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Original Research Article

Anticonvulsant effect of nifedipine, dizepam and in combination on pentylenetetrazol induced experimental models of epilepsy on albino rats

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ABSTRACT

Background: In many patients, the presently available antiepileptic drugs such as phenobarbital, phenytoin, benzodiazepines, sodium valproate, etc., are unable to control seizures efficiently and the problem of adverse effects has also not been circumvented completely and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy. Hence, search should continue to develop newer, more effective, and safer neuro-protective agents for treatment of epilepsy. Aim of the study was to investigate the activity of nifedipine, the dihydropyridine calcium channel blocker, diazepam, the benzodiazepine anti-convulsant of established efficacy and their combinations against rat models of pentylenetetrazol (PTZ) induced convulsions. Method: Wister albino rats of either sex, weighing between 150-220gm were used. Rats were divided into 10 groups, in each group n=6 total N=60.
Methods: PTZ was administration 30 min after test drug administration. Intraperitoneal injection of PTZ at the dose of 80mg/Kg body weight were administered to the rats to produce chemically-induced seizure. The effect of nifedipine and diazepam were assessed on such seizure model. The onset and duration of clonic convulsion were recorded.
Results: The onset time of PTZ-induced clonic convulsion was significantly prolonged with the Nifedipine in the doses of 4mg and 8mg per Kg. in comparison to nifedipine in dose of 2mg per Kg. The interesting observation was that while Diazepam in 1mg/Kg. dose significantly ($P<0.05$) prolonged the onset time, there was significant decrease ($P <0.001$) in the onset time of PTZ-induced clonic convulsion with diazepam in doses of 2 and 4mg per Kg. in comparison to Diazepam 1mg per Kg. But the combination of diazepam 2.5 mg and Nifedipine 2.6mg and 5.3mg exhibited significant prolongation of the onset time. Diazepam 1 and 2mg per Kg was found to be equally effective in reduction of convulsion time, while 4mg dose showed more reduction of convulsion time. The combination of diazepam and nifedipine showed no better reduction in the convulsion time and also valproic acid in doses of 135mg. Kg.
Conclusions: Nifedipine (3-5mg/Kg) and diazepam (2.5mg/Kg.) combination delayed the onset of convulsion. Diazepam 2mg / Kg. alone was effective in reduction of duration of convulsion. The combination dose having 2.6mg of nifedipine showed comparable protection with valproic acid 135mg per Kg. while the combination having 5.3mg of nifedipine showed significantly better protection.

Keywords: Calcium channel blockers, Diazepam, Epilepsy, Sodium valproate, Pentylenetetrazol

INTRODUCTION

Epilepsy has afflicted human beings since the dawn of our species and has been recognized since the earliest medical writings. An Akkadian text from 2000 BC described a person in an epileptic attack. The attack was attributed to the god of sin and treated by exorcism. Recognized from the dawn of history as "disease of lightening" it was correctly described by Jackson little over a century ago. John Huglings Jackson established a neuropathological basis for the epilepsies that earned him the reputation as the "Father of modern concept of Epilepsy.[1,2]

A large number of agents have been used to treat epileptic patients. Herbs and simple inorganic compound including sulphuric acid have been used in medieval times. In early part of 19th century salts of iron, iodine, zinc, and silver as well as Digitalis, Belladonna, Opium, Mistle toe and oil of wintergreen were among the agents prescribed to epileptic patients.[3] The 1st anti-epileptic drug, potassium bromide was used in late 19th Century.[4] Discovery of the next antiepileptic agent Phenobarbital came about in 1912 by chance.[3]

In the course of screening a variety of drugs, they discovered that Diphenylhydantion, suppressed seizures in the absence of sedative effect. The chemical structures of most of the drugs introduced before 1965 were closely related to Phenobarbital. These included the hydantoins and the succinimides.

