ORIGINAL ARTICLE

Comparison of Anticonvulsant Effects of Combined Regimens of Gabapentin and Verapamil with their Individual Effects

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ABSTRACT:

Objective: To evaluate and compare in-vivo anticonvulsant effects of combined regimens of gabapentin and verapamil with their individual effects on kindled model of epilepsy in mice.

Materials and Methods: This experimental study was carried out in H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, from May 2009 to July 2011.Gabapentin (GBP) and Verapamil (VP) were used as tested drugs both as combined regimens as well as individual drugs, Phenytoin was used as reference drug. Kindling was produced by repeated administration of Pentylenetetrazole in a dose of 50 mg/kg by subcutaneous route every 48 hours for 20 days. Three doses of Gabapentin from 100 mg/kg to 300 mg/kg and three doses of Verapamil from 10 mg/kg to 30 mg/kg in combination regimens and individual drugs were administered by intraperitoneal route. Phenytoin was administered by intraperitoneal route in a dose of 50 mg/kg. Gabapentin, Verapamil and Phenytoin were administered once daily, however, on the day of Pentylenetetrazole treatment, the tested and reference drugs were injected 40 minutes before injecting Pentylenetetrazole.The anticonvulsive effects of combined regimens of tested drugs were compared to their individual effects as well as to reference drug Phenytoin.

Results: Combination regimens of GBP and VP demonstrated dose dependent anti- seizure effects up to 100%. The different doses of combined regimens of GBP and VP demonstrated anti-seizure effects which were superior to their individual effects.

Conclusion: Combination regimens of gabapentin and verapamil showed synergistic effects which were superior to their individual effects on kindled model of epilepsy in mice.

Keywords: Antiepileptic drugs, Phenytoin, Gabapentin, Verapamil , Pentylenetetrazole

INTRODUCTION:

Epilepsy is the most complex chronic and common neuronal disorder throughout the world. International League Against Epilepsy (ILAE) has defined Epilepsy as: "A transient occurrence of sign and or /symptoms due to abnormal, excessive or synchronous neuronal activity in the brain.[1] Around the world about 50 million people are suffering from epilepsy.[2]In Pakistan approximately 1.4 million have been suffering from Epilepsy.[3] About 9.9 per 1000 of the general population in Pakistan suffers from this disease.[4] Epilepsy is generated when in brain groups of neurons undergoes process of epileptogenesis. The common causes of epileptogenesis are genetics, neoplasm, abscesses, cysts, stroke, trauma, chronic degenerative diseases and infections. The inhibitory and excitatory balance in neurons are adversely affected, the affects of inhibitory neurotransmitters declines and the epileptogenic neurons becomes hyper excitable. Epileptic seizures are classified according to affected cortical areas of brain. Epileptic seizures are different from acute non-epileptic seizures such as, hypoglycemia, alcohol and narcotic withdrawal, poisonings, diabetic ketoacidosis and high grade fever. In these conditions the neurons are hyper excitable for limited period of time and not permanent like in epileptic foci. Genetic mutations are the common causes of inherited epileptic syndromes.[5]These genetic mutations mostly causes defective voltage gated channelopathies.[6-7] Thirty percent of the epileptic patients suffer from pharmacoresistant epilepsy.[8]These patients are most difficult to be treated as there are no specific drug regimens for their management.[9-10] Newer antiepileptic drugs like GBP and a calcium channel blockers like Verapamil both in randomized studies have shown antiepileptic effects to control resistant epilepsy. GBP(Gabapentin)is FDA approved AED for partial as well as for generalized seizures. Gabapentin needed in higher therapeutic doses for seizure control, however, the high doses are mostly tolerable and the drug rated as tolerable and safe.[11,12,13,14] While VP (Verapamil) is approved for hypertension, angina and cardiac arrhythmias. In CNS it acts on L-type rapidly firing voltage gated calcium channels and blocks T-type voltage gated calcium channels. Though VP is not FDA approved drug for seizures, however, in some clinical randomized trials in pharmaco-resistant epilepsies it has given antiseizure effects.[15,16,17]VP when used as adjunctive therapy in patients of Pharmaco-resistance epilepsy had successfully controlled the seizures. It has been established that VP has modulating effects on calcium

