ORIGINAL ARTICLE

In- Vivo Comparison of Anticonvulsant Effects of Gabapentin and Verapamil alone and in Combination with Diazepam on Acute Seizure Model

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ABSTRACT:

Objective: To compare in-vivo anticonvulsant effects of gabapentin and verapamil alone and in combination with diazepam in acute seizure model of mice.

Methodology: This experimental study was conducted in H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, Karachi University, from May 2009 to July 2011.

Pentylenetetrazol (PTZ) was used in the dose of 90 mg/kg subcutaneously to induce acute seizures in mice. The test drugs Gabapentin (GBP) and Verapamil (VP) were administered by intraperitoneal route in six doses individually as well as in combination. The reference drug Diazepam (DZ), and test drugs were administered 40 minutes before PTZ intraperitoneally. The acute anticonvulsive activities of test drugs individually and in combinations were evaluated in-vivo by comparing with anti-seizure effects of reference drug DZ. After administration of PTZ, mice were observed for next 40 minutes for latency to onset of threshold seizures and for the presence or absence of seizure behaviors. The duration of seizures was divided into Rearing and falling (R & F) and Hind limbs tonic extensions (HLTE). R & F was the time calculated from beginning of seizure phase to rearing and falling of mice. HLTE was the time recorded from rearing and falling to development of generalized tonic clonic phase of seizure. The cut off time was 40 minutes.

Results: As individual treatment regimens the anti-seizure scores and mortality protection of GB: PTZ as well as VP: PTZ were significantly inferior to DZ in all seizure patterns, however, combination regimen of GBP:VP:PTZ in the last two higher doses exhibited highly significant antiseizure effects with 100% mortality protection which were equivalent to reference drug DZ.

Conclusion: The Combination regimen was novel and at higher doses exhibited potent acute anti-seizure activities equal in efficacy to DZ.

Keywords: Antiepileptic drugs, Gabapentin, Verapamil, Diazepam, Status epilepticus

Introduction:

Status epilepticus is a common neurological and life threatening medical emergency[1]. The patient is labeled as under status epilepticus when the patient has continuous rapidly repeated attacks of seizures without gaining consciousness between them. It must be treated or else it may cause serious damage to the brain and even death in many cases.[2,3] Despite the improvements made in managing status epilepticus patients, mortality is still very high, thus indicating that there is a substantial need to improve measures for both the prevention and effective management of this syndrome. There are various causes of status epilepticus including sudden withdrawal of antiepileptic drugs, central nervous system infections, high grade fever, hypoglycemia, brain tumors, refractory epilepsy, hypocalcemia, vitamin B deficiency and various metabolic abnormalities.

Lorazepam and DZ are the first line drugs, and only benzodiazepines recommended for the acute short term management of status epilepticus.[4,5] If the seizures are uncontrolled then Phenytoin, Phenobarbitone and Valproate are given intravenously for long term control of status epilepticus.[6,7] Many anti-epileptic drugs (AED) share potential drug-drug interactions and various harmful short term and long term side effects.[8,9]

GBP has established antiepileptic effects when used as an adjunct or monotherapy for partial as well as for generalized tonic clonic seizures. High doses of GBP are needed for improvement in seizure control, however, the high doses are mostly tolerable and its safety and tolerability is rated as good to excellent. Its major side effects are tremors, headache, ataxia, dizziness and somnolence.[10,11] Though VP is basically anti-hypertensive and anti-arrhythmia drug but its unique anti-seizure effects have been noted in pharmaco-resistance epilepsy and in patients of refractory epilepsy suffering from severe myoclonic epilepsy of infancy.[12,13] Verapamil when used as adjunctive therapy, controlled seizures including status epilepticus.[14] The present study was aimed to studyin-vivo comparison of anticonvulsant effects of gabapentin and verapamil alone and in combination with diazepam on acute seizure model of mice.

