarly, kainic acid at the subconvulsive dose synergised with the subconvulsive dose of PTZ resulting in frank seizures in mice. The *per se* subconvulsive dose for PTZ (i.e. 30.00 mg/kg, ip) and that of kainic acid (1.00 mg/kg, ip) were derived from previously conducted dose-response studies (unpublished observation). In those studies, the CD50±SEM value for PTZ per se and that of kainic acid per se were found to be 50.00±2.67 mg/kg, ip and 2.50±0.32 mg/kg, ip respectively. However, in the present study, similar values (i.e. CD50±SEM) for PTZ in kainic acid pretreated animals and that of kainic acid in PTZ pretreated animals were found to be 20.00±0.92 mg/kg, ip and 0.30±0.08 mg/kg, ip respectively. The latter CD50±SEM values for PTZ and kainic acid were significantly less as compared to the per se CD50±SEM values for PTZ and kainic acid as observed in the previous studies (unpublished observation). Thus, pretreatment with subconvulsive dose of PTZ or kainic acid to mice, significantly attenuates the CD50 value for the other agent signifying synergistic interaction between the two chemoconvulsants. The convulsant role of PTZ (7) and kainic acid (8) have been well characterized, however, there are very few published documents on the proconvulsant role of these two chemoconvulsants at subconvulsive doses. However, the exact mechanism behind the synergistic interaction between PTZ and kainic acid is unclear. The nature of seizure, as observed in both the groups of mice (i.e. PTZ induced seizure in kainic add pretreated mice and

kainic acid induced seizure in PTZ pretreated mice) were qualitatively similar. After administration of PTZ and kainic add, the animals became less active. After sometime, the animals showed greater locomotor activity and erection of body hair. All the mice that showed convulsions had increased salivation, defecation and urination. Some of the mice showed 'Straub's tail' phenomenon, writhing movement, rearing and falling down episodes. Clonic convulsions were noted in mice in a dose-dependent manner.

PTZ is reported to be a selective blacker of the chloride ionophore coupled to the GABA-A receptor (9). GABA-A receptor through chloride anion influx renders the neurons hyperpolarized and thus less excitable. GABA is the natural ligand for GABA-A receptor and is reported to be an inhibitory neurotransmitter and offers antiseizure activity (10). At subconvulsive doses, PTZ couples to the chloride ionophore to a limited extent leading to a state of neuronal hyperexcitability by antagonizing the effect of GABA. It might be possible that such hyperexcitable neurons respond to subthreshold dose of another proconvulsant through summation of excitatory potential leading to the origin of depolarization shift and spread of excitable impulse. Kainic acid, on the other hand, excites kainate receptor which belongs to a subgroup of the ionotropic glutamate receptor and is widely distributed in mammalian central nervous system (11). Excitation of kainate receptors leads to GABAergic cell loss (12) and rapid influx of calcium ion to overwhelm the cell's Ca^{2+} homeostatic capabilities (13). The synergistic interaction between PTZ and kainic acid as observed in the present study could be therefore, due to blockade of inhibitory GABA-A receptor mediated hyperpolarisation coupled to excitation of neurons due to kainic acid induced stimulation of excitatory glutaminergic system.

Earlier several groups of workers had reported the protective efficacy of nimodipine in different seizure models (14, 15). Nimodipine is a centrally selective dihydropyridine calcium channel blocker. In the present study, Nimodipine was found to offer significant protection to the mice from seizures evoked through combined use of PTZ and kainic acid i.e. in the two models of seizures developed in the present study namely PTZ induced seizures in kainic acid primed mice and kainic acid induced seizure in PTZ primed mice. Nimodipine administration did not cause sedation to the animals. The seizure protective efficacy of nimodipine could be related to the blocking of Ca^{2+} influx through voltage gated L-channels on the neurons (16). It might also be possible that nimodipine interacted with CaR (neuronal membrane bound receptor that are sensitive to the concentration of extracellular Ca^{2+}) and thereby regulated the activity of Ca^{2+} permeable non-selective cation channel (NCC). CaR has been postulated to modulate key neuronal functions including neuronal excitability and neurotransmission (17).

Thus, this study has established two *in vivo* models of seizures. It has demonstrated the synergism between kainic acid and PTZ in producing seizures in mice. Further, the study observed the protective rote of *in vivo* administration of 5.00 mg/kg dose of nimodipine in both the seizure models, thereby highlighting the role of Ca^{2+} in the genesis of seizure (18).

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