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channels of CNS. [18-19]Certain epilepsies are due to genetic mutations causing defective calcium channels with hyperactivities in neurons. [20-21] Pharmaco-resistant epilepsies are without any proper recommended FDA regimen. [22] VP can control such epilepsies.GBP and VP may provide successful results as both are blockers of calcium channel. Hence both the drugs were employed in combination novel regimens keeping in mind their synergistic effects.[23,24,25,26,27,28]The present study was designed to compare the effects of combined regimens of GBP/VP with their individual effects on kindled model of epilepsy in mice

MATERIALS AND METHODS:

This experimental animal study was carried out in International Center for Chemical and Biological Sciences, at Hussain Ebrahim Jamal (H.E.J.) Research Institute of Chemistry, University of Karachi, Karachi. NMRI Albino mice with standard weight were used. The group size of 12 mice was used for standard statistical analyses.The use of animals in this Project was approved by the Scientific Advisory Committee on Animal Care, Use, and Standards, International Center for Chemical & Biological Sciences, University of Karachi.GBP and VP anticonvulsive effects were studied by Internationally recognized chemically-Induced Kindled Model of Epilepsy. Twenty alternate day administration of sub-convulsive dose of PTZ (50 mg/kg, s.c) was used to produce kindling. The Kindling seizure scores were classified as outlined in Table 1 which is an internationally recognized kindling score.On 20th treatment day the animals receiving PTZ which only showed the score 4-5 were considered to be fully kindled. The effects of the tested drugs and reference drug on inhibiting the kindling/convulsions were recorded with reference to kindling scoring mentioned in Table 1. The tested drugs GBP and VP were given daily, however, on the day of PTZ-treatment the drugs were administered 40 minutes before injecting PTZ. The animals were closely observed for one hour on every day. Total duration of study was forty days.

Statistical Analysis: The statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 10 and Graph Pad Prism. Results were reported as mean ±SEM. Data of seizure activity was analyzed by non-parametric Student's t-test and ANOVA statistical tests with post-hoc Dennett's multiple comparison tests. The sequential differences among means were calculated at the level of $p < 0.05$.

RESULTS:

It was observed that 100, 200 and 300 mg/kg doses of GBP when used as a single agent therapy in PTZ-kindled animals in a 20 day alternate day treatment elicited mean seizure scores of 3.08 ± 0.49, 2.66 ± 0.25 and 1.83 ± 0.37 with seizure percentage of 62%, 53%, 37% and seizure safety or protection of 38%, 47% and 63 % respectively (Table 2).The effects were inferior to reference drug PHT. On the other hand, the treatment of VP as a single agent therapy at the dose of 10, 20 and 30 mg/kg in 20 day alternate day treatment in kindled animals exhibited mean seizure scores of 4.16 ± 0.37, 3.91 ± 0.49, and 3.25 ± 0.43 with seizure percentage of 83%, 78%, 65% and seizure safety of 17%, 22% and 35% respectively (Table 3).

When GBP and VP were used as combination therapy in doses of 100: 10 mg/kg;200: 20 mg/kg; and 300:30 mg/kg in kindled animals,the drugs elicited mean seizure scores of 2.91 ± 0.49, 2.25 ± 0.82 and 0.00 ± 0.00 with seizure percentage of 58%, 45%, 0.00% and seizure protection of 41.8%, 55% and 100 % in three different doses regimens respectively(Table 4a).The maximum synergistic action was seen in the groups receiving 300 mg/kg GBP / 30 mg/kg VP with the mean seizure scores of 0.00 0.0 with seizure percentage 0.00% and seizure protection of 100% respectively. A significant difference i.e. $p < 0.05$ was observed i.e. the difference of 25 %, 33 % and 65 % in seizure protection between these three combined doses and VP treatment and difference of alone was seen (Table 4b). When the same doses were compared against GBP-treatment alone, difference of 3 %, 8 % and 37 % in seizure protection was observed. The effects of maximum dose were found superior to PHT. The combination regimen at maximum dosage employed was 10 percent more efficacious to PHT. Thus, we are inclined to hold that the combination regimen of GBP and VP exhibited superior anti-epileptic activity in terms of seizure protection capability compared to their individual effects on same dosage with almost zero seizure score at the maximum dosage (100 % seizure inhibition) compared to their individual effects (Figure 1)

Figure 1

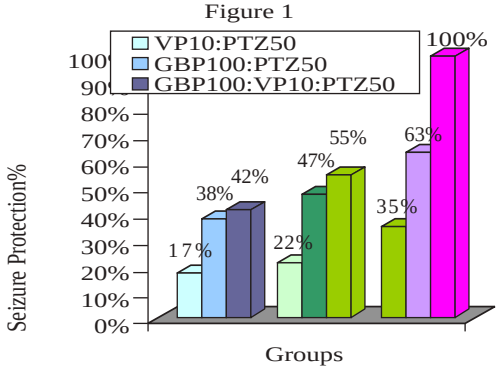

Table: 1
Five distinct seizure patterns used for scoring of kindling stages

Seizure Scoring in Kindling by ptz	Seizure Pattern No
0	Response
1	Ear and Facial Twitching
2	Convulsive Wave through the body
3	Myoclonic Jerks
4	Clonic-Tonic Convulsions, Turnover into Side Position
5	Gerneralized Clonic-Tonic Seizures, Loss of Postural Control.