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METHODOLOGY:

This experimental study was carried out in Hussain Ebrahim Jamal (H.E.J.) Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, from May 2009 to July 2011. The use of animals was approved by the Institutional Scientific Advisory Committee. Male NMRI albino mice weighing 20-25 g, in a group of 12 were used,which had 80% power to detect differences in the means.

Experimental animals were divided into three sections, i.e. A, B and C. In each section, animals were divided into ten groups comprising of 12 mice each. In each section, Group I served as control and was given normal saline, group II received only PTZ 90mg/kg subcutaneously; groups III to VIII were treated with six different doses of tested drugs intraperitoneally.[15,16] Group IX, treated with DZ served as standard antiepileptic drug for status epilepticus, a single dose of 7.5mg/kg was given 40 minutes before administration of 90 mg/kg of PTZ.[17,18] Six groups of section-A received GBP in doses of 100, 200, 300, 400, 500 and 600mg/kg intraperitoneally.[19,20] Section B received VP in doses of 5, 10, 15, 20, 25 and 30 mg/kg by intraperitoneal route. Section C received combined GBP: VP in doses of 100:5, 200:10, 300:15, 400:20, 500:25 and 600:30mg/kg respectively, forty minutes before administration of 90 mg/kg of PTZ,as per recommended dose for international animal studies.[21,22] After injecting PTZ, mice were isolated and closely observed for next 40 minutes (2400 seconds) for latency to onset of threshold seizures (LOTS) and for the presence or absence of seizure behaviors. The mortality protection (number of mice survived) was recorded in percentage. This model of epileptic seizure was employed to induce status epilepticus. After administration of PTZ latent time (LOTS) was recorded, i.e., period (time in seconds or minutes) immediately after administration of PTZ or combination of PTZ with test drugs and before the beginning of first sign of seizure phase in mice, in order to determine threshold of seizures affected by PTZ and test drugs.Then, total duration of seizure behavior from the beginning to tonic clonic phase was recorded. We had split the complete duration of seizures into two behaviors; Rearing and falling (R & F) and Hind limbs tonic extensions (HLTE) for complete analysis of our results. R & F(time calculated from beginning of seizure to rearing and falling of mice) and HLTE(time recorded from rearing and falling to development of generalized tonic clonic seizure) were the actual duration of seizures induced by PTZ with or without test drugs. Period of 40 minutes was taken as seizure protection after administration of PTZ with or without tested drugs. The anticonvulsive effects of GBP and VP alone, or in combination regimen was evaluated in-vivo by recording durations of LOTS, R& F, HLTE and seizure protection in percentage, and then comparing with anti-seizure effects of reference drug DZ.

STATISTICAL ANALYSIS:

The statistical analysis was performed using SPSS version 17. Results were reported as mean±SEM.Data of seizure activity was analyzed by nonparametric Student's t-test and ANOVA with post hoc Dunnett's multiple comparison tests. The sequential differences among means were calculated at the level of $p<0.05$.

RESULTS:

Table-1 shows the results of section A experimental animals with the treatment of GBP: PTZ as a monotherapy from 100: 90 to 600: 90 mg/kg six doses. GBP: PTZ exhibited mortality protection from 41.66% in lower doses while the mortality protection increased to 66.66% in higher doses. Anti-seizure effects recorded in LOTS, R&F and HLTE of GBP: PTZ compared to PTZ induced seizures by t test have shown that results were highly significant. The reference drug DZ with PTZ exerted 0.00 anti-seizure score at the cutoff time of 2000 seconds in all three patterns of seizure behaviors

Table: 1
Seizure patterns recorded in the acute model of PTZ-induced seizures in mice following treatment with GB. Each value represents the Mean ± SEM of 12 animals per group