Between 1965 and 1990, the chemically distinct structures of Benzodiazepines, an Iminostilbene (Carbamazepine) and a branched - chain carboxylic acid (Valproic acid) were introduced followed in the 1990's by a Phenyltriazine (Lamotrigine), a cyclic analog of GABA (Gabapentin), a sulfamate substituted monosachride (Tiagabine) and a Pyrrolidine derivative (Levetiracetam).[4]

Epilepsy in India

Based on the total projected population of India in the year 2001, the estimated number of people with epilepsy is 5.5 million. The prevalence rate of epilepsy in India is 5.35 / 1000 population.[5] This is the percentage of persons with active epilepsy who are not receiving treatment as per the standard guidelines. This gap is very wide in India, esp. in rural India. As rural population constitutes 74% of Indian population, the number of people with epilepsy in rural areas is nearly 4 million, three fourth of them are not getting any specific treatment as per the present standard.[6]

In many patients, the presently available antiepileptic drugs (AED) such as phenobarbital, benzodiazepines, sodium valproate, carbamazepine, ethosuximide, trimethadione etc., are unable to control seizures efficiently and the problem of adverse effects has also not been circumvented completely and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy.[7,8] Hence, search should continue to develop newer, more effective, and safer neuroprotective agents for treatment of epilepsy.

Calcium channel blockers, especially diahydropyridine type of calcium channels blockers have been shown to block various aspects of epileptogenesis and are effective anti-convulsants in a number of in vivo models.[9]

Several other mechanisms of action in neuropsychiatric disorders have been proposed for valproic acid in recent years.[10] However Valproic acid blocks the voltage-gated sodium channels and T-type calcium channels. These mechanisms make valproic acid a broad spectrum anticonvulsant drug.[11]

Injection of pentylenetetrazol **(**PTZ) in rats or mice produces clonic convulsions which are prevented by drugs effective in absence seizures.[12]

The present study was undertaken to investigate the activity of nifedipine, Dizepam, in combination on PTZ induced convulsion against rat models.

METHODS

Design of the Study was Experimental animal based study.

In each group Sample size was n=6 total N=60.

Rearing of animal

Rats were kept at the animal house in polypropylene cages at controlled temperature of 23°±2C° and humidity of 50% with standard 12 hour light-dark cycle beginning at 6.00 AM. They received standard diet and water ad libitum.[13]

Inclusion criteria

Wister albino rats of either sex, weighing between 150-220 gm were used for PTZ chemical induced seizures. Rats were screened before the experiment first with Rota rod test. Only those rats showing characteristic course of convulsions were selected for the experiment.[14]

Exclusion criteria

Those rats showed abnormality and behavioural changes were excluded

Drugs preparation

Nifedipine

Himedia Laboratories

25ml of standard solution of Nifedipine was prepared by dissolving 25mg of Nifedipine in a solution containing 9ml of polyethylene glycol, 14.5ml of glycerine, and

1.5ml of distilled water in the ratio of 6:10:1 making the concentration of Nifedipine 1mg/ml. Dose of Nifedipine given to rat is 2mg, 4 and 8mg/Kg of body weight.[15]

Diazepam

Stock solution of diazepam in the concentration of 1mg/ml was prepared by adding 8 ml of distilled water to one ampoule of diazepam containing 10mg/2ml. It was administered to the rats in the dose of 1, 2 or 4mg/Kg of body weight.[16]

Sodium valproate (S. S. Pharmaceuticals)

One ampoule of sodium Valproate contains 40mg/ml concentration. 3ml of distilled water was added to make a concentration of 10mg/ml. It was administered to the rats in the dose of 135mg/Kg of body weight.[17]

Dose and route of administration

Individual doses were calculated for each rat according to their body weight, combination was selected as ED_{25} and ED_{50} of Nifedipine (2.6 and 5.3mg) with ED_{50} of Diazepam (3mg) and injected intraperitonelly using insulin syringe.