Table:2
Effect of gabapentin treatment on the seizure score and seizure protection against PTZ-induced kindling

Group ID	Treatments	Dosage (mg/kg)	Mean ± SEM of Seizure Score (20^{th} treatment) (Seconds)	Seizure Score (%)	Seizure Protection (%)
GI	Normal Control	Saline 0.9%	0.00	0.00%	0.00%
GII	PTZ	50			
	GBP	100	4.25 ± 0.59	85%	15%
GIII	PTZ	50			
	GBP	200	3.08 ± 0.49	61.6%	38.4%
GIV	PTZ	50			
	GBP	300	2.66 ± 0.25	53.2%	46.8%
GV	PTZ	50			
	PHT	50	1.83 ± 0.37	36.6%	63.4%
GVI	PTZ	50			
			0.41 ± 0.19	10%	90%

Both the seizure score and seizure protection were calculated in % and the data is represented as a Mean SEM of n = 12 animals per group

Table: 3
Effect of verapamil treatment on the seizure score and seizure protection against PTZ-induced kindling

Group ID	Treatments	Dosage (mg/kg)	Mean ± SEM of Seizure Score (20th treatment)	Seizure Score (%)	Seizure Protection (%)
GI	Normal Control	0.9% Saline	0.00	0.00%	0.00%
GII	PTZ	50	4.25 ± 0.43	85%	15%
	VP	10	4.16 ± 0.37	83%	17%
GIII	PTZ	50			
GIV	VP	20	3.91 ± 0.49	78%	22%
	PTZ	50			
GV	VP	30	3.25 ± 0.43	65%	35%
	PTZ	50			
GVI	VPT	100	0.5 ± 0.23	10%	90%
	PTZ	50			

Both the seizure score and seizure protection were calculated in % and the data is represented as a Mean SEM of n = 12 animals per group.

Table: 4a
Synergistic effect of gabapentin and verapamil treatment on the seizure score and seizure protection against PTZ-induced kindling.

Group ID	Treatments	Dosage (mg/kg)	Mean ± SEM of Seizure Score (20th treatment)	Seizure Score (%)	Seizure Protection (%)
GI	Normal Control	0.9% Saline	0.00	0.00%	0.00%
GII	PTZ	50	4.08 ± 0.64	81.6%	18.4%
GIII	GBP: VP	100: 10			
	PTZ	50	2.91 ± 0.49	58.2%	41.8%
GIV	GBP: VP	200: 20			
	PTZ	50	2.25 ±0.82	45%	55%
GV	GBP: VP	300: 30	0.00	0%	100%
	PTZ	50			
GVI	PHT	50	0.41 ± 0.19	8.2%	91.8%
	PTZ	50			

Both the seizure score and seizure protection were calculated in % and the data is represented as a Mean SEM of n = 12 animals per group.

Table: 4b
GBP and VP and their effects on kindled model of epilepsy when compared to PTZ

Treatment Group	Dose (mg/kg)	GBP, VP and GBP: VP vs. PTZ Control Group		
		P value	Are Means Significant different? $p < 0.05$	Difference between the means
GBP:PTZ	100 : 50	0.0005	YES	1.167
GBP:PTZ	200 : 50	0.0001	YES	1.417
GBP:PTZ	300 : 50	0.0001	YES	2.417
VP:PTZ	10:50	0.633	NO	0.083 0.172
VP:PTZ	20:50	0.106	NO	0.333 0.197
VP:PTZ	30:50	0.0001	YES	1.00 0.184
GBP:VP:PTZ	100:10:50	0.0001	YES	1.33 0.263
GBP:VP:PTZ	200:20:50	0.0001	YES	1.33 0.263
GBP:VP:PTZ	300:30:50	0.00	YES	0

Statistical analysis of data represented by P-values, means significant difference ($p<0.05$) and difference between the means of test drugs