Group	Dose (mg/kg)	LOTS (sec)	R & F (sec)	HLTE (sec)	Mortality (%)	% of Mice Suffering from R & F	Mortality Protection %
Normal Control	0.9 % Saline	0.00	0.00	0.00	0.00	0.00	0.00
PTZ	90	184 ± 46	330 ± 50	686 ± 66	100	100	0.00
GBP : PTZ	100 : 90	230 ± 56 [d]	430 ± 77 [b,d]	0.00 [b,d]	0.00	75.00	100
GBP : PTZ	200 : 90	260 ± 62 [b,d]	480 ± 71 [b,d]	0.00 [b,d]	0.00	75.00	100
GBP : PTZ	300 : 90	330 ± 80 [b,d]	590 ± 125 [b,d]	0.00 [b,d]	0.00	66.66	100
GBP : PTZ	400 : 90	360 ± 73 [b,d]	760 ± 229 [b,d]	0.00 [b,d]	0.00	50	100
GBP : PTZ	500 : 90	590 ±76 [b,d]	930 ± 280 [b,d]	0.00 [b,d]	0.00	50	100
GBP : PTZ	600 : 90	740 ± 74 [b,d]	0.00 [b,d]	0.00 [b,d]	0.00	0.00	100
DZ : PTZ	10 : 90	0.00	0.00	0.00	0.00	0.00	100

n= 12
Values are mean ± S.E.M
LOTS= latency to onset of threshold seizures
R & F = rearing and falling
HLTE=hind-limbs tonic extension
b P ≤ 0.005 highly significant as compared to PTZ
d P ≤ 0.005 highly significant as compared to DZ

(Table-1).
Table-2 shows the results of section B experimental animals treated by VP: PTZ as a single agent therapy at six different doses from 5:90mg/kg to 30:90mg/kg.Values of LOTS, R&F and HLTE are shown. VP: PTZ groups in acute model of epilepsy exhibited 100% mortality in three lower doses however, in higher doses mortality reduced to 66.67%. The maximum mortality protection was 33.33% in higher doses and nil in lower three doses. Anti-seizure mean scores and mortality protection of VP: PTZ as a single agent when compared to reference drug revealed them to be significantly inferior to it in all seizure patterns (Table-2).

Table: 2
Seizure patterns recorded in the acute model of PTZ-induced seizures in mice following the treatment with VP.
Each value represents the Mean ± SEM of 12 animals per group

Group	Dose (mg/kg)	LOTS (sec)	R & F (sec)	HLTE (sec)	Mortality (%)	% of Mice Suffering from R & F	Mortality Protection %
Normal Control	0.9 % Saline	0.00	0.00	0.00	0.00	0.00	0.00
PTZ	90	186 ± 44	340 ± 50	700 ± 57	100	100	0.00
VP : PTZ	5 : 90	200 ± 19 **d**	400 ± 24 **d**	770 ± 45 **d**	100	100	0.00
VP : PTZ	10 : 90	230 ± 23 **b,d**	440 ± 32 **d**	880 ± 34 **d**	100	100	0.00
VP : PTZ	15 : 90	290 ± 19 **d**	500 ± 19 **d**	910 ± 19 **d**	83.33	83.33	16.33
VP : PTZ	20 : 90	320 ± 34 **a,d**	560± 39 **a,d**	940 ± 24 **a,d**	83.33	83.33	16.33
VP : PTZ	25 : 90	400 ± 48 **a,d**	630 ± 109 **a,d**	1010 ± 122 **a,d**	75.00	75.00	25.00
VP : PTZ	30 : 90	480 ± 54 **a,d**	700 ± 122 **a,d**	1100 ± 138 **a,d**	66.66	75.00	33.33
DZ : PTZ	10 : 90	0.00	0.00	0.00	0.00	0.00	100

n= 12
Values are mean ± S.E.M
LOTS= latency to onset of threshold seizures
R & F = rearing and falling
HLTE=hind-limbs tonic extension
a P ≤ 0.05 significant as compared to PTZ
b P ≤ 0.005 highly significant as compared to PTZ
c P ≤ 0.05 significant as compared to DZ
d P ≤ 0.005 highly significant as compared to DZ

Table-3 demonstrates section C experimental animal results. The Combined regimen of GBP: VP: PTZ exhibited mortality protection of 58%- 83.33% in first four doses, whereas, in 5th and 6th doses, the mortality protection was 100% in cut off time of 2400 seconds. The effect of last two doses was equivalent to reference drug DZ (Table-3).