Grouping: Group 1: Control, Group 2-4: Nifedipine 2, 4 and 8 mg/kg accordingly, Group 5-7. Diazepam 1, 2 and 4 mg/kg accordingly, Group 8: Nifedipine (2.6 mg) + Diazepam (2.5mg), Group 9: Nifedipine (5.3 mg) + Diazepam (2.5mg), Group 10: Sodium valproate (135mg/kg)

Animals successful with screening tests were selected randomly using random number table. PTZ was administration 30 min after test drug administration. Intraperitoneal injection of PTZ at the dose of 80mg/Kg body weight were administered to the rats to produce chemically-induced seizure. The seizures were clonic in nature and analogous to petitmal (absence) type of seizures. The effect of nifedipine and diazepam were assessed on such seizure model. The onset and duration of clonic convulsion were recorded. These are the parameters was studied.

Statistical analysis

SPSS statistical software was used for analysis. Post hoc test and one way ANOVA were applied for comparison between the groups. Data was presented as Mean±SE.

RESULTS

Clonic seizure

The onset of clonic convulsion was significantly (P <0.001) delayed by the 3 doses of nifedipine (2, 4 and 8mg/Kg.), 3 doses of diazepam (1, 2 and 4mg/Kg.), combinations of diazepam ED50 (2.5mg) with nifedipine ED25 (2.6mg) and ED50 (5.3mg), valproic acid (135 mg/Kg.) doses in comparison to placebo (normal saline). One interesting observation was that the delay in the onset time was less with the diazepam in the dose of 2 and 4mg per Kg. in comparison with 1mg per Kg.

Table 1: Effect of anti convulsant on onset, duration of actions.

Group	Body weight (gm)	Time duration in seconds	
		Onset (Sec)	Duration (Min)
Group 1	172.8±3.49	75.17±2.76	15.17±1.28
Group 2	172.83±3.49	222.5±13	12.6±0.48
Group 3	173.3±4.58	338±12.2	7±0.37
Group 4	178.5±6.32	410±4.58	5.5±0.34
Group 5	172.83±4.93	427.2±10.2	3.4±0.33
Group 6	182.50±5.78	223.4±8.85	2±0.45
Group 7	177.83±4.35	185.25±5.12	1±0.37
Group 8	181.33±4.06	466.2±35.12	1.5±0.34
Group 9	186±3.19	488.8±33.53	0.83±0.31
Group 10	176±2.31	591.7±38.31	1.17±0.31

Table 2: Statistical One-way ANOVA.

Source	S.S.	df	MS	F	P	Sig
Between	1880257.10	9	208917.46	55.07	P <0.001	***
Within	166918.40	44	3793.60			
Total	2047175.50	53				

There was highly significant (P <0.001) variation among the study groups

The onset time of PTZ-induced clonic convulsion was significantly prolonged with the Nifedipine in the doses of 4mg and 8mg per Kg. in comparison to nifedipine in dose of 2mg per Kg. The interesting observation was that while Diazepam in 1mg/Kg. dose significantly prolonged the onset time, diazepam in higher doses (2mg/Kg and 4mg/Kg.) reduces the onset time and it became comparable with that of Nifedipine at the dose of 2mg/Kg. Again, when nifedipine (ED25 =2.6mg/Kg and ED50 = 5.3mg/Kg) was co-administered with diazepam (ED50 =2.5mg/Kg.), the onset time of clonic convulsion was significantly increased. Of course, there was significant prolongation (P <0.001) of onset time for clonic convulsion with valproic acid, the reference standard drug.

Nifedipine (4mg/Kg.) and Nifedipine (8mg/Kg.) both showed almost equal prolongation of onset time, the difference between them statistically insignificant (P >0.05), indicating Nifedipine 4 mg showed the maximal effect. Again, Diazepam 1mg/Kg. dose showed significant prolongation of effect in comparison to Nifedipine 4mg/Kg, while the higher doses i.e. 2 and 4mg/Kg. progressively decreased the onset time in comparison to

Nifedipine 4mg/Kg. With the combination doses, there was significant prolongation of onset time and so also with valproic acid.

