

Effects of Combined Regimens of Gabapentin and Verapamil with Diazepam on Kindled Model of Epilepsy in Mice

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ABSTRACT:

Objective: To compare the in vivo effects of anticonvulsant combined regimens of Gabapentin / Verapamil with diazepam on kindled model of Epilepsy in Mice.

Materials and Methods: 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, Karachi from May 2009 to July 2011. Gabapentin and Verapamil were used as tested drugs while Diazepam was used as a reference drug. Kindling was produced by repeated administration of Pentylentetrazole in a dose of 50 mg/kg by subcutaneous route every 48 hours for 20 days. Six doses of Gabapentin from 50mg/kg to 300mg/kg and six doses of Verapamil from 5mg/kg to 30mg/kg in combination regimen were administered by intraperitoneal route. Diazepam was administered by intraperitoneal route in a dose of 7.5mg/kg. Both tested drugs Gabapentin and Verapamil with reference drug Diazepam were administered once daily, however on the day of Pentylentetrazole treatment the tested and reference drugs were injected 40 minutes before injecting Pentylentetrazole. The anticonvulsive effects of tested drugs were then compared to reference drug Diazepam.

Results: Combination regimens of Gabapentin and Verapamil exhibited synergistic dose dependent anti-seizure effects up to 100%. The maximum dose of combined regimen exhibited antiseizure effects which were superior to the reference drug Diazepam.

Conclusion: Combination regimens of Gabapentin and Verapamil showed synergistic effect superior to diazepam on kindled model of epilepsy in mice.

Keywords: Antiepileptic drugs (AED), Diazepam (DZ), Gabapentin (GBP), Verapamil (VP), Pentylentetrazole (PTZ)

INTRODUCTION:

Epilepsy is one of the most common neurological disorders which has no age, racial, social, sexual and geographical boundaries. The International League Against Epilepsy (ILAE) defines epilepsy as: "A transient occurrence of signs and symptoms due to abnormal excessive or synchronous neuronal activity in the brain."¹ About 50 million people around the world are suffering from Epilepsy.² In Pakistan 1.38 million epileptic patients have been reported.³ The incidence of Epilepsy in Pakistan is 9.9 per 1000 of the general population.⁴ Epileptic foci are a potential site for the generation of epileptic seizures and have high levels of neuronal activity either due to abnormally increased excitatory neurotransmitters or abnormally decreased levels of inhibitory neurotransmitters. The clinical manifestation of epileptic seizures depends upon the affected cortical area from where the seizures originate. There are various pathological causes of Epilepsy resulting in abnormal neuronal discharge or neuronal

and synchronicity such as trauma, infections, neoplasm, abscesses, cysts, metabolic abnormalities, stroke, chronic degenerative disease of the central nervous system and genetics.

It is essential to differentiate between epileptic seizures and general non-epileptic seizures like febrile seizures, hypoglycemic seizures, alcohol and narcotic withdrawal seizures, seizures due to poisoning and diabetic ketoacidosis. In non-epileptic seizures the mechanism of initiation of seizures is almost the same that is hyperexcitability; however, the neurons are hyperexcitable for a limited period of time and not permanent like epileptic foci. The genetics of epilepsy is a very complex phenomenon and new progress has been made in the last 2 decades which shall provide new possibilities for the diagnosis and treatment of inherited disorders of epilepsy. Mutations in the genes which code for the different ionic channels are one of the main causes of inherited epileptic syndromes.⁵ These mutational channelopathies result in defective voltage gated ionic channel formation which control flow of sodium, potassium and calcium ions in and out of the neuronal cells.^{6,7}

The Pharmac-resistant or refractory epilepsy presents in about 30 percent of the epileptic patients.⁸ This pharmac-resistant epilepsy is the most difficult type of epilepsy to be treated.^{9,10} The clinical condition can be improved by add-on therapy with newer antiepileptic drugs like GBP and calcium channel blockers like Verapamil (VP). Gabapentin (GBP) is approved as adjunct therapy 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

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to excellent.^{11,12,13,14} VP is a calcium channel blocker which is widely used as antianginal, antiarrhythmic and antihypertensive drug in patients of coronary heart disease. In heart it acts on rapidly firing L-type voltage gated calcium channels and blocks T-type voltage gated calcium channels in central nervous system. Antiseizure effects of VP have been noted in some clinical randomized trials in pharmaco-resistant epilepsy.^{15,16,17} It affects P-glycoprotein expression at various sites including blood brain barrier. Some patients of refractory epilepsy were also found suffering from severe myoclonic epilepsy of infancy. VP when used as an adjunctive therapy successfully controlled seizures. Even on long term usage it has given promising results by its modulating effects on calcium channels.^{18,19} Defective calcium channels due to inherited genetic mutation causes various types of epilepsies.^{20,21} Until today, there are no satisfactory and approved treatment regimens for pharmaco-resistant refractory epilepsy.²² GBP and VP are voltage-gated calcium channel blockers; therefore, they can be a potential candidate for the treatment of different kinds of epilepsies.^{23,24,25,26,27,28} Present study was designed to compare the anticonvulsant effects of combined regimens of GBP/ VP with DZ on kindled model of epilepsy in mice.