Table: 3
Seizure patterns recorded in the acute model of PTZ-induced seizures in mice following the treatment with GBP: VP

Group	Dose (mg/kg)	LOTS (sec)	R & F (sec)	HLTE (sec)	Mortality (%)	% of Mice Suffering from R & F	Mortality Protection %
Normal Control	0.9 % Saline	0.00	0.00	0.00	0.00	0.00	0.00
PTZ	90	190 ± 49	365 ± 50	700 ± 55	100	100	0.00
GBP : PTZ	100 : 5 : 90	360 ± 30 [a,d]	510 ± 108 [a,d]	0.00 [b]	0.00	66.66	100
GBP : PTZ	200 : 10 : 90	420 ± 45 [b,d]	650 ± 196 [b,d]	0.00 [b]	0.00	50	100
GBP : PTZ	300 : 15 : 90	570 ± 37 [b,d]	980 ± 374 [b,c]	0.00 [b]	0.00	41.66	100
GBP : PTZ	400 : 20 : 90	750 ± 70 [b,d]	1230 ± 524 [b,c]	0.00 [b]	0.00	25	100
GBP : PTZ	500 : 25 : 90	1060 ± 72 [b,d]	0.00 [b]	0.00 [b]	0.00	0.00	100
GBP: PTZ	600 : 30 : 90	0.00 [b]	0.00 [b]	0.00 [b]	0.00	0.00	100
DZ : PTZ	10 : 90	0.00	0.00	0.00	0.00	0.00	100

n= 12
Values are mean ± S.E.M
LOTS= latency to onset of threshold seizures
R & F = rearing and falling
HLTE=hind-limbs tonic extension
a $P \leq 0.05$ significant as compared to PTZ
b $P \leq 0.005$ highly significant as compared to PTZ
c $P \leq 0.05$ significant as compared to DZ
d $P \leq 0.005$ highly significant as compared to DZ

DISCUSSION:

The rationale for selecting GBP and VP combination had many reasons including their reported characteristics of having inhibitory and modulating effects on the voltage-gated calcium channels of CNS.[23,24] GBP has inherent potential of antiepileptic properties which can be augmented if given in combination with other drugs like calcium channel blockers i.e. VP. GBP has been approved by the FDA as monotherapy for partial and complex partial seizures with or without generalized tonic-clonic seizures.[25,27] VP is a typical calcium channel blocker which is not an approved AED for the treatment or add-on therapy for epileptic disorders. However, in various research studies it has proved its blocking and inhibitory effects on voltage-gated calcium channels of CNS.[28,30]

We proposed that anti-seizure actions of GBP can be augmented or modified if given in combination with VP. Our study is supported by various animal and clinical studies. It was revealed that calcium channel antagonists possess anticonvulsant potential in experimental models of epilepsy and potentiate the protective activity of some AEDs.[31,32] Influx of Ca^{2+} into the neuron plays an important role in the genesis of epileptic seizures, and current research suggests that calcium entry blockers such as VP which blocks N- and P/Q-type calcium channels may have anticonvulsant activity by blocking effects on both these channels.[33,34] Amlodipine (at 10 mg/kg) reduced PTZ-induced clonic and tonic convulsions in mice and enhanced the anticonvulsant properties of Valproate and Phenobarbitone.[35] Nimodipine showed a decrease in seizure frequency in patients with intractable epilepsy caused by organic brain lesions when used in combination with other AEDs.[36,37] Modulating effects of Nimodipine and Nifedipine were observed in experimental convulsions in acute model of epilepsy in mice.[38] VP as a calcium channel blocker possessed anticonvulsant activity in acute model of epilepsy in mice.[39] Various studies observed significant enhancing anti-convulsant effects of calcium channel blockers on AEDs.[40] One clinical study showed successful treatment with intravenous calcium channel blockers in patients with continuous focal epileptic seizures intractable to conventional antiepileptic therapy.[41] Hence Ca^{2+} antagonists which penetrate the blood-brain barrier and bind to neuronal tissue may emerge in future as a novel class of anticonvulsants.[42] Hence, there are compelling reasons to state that present study has significant clinical potential. Our proposed objective was to compare the anti-seizure effects of GBP and VP as individual and combination regimens with DZ in acute seizure model in mice. We examined and analyzed the combination therapy from multiple dimensions in acute model of seizures.