The effect of diazepam (1mg/Kg.) and Nifedipine (8mg/Kg.) showed comparable effect on onset time, the difference between them being statistically insignificant (P > 0.05). Diazepam in doses of 2 and 4 mg per Kg. showed significant (P <0.001) decrease in the onset time. The two combination doses and Valproic acid exhibited significant prolongation in the onset time that indicated better protection from the PTZ induced seizure.

There was significant decrease (P <0.001) in the onset time of PTZ-induced clonic convulsion with diazepam in doses of 2 and 4mg per Kg. in comparison to Diazepam 1mg per Kg. But the combination of diazepam 2.5mg and Nifedipine 2.6mg and 5.3mg exhibited significant prolongation of the onset time. However, the prolongation of onset time by Valproic acid was significantly more (P <0.001) in comparison to diazepam 1mg/Kg.

The effect of diazepam 2 and 4mg per Kg dose on were found to be comparable. The combination of diazepam and nifedipine were having significantly more prolongation of onset time as well as the Valproic acid.

Both the doses of nifedipine i.e. 2.6mg and 5.3mg when combined with diazepam 2.5mg per Kg. was showed to prolong the onset time of PTZ-induced convulsion in comparison to diazepam 4mg per Kg. The same trend was also shown by Valproic acid.

The prolongation of onset time was significantly more (P <0.01) when 5.3mg of nifedipine was combined with 2.5mg of diazepam than when 3.0mg was combined with the same dose of diazepam. But the delay afforded by combination of 2.5mg of diazepam with 2.6mg of nifedipine was found to be comparable to valproic acid in the dose of 135mg/Kg.

Duration of clonic convulsion

There was significant (P <0.001) difference in the duration of clonic convulsion between the study groups.

The effect of nifedipine, diazepam in all the doses employed in this study as well as their combination exhibited significant reduction of duration of PTZ-induced clonic convulsion than placebo. The reduction of duration of PTZ-induced clonic convulsion was significantly less with nifedipine 4mg and 8mg, diazepam 1, 2 and 4mg, the combination of diazepam and nifedipine than nifedipine in 2mg/Kg. dose.

The reduction of duration of PTZ-induced clonic convulsion was comparable with 4 and 8 mg of nifedipine dose, the difference between them were not significant. So the maximal effect was seen in the dose of 2-4mg of diazepam. However, diazepam in all doses (1, 2 and 8mg/Kg.) showed significant reduction of convulsion time as well as the combinations and valproic acid from the nifedipine 4mg/Kg.

The reduction of duration of convulsion was statistically more significant with the diazepam in all 3 doses, the combination of nifedipine and diazepam and valproic acid than the highest dose of nifedipine (8mg/Kg.) employed in this study.

Diazepam 1 and 2mg per Kg was found to be equally effective in reduction of convulsion time, while 4mg dose showed more reduction of convulsion time. Diazepam 2 and 4mg showed equal reduction in convulsion time, their difference was statistically not significant. The combination of diazepam and nifedipine showed no better reduction in the convulsion time and also valproic acid in doses of 135mg/Kg. It indicated the optimum dose of nifedipine that caused reduction of convulsion time was 2mg. which was comparable to valproic acid. The combination dose of nifedipine and diazepam was not found to superior in reduction of convulsion time.