MATERIALS AND METHODS:

This experimental study was carried out at Hussain Ebrahim Jamal (H.E.J) Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi from May 2009 to July 2011. The use of animals in this study was approved by the Scientific Advisory Committee on Animal Care, Use, and Standards, International Center for Chemical & Biological Sciences, University of Karachi, Pakistan, in accordance with the international guidelines for the care and use of laboratory animals. Male NMRI albino mice weighing 20-25 g were used. The group size of 12 were used which had 80% power to detect differences in the means.

The anti- epileptic activity of the GBP and VP were evaluated in vivo by chemically-kindled model of epilepsy. Total duration of study was forty days. The mice were divided into nine groups that is G-I to G-IX. Each group had twelve mice. G-I (normal control) was given only 0.9 percent normal saline. G-II was given PTZ only. G-III to G-VIII was given tested drugs GBP and VP. G-IX was given DZ and PTZ. The kindling was produced in GII group by repeated administration of sub-convulsive dose of PTZ (50 mg/kg, s.c.) every 48 hours for 20 days. The test drugs VP and GBP were administered to the mice intraperitoneally (i.p.) in six different doses. The reference drug DZ was also administered intraperitoneally (i.p.) in a dose of 7.5mg/kg in G-IX. The test drugs GBP and VP and the reference drug were given daily, however, on the day of PTZ-

treatment which was given on every alternate day, the drugs were administered 40 minutes before injecting PTZ. The resultant kindling scores were classified as numerical 1 to 5. The animals showing the score 4-5 on 20th day of treatment in G-II PTZ treated were considered to be fully kindled. The mean of the seizure scores were calculated showing results of all groups mean of seizure scores of kindling standard scores from 1 to 5 \pm SEM in 12 mice (n = 12 per group). The mean of the seizure scores were converted into percentage of seizure scores and seizure protection in all nine groups.

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 \pm SEM. Data of seizure activity was analyzed by nonparametric Student's t-test and ANOVA with post hoc Dennett's multiple comparison tests. The sequential differences among means were calculated at the level of $p < 0.05$.

RESULTS:

The results of kindling scores were classified as numerical 1 to 5 (Table 1). The data from combined usage of GBP and VP when analyzed showed a dose dependent synergistic anti-epileptic activity exhibiting 33.4%, 41.8%, 45%, 55%, 77% and 100% seizure protections in six different dose regimens respectively (Table 2). Thus, at the maximum dose employed, the combination regimen of GBP and VP exhibited superior anti-epileptic activity in terms of seizure protection capability compared to the reference drug DZ. (Figure 1). GBP and VP at the maximum dose at GVIII group exhibited 100% seizure inhibition and seizure score was 0.00%, while DZ exhibited 91.8% seizure inhibition. Combination regimen of GBP and VP exhibited 8.2% more seizure protection with almost zero seizure score (100% seizure inhibition) compared to DZ. Though the effect is dose dependent, however, the therapeutic index of GBP is much higher than DZ and human maximum dosage of GBP is 4.5 gm per day (Table 2, Figure 1).

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

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

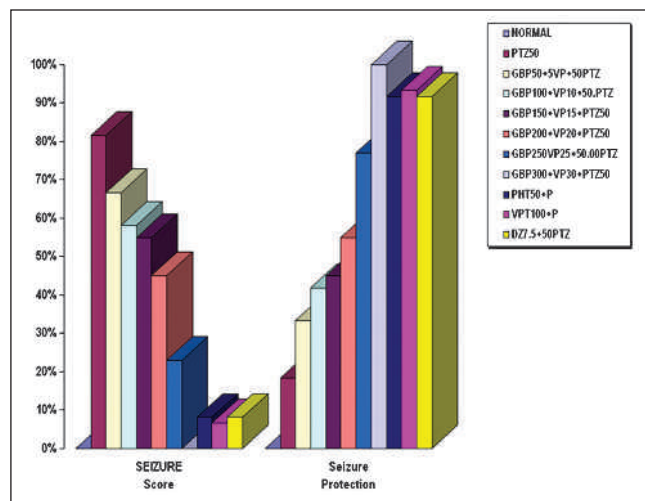
Table: 2
Effect of Gabapentin and Verapamil treatment on the seizure score and seizure protection against PTZ-induced kindling

Group ID	Treatments	Dosage (mg/kg)	Mean ± SEM of Seizure Score	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:PTZ	50: 5:50	3.33 ± 0.74	66.6%	33.4%
GIV	GBP: VP:PTZ	100: 10:50	2.91 ± 0.49	58.2%	41.8%
GV	GBP: VP:PTZ	150: 15:50	2.75 ± 0.43	55%	45%
GVI	GBP: VP:PTZ	200: 20:50	2.25 ± 0.82	45%	55%
GVII	GBP: VP:PTZ	250: 25:50	1.16 ± 0.89	23%	77%
GVIII	GBP: VP:PTZ	300: 30:50	0.00	0%	100%
GIX	DZ:PTZ	7.5:50	0.41 ± 0.14	8.2%	91.8%

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

Figure: 1

Graphical representation of synergistic effect of gabapentin and verapamil treatment on the seizure score and seizure protection against PTZ-induced kindling



The mean SEM of seizure score and seizure protection are shown in %

DISCUSSION:

Current treatment and management of epilepsy by antiepileptic drugs is not satisfactory and though, availability of newer antiepileptic drugs have widened the choices of the clinicians for the treatment of epilepsy, however; the prognosis and efficacy of these new drugs are still disappointing.²⁹The results of our study showed that combined regimens of GBP and VP when analyzed and compared exhibited a dose dependent synergistic anti-epileptic activity starting from 150:15 mg/kg of GBP: withVP exhibiting 45 % seizure protection. This further increased to 55 % seizure protection at the dose of 200: 20 mg/kg of GBP:VP reached maximum that is 100 % seizure protection at 300:30 mg/kg of GBP: VP. When the synergistic effects of GBP and VP were compared to DZ we observed the differences in seizure inhibition of 8.2 % at the maximum dose of combination regimen which was superior to the anti-seizure effects of DZ. Though the effect is dose dependent, however,

the therapeutic index of GBP is much higher than DZ and human recommended maximum dosage of GBP is 4.5 g per day. We are therefore, inclined to hold that the dose dependent superior anticonvulsant synergistic effects of GBP and VP compared to antiseizure effects of standard single dose of DZ had insignificant chances of error. When the synergistic effects of GBP and VP were compared to PTZ control we observed that the seizure inhibition was 17%, 26%, 29%, 39%, 61% and 84% in six dose regimens of combination therapy, demonstrating inhibition of PTZ seizure effects of 67%, 58%, and 55%, 45%, 23% and 0.00 % respectively. At the maximum dose of combination regimen we observed complete inhibition of PTZ induced seizures, which was beyond doubt, superior to the antiseizure effects of reference drug given with PTZ.

In one study, it was observed that animal models using subcutaneous Pentylenetetrazole, is a common model to study the antiseizure effects and mechanism of action of antiepileptic drugs. It was further observed in the same study that animal models can be used to evaluate different combinations of AEDs before their use in humans.³⁰In another study it was observed that GBP as monotherapy had not exhibited effective antiseizure effects however, the study revealed that combinations of GBP with other antiepileptic drugs generally exhibited synergistic interactions. The study concluded that GBP had exhibited synergistic effects with other antiepileptic drugs in same dosage.³¹Our results are coinciding with the findings of these studies.

The most important question is how the instant combined regimen of GBP with VP is justified for providing better, safer, synergistic, effective and broader spectrum therapeutic choice for the treatment of various types of epilepsies as compared to other AEDs. Firstly, the mode of action of GBP can be augmented or modified if given in combination with other drugs like calcium channel blockers i.e. verapamil.³²Secondly, GBP is a very safe drug as compared to other AEDs.³³Thirdly, the GBP has a very high therapeutic index and safety profile.³⁴Fourthly, instant novel regimen of GBP and VP can be employed with adjustment of their doses from minimum to

maximum doses for the treatment of different types of epilepsies without any significant adverse effects. Fifthly, doses of GBP up to 4.5 g/day can be given with low doses of VP to achieve the therapeutic goals which are not possible with other antiepileptic drugs. Sixthly, GBP in various studies has demonstrated its efficacy as monotherapy equivalent to that of carbamazepine (CMZ) for partial and generalized seizures. GBP has also established its efficacy in refractory epilepsy, therefore the combination regimen would have wider spectrum to treat various types of epilepsies.³⁵ Seventhly, the side effects of GBP are few, tolerable and short term. Eighthly, GBP has been approved by the drug agency FDA as a monotherapy for partial and complex partial seizures with or without generalized tonic-clonic seizures.³⁶ Lastly most of the antiepileptic drugs have potential for causing hepatitis and bone marrow suppression like Valproate and Carbamazepine.

CONCLUSION:

The novel combination regimen of GBP with VP has potential for alternate regimen of treatment for different types of seizures including epileptic seizures and would provide better, safer, synergistic and effective therapeutic effect because of its synergistic and channel modulating effects not only in drug resistant epilepsy but also in various other types of epilepsies. This study has provided basic ground-work guidelines for the future clinical use of combination therapies of GBP and VP in different doses combinations in various forms of epilepsies for the long term management of epilepsy.

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