In the present study, GBP as monotherapy exhibited mild to moderate anti-epileptic effect. Seizure protection by GBP at the doses of 100–200mg/kg, 300-400mg/kg and 500-600mg/kg was 41.66%, 50%-58.33% and 66.66% respectively. This shows that maximum seizure protection was 33.34% inferior to reference drug DZ.

Thus, GBP as individual drug failed to show significant anticonvulsant effects at all tested doses compared to DZ. VP alone demonstrated poor anti-epileptic effects in lower doses and mortality was 100%, however, in higher doses it exhibited insignificant dose dependent anti-seizure effects, much inferior to DZ. VP-treated group showed 33.33 % maximum seizure protection at the dose of 25-30mg/kg, which was very weak compared to DZ. When anti-seizure effects of combination therapy were compared to reference drug DZ, LOTS demonstrated that seizure inhibition was equal to the effects of DZ. However, in case of R&F, combination therapy of GBP and VP completely inhibited the seizure behavior at the dose of 500-600 mg/kg GBP and 25-30 mg/kg VP thereby, demonstrating that combination therapy in higher two doses was equivalent in efficacy to DZ.

Combination therapy of GBP with VP in six different doses in PTZ-induced acute seizures elicited seizure protection of 58.33%, 66.66%, 83.33%and 100 % respectively. The maximum anticonvulsant effects were seen in the groups receiving 500-600 mg/kg GBP / 25-30 mg/kg VP where in seizure protection of 100% was observed. While reference drug DZ exhibited 100% seizure protection/mortality protection and no seizure scores were observed in all three seizure behavior.DZ completely abolished the effects of PTZ. We compared the combined regimens of GBP/VP groups receiving 500-600 mg/kg GBP / 25-30 mg/kg VP doses with reference drugs D.Z. We observed no difference in seizure/ mortality protection. From the above discussion we are inclined to hold that the combination regimens exhibited zero seizure score and 100 % seizure/mortality protection at the last two higher doses and were equivalent to reference drug diazepam.

CONCLUSION:

In acute Model of epilepsy we observed that GBP as single therapy exhibited mild to moderate anti-epileptic effects. Our data has demonstrated that none of the doses of GBP as individual treatment regimens demonstrated 100 percent seizure protection. VP alone demonstrated poor anti-epileptic effects in lower doses and mortality was 100%, however, in higher doses it exhibited dose dependent anti-seizure effects and those were much far inferior to reference drug DZ and were insignificant. The combined regimen of GBP/VP regimen groups receiving higher doses when compared with reference drugs DZ we observed no difference in seizure protection. The mortality protection was 100 percent as exhibited by DZ, while all three seizure behavior characteristics results were equivalent to DZ. Combined regimens anti-seizure effects at higher doses were equivalent to reference drug DZ. It can reasonably be presumed that the instant regimens of GBP: VP may probably contribute to be the alternative regimens for the management of both status epilepticus and in resistance/refractory cases of status epilepticus. The combination regimens may have significant potential for short term and long term management of status epilepticus. Such query requires elaborate further in vitro animal studies as well as clinical trials.

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