DISCUSSION

The prolongation in the onset time of PTZ-induced convulsion indicated protection from clonic seizure which was seen with absence seizure. Nifedipine caused dose-dependent prolongation of onset time, but it was significantly less than valproic acid 135mg/Kg. employed in this study, with 4-8mg showed maximal effect. Diazepam 1mg/Kg dose showed prolongation of onset time, but the higher dose i.e. 2 and 4mg/Kg showed progressively decrease in the onset time indicating lower degree protection from PTZ-induced clonic convulsion. The combination of Nifedipine (2.6mg and 5.3mg) and diazepam (2.5mg) showed significant prolongation of onset time, which increased with the increased dose of nifedipine in the combination, indicating synergistic effect of both drugs. The combination dose having 2.6mg of nifedipine showed comparable protection with valproic acid 135mg per Kg. while the combination having 5.3mg of nifedipine showed significantly better protection PTZ-induced clonic convulsion it was found that- The optimum dose of nifedipine was 2-4mg, but its effect was less than diazepam. The optimum dose of diazepam was 2 mg which was comparable to valproic acid (135mg/Kg.). Combination of diazepam and nifedipine showed no increased reduction of convulsion time. So, the overall effect of this study on PTZ-induced clonic convulsion showed that- Nifedipine (3-5mg/Kg) and diazepam (2.5mg/Kg.) combination delayed the onset of convulsion. Diazepam 2mg/Kg. alone was effective in reduction of duration of convulsion.

PTZ is a noncompetitive GABA receptor antagonist that produces generalized seizures at high doses.[18] It has also been demonstrated that the administration of PTZ could up-regulate NMDA receptors in several regions of the rat brain.[12] There is some other evidence that free radicals are

actively involved in physiological processes during oxidative stress induced by administration of convulsants. Different mechanisms may lead to in PTZ-induced convulsions.[19]

The efficacy of CCBs to change the parameters in PTZ test in the present study. Calcium ion influx may be involved in the origin of seizures.[20]

The anticonvulsant activity elicited by nifedipine observed in our study is in keeping with previous studies. The hippodcampus regions CA1 and CA3 of rat brain are also known to possess one of the higher densities of the dihydropyridine receptors. The results of many experimental studies have shown that calcium channel blockers are effective against several different types of seizures Hence, nifedipine proved its effect in this model.[21]

Czuczwar SJ et al, study concluded that calcium channel inhibitors studied influenced the action of diazepam (0.2mg/kg). It may be concluded that combinations of ethosuximide, with either nifedipine or diltiazem, may be promising for the treatment of absence epilepsy.[22]

A study by Luisa Rocha found that there is an aggravation of PTZ-induced seizures following the repetitive administration of diazepam. The shorter latency to all the components of the PTZ-induced seizures after subchronic administration with diazepam could result from a higher activation of forebrain areas.[18]

Results of papazova et al, suggest that nifedipine and diltiazem might be useful in the treatment of cognitive disorders in epileptic patients.[23]

Limitations of the study was as it is experimental animal study further clinical studies are required to prove the effect and more concentration required on combination interactions.

CONCLUSION

The present study was undertaken to investigate the activity of nifedipine, the dihydropyridine Ca^{++} channel blocker, diazepam, and their combinations against rat models of pentylenetetrazol induced clonic seizures. Nifedipine (3-5mg/Kg) and diazepam (2.5mg/Kg.) combination delayed the onset of convulsion. Diazepam 2mg/Kg. alone was effective in reduction of duration of convulsion. The combination dose having 2.6mg of nifedipine showed comparable protection with valproic acid 135mg per Kg. while the combination having 5.3mg of nifedipine showed significantly better protection.