DISCUSSION:

We observed that GBP as a single therapy exhibited mild to moderate anti-epileptic effects. The maximum anti-epileptic effects of GBP were seen at the doses of 200-300 mg/kg where GBP demonstrated 47% and 63% seizure protection respectively. However, GBP demonstrated mild anti-epileptic activities at the doses of 100- 200 mg/kg and moderate anti-epileptic activities at the dose of 300 mg/kg. Likewise, VP demonstrated dose dependent anti-epileptic effects but those were much far inferior to GBP and were insignificant. The VP treated group demonstrated 35% maximum seizure protection at the maximum dose of 30 mg/kg. We are, therefore, inclined to hold that VP exhibits weak antiepileptic activity at lower doses and has no potential to be used as a single agent for the treatment of epileptic seizures as an individual drug. Our data has shown that none of the doses of GBP and VP as individual treatment regime demonstrated 100 percent seizure protection. When GBP and VP were used as combination therapy in three different doses in PTZ-kindled animals both the drugs in combination regimens elicited seizure protection of 41.8%, 55% and 100% respectively (Table 4).The maximum synergistic action was seen in the groups receiving 300 mg/kg GBP/30 mg/kg VP with the mean seizure scores of 0.00 0.0 with seizure percentage 0.00% and seizure protection of 100% respectively. A significant difference that is the difference of 25%, 33% and 65% in seizure protection between these three combined regimens and VP treatment alone was seen. When the same combined regimens were compared against GBP-treatment alone, a difference of 3%, 8% and 37% in seizure protection was observed. Thus, we are inclined to hold that the combination regimen of GBP and VP exhibited superior anti-epileptic activity in terms of seizure protection capability compared to their individual effects and reference drug PHT.The combination regimen exhibited almost zero seizure score at the maximum dosage (100% seizure inhibition) compared to their individual effects. The P values and the student's t-test analysis exhibited significant difference in combination regimens in first two doses and with GBP alone, two initial doses but the most significant effects were seen at the maximum combination doses of GBP and VP where, 100 percent results were achieved. (Table 5 and Figure 1) We therefore have found that the combination therapy exhibited synergistic effects up to 100 percent seizure protection which cannot be achieved by individual higher doses.

The efficacy of new AED are still disappointing though it has provided more options for clinicians for management of epilepsy.[29] Our study demonstrated that anti-seizure actions of GBP can be augmented or modified if given in combination with calcium channel blocker verapamil.[30] GBP is a relatively safer drug interms of its pharmacokinetics and dynamics profiles.[31,32] GBP is a multipurpose drug and it has exhibited its antiseizure effects in refractory epilepsy.[33]GBP has been approved recently by FDA as amonotherapy for partial and complex partial seizures with or without generalized tonic-clonic seizures.[34] GBP in various studies has demonstrated its efficacy as monotherapy equivalent to that of carbamazepine (CMZ) for partial and generalized seizures.[35]GBP has also established its efficacy in refractory epilepsy, therefore the combination regimen would have wider spectrum to treat various types of epilepsies.GBP commonly is employed as an add-on therapy for different types of Epilepsies.

Our results has been supported by another animal study whereby it was revealed that combinations of GBP with other AEDs generally results in synergistic interactions, though as an individual drug its anticonvulsive effects were not significant. It was further observed in the same study that GBP appeared to act synergistically with Carbamazepine, Valproate and Phenytoin. Thus, GBP has potential of synergistic action if used with other AED, keeping this idea in our mind, we conducted

the present study and used VP which is a calcium channel blocker. The purpose of using PHT reference drug was two fold firstly, PHT is not only approved for long term management of different epilepsies but it is also recommended for the long term management of status epilepticus. Secondly, in another animal study it was observed that GBP was more efficacious than PHT both in refractory partial epilepsy and in PHT non responders. After the results of the present study we are inclined to hold that the regimens of GBP and VP would provide better option for the use of these combina-tions regimens having synergistic effect for the treatment of typical and atypical epilepsies.

CONCLUSION:

Combination regimens of GBP and VP provided synergistic and effective therapeutic choice for the treatment of epilepsies.The combination regimens of GBP and VP has significant potential for different types of epilepsies including resistant epilepsies because of synergistic and channel modulating effects.The most significant point for future studies and trials is that what would be cut off dose of GBP and VP whereby both the drugs would exhibit 100 percent seizure protection for use in long term and short term management of epilepsies and such query requires elaborate further animal as well as clinical studies.

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