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Effect of pregabalin, nimodipine and their combination in the prevention of status epilepticus and death protection in mice

Pregabalin, nimodipin ve kombinasyonlarının farelerde status epileptikus ve ölüm korumasının önlenmesinde etkisi

Short title: synergistic effect of pregabalin and nimodipine on mortality rate

pregabalin ve nimodipinin ölüm oranı üzerine sinerjistik etkisi

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ABSTRACT

Objective: The objective of this study was to assess the cumulative anti-epileptic effects of PGB and NMD in acute seizure model of epilepsy in mice

Materials and Method: This study was planned to explore cumulative antiepileptic effects of pregabalin with nimodipine on mortality protection in mice. Pentylenetetrazole was employed to prompt seizures. Both drugs were used separately as well as in combination to assess anticonvulsant effects on acute seizure model of epilepsy in mice. Diazepam and valproate were employed as standard antiepileptic drugs.

Results: The mortality protection exhibited in mice by both the drugs were noted in percentage and considered as marked change when the response of the tested drugs was equal to ED₅₀ of PGB and considered highly marked when the response was more than ED₅₀ for PGB.

Combination treatment of pregabalin and nimodipine exhibited substantial mortality protection at 30+2.5 mg/kg dose and highly marked at doses from 35+5mg/kg to 55+15mg/kg, these effects were superior to individual effects of PGB, showing synergism, but lesser than standard drugs valproate and diazepam. **Conclusion:** Nimodipine exhibited synergistic anticonvulsant effect with pregabalin; however clinical studies are required to ascertain the efficacy of combination in humans.

Keywords: Pregabalin, Nimodipine, Valproate, Diazepam, Pentylenetetrazole

ÖZET

Amaç: Bu çalışmanın amacı, farelerde akut nöbet modelinde PGB ve NMD'nin kümülatif anti-epileptik etkilerini değerlendirmektir.

Gereç ve Yöntem: Bu çalışmada, pregabalinin nimodipin ile kümülatif antiepileptik etkilerinin farelerde mortalite koruması üzerindeki etkileri araştırıldı. Pentylenetetrazole nöbetleri hızlandırmak için kullanıldı. Her iki ilaç da, farelerde akut nöbet epilepsi modeli üzerindeki antikonvülsan etkilerini değerlendirmek için kombinasyon halinde kullanıldı. Diazepam ve valproat standart antiepileptik ilaçlar olarak kullanıldı.

Sonuçlar: Her iki ilacın farelerde sergilediği mortalite koruması yüzde olarak not edildi ve test edilen ilaçların tepkisi PGB'nin ED50'sine eşit olduğunda ve yanıtın PGB için ED50'den fazla olduğu zaman yüksek derecede işaretlenmiş olarak kabul edildiğinde belirgin değişiklik olarak kabul edildi. Pregabalin ve nimodipinin kombinasyon tedavisi 30 + 2.5 mg / kg dozda önemli derecede ölümcül koruma sergilemiştir ve 35 + 5mg / kg ila 55 + 15mg / kg arasındaki dozlarda oldukça belirgindir, ancak bu etkiler sinerjiyi gösteren PGB'nin bireysel etkilerinden daha üstündür, ancak daha az standart ilaçlar valproat ve diazepam. **Sonuç:** Nimodipin pregabalin ile sinerjistik antikonvülsan etki göstermiştir, ancak kombinasyonun