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REFERENCES

1. Bradley WG. Neurological disorders: Neurology in clinical practice. Butter worth - Heinemann Medical, England. 1991;2:1444-6.
2. Allan H, Roopper, Maurice Victor. Adams and victor's principles of Neurology. 10th Edition; McGraw Hill Text, New York; 2014:249-294.
3. Kutt H, Mcdowell F. Clinical Neuropharmacology: Churchill Living Stone, London. 4: 15-18.
4. James O, Namara Mc. Pharmacotherapy of the Epilepsy: Goodman and Gilman's Pharmacological basis of Therapeutics. 2005;11:501-698.
5. Pumani R, Rurlewizl LJ. Fast Extracellular transients: involvement in epileptic processes. Indian J of Pharma. 2000;32:347-52.
6. Tortella FC, Robles L, Holaday JW. U50 488 a highly selective Kappa opioid anticonvulsant effect in rats. Indian J of Pharma. 1997;29:194-7.
7. Ezekiel I, Mabrouk MA, Ayo JO, Goji AD, Okpanachi AO, Mohammed A, et al. Study of the effect of hydro-aqueous extract of Commiphoraafricana (stembark) on sleeping time and convulsion in mice. Asian J Med Sci. 2010;2:85-8.
8. Bhosle V. Anticonvulsant and antioxidant activity of aqueous leaves extract of Desmodiumtriflorum in mice against pentylenetetrazole and maximal electroshock induced convulsion. RevistaBrasileira de Farmacognosia Brazilian Journal of Pharmacognosy. 2013;23(4):692-8.
9. Cain SM, Snutch TP. Voltage-Gated Calcium Channels in Epilepsy. Jasper's Basic Mechanisms of the Epilepsies. 4th Edition. National Center for Biotechnology Information (US); 2012.
10. Rosenberg G. The mechanisms of action of valproate in neuropsychiatric disorders: can we see the forest for the trees? Cell Mol Life Sci. 2007;64(16):2090-103.
11. Michael A. Rogawski, José Enrique Cavazos. Mechanisms of action of antiepileptic drugs. Available at: http://mr.ucdavis.edu/uploads/1/1/7/5/11757140/ch43.pdf
12. Keshavarz M, Showraki A, Emamghoreishi M. Anticonvulsant Effect of Guaifenesin against Pentylenetetrazol-Induced Seizure in Mice. Iran J Med Sci. 2013;38(2):116-21.
13. Sangishetty VP, Anthireddy S, Ambareesh K, Nayak BB, Shinde BB, Ambadas B. Protective Role of Oxitard in Drug Induced Ulcers in Rats. Int J Biol Med Res. 2012;3(3):1948-51.
14. Phulen S, Bhattacharyya A. Models of epilepsy used in antiepileptic drug discovery: A review. Int J Pharm Pharm Sci. 2014;6(11):1-7.
15. Khanna N, Bhalla S, Verma V, Sharma KK. Modulatory effects of nifedipine and nimodipine in experimental convulsions. Indian J of Pharma. 2000;32:347-52.
16. Hanada T, Ido K, Kosasa T. Effect of perampanel, a novel AMPA antagonist, on benzodiazepine-resistant status epilepticus in a lithiumpilocarpine rat model.

Pharmacology Research and Perspectives. 2014;2(5):63.
17. Niquet J, Baldwin R, Norman K, Suchomelva L, Lumley, Wasterlain CG. Midazolam ketamine dual therapy stops cholinergic status epilepticus and reduces morris water maze deficits. Epilepsia. 2016;57(9).
18. Rocha L. Subchronic treatment with antiepileptic drugs modifies pentylenetetrazol-induced seizures in mice: Its correlation with benzodiazepine receptor binding. Neuropsychiatr Dis Treat. 2008;4(3):619-25.
19. Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. Science. 1993;262(5134):689-95.
20. Sahadevan P, Rema MN. A comparative experimental study of the anticonvulsant effect of three calcium channel blockers in albino mice. Indian J of Pharma. 2002;34:52-5.
21. Mante PK, Adongo DW, Woode E. Anticonvulsant effects of antiaris toxicaria aqueous extract: investigation using animal models of temporal lobe epilepsy. BMC Research Notes. 2017;10:167.
22. Czuczwar SJ, Małek U, Kleinrok Z. Influence of calcium channel inhibitors upon the anticonvulsant efficacy of common antiepileptics against pentylenetetrazol-induced convulsions in mice. Neuropharmacology. 1990;29(10):943-8.
23. Genkova-Papazova MG, Petkova B, Shishkova N, Lazarova-Bakarova. Effect of the calcium channel blockers nifedipine and diltiazem on pentylenetetrazole kindling-provoked amnesia in rats. European Neuro Psychopharmacology. 2001;11(2):91-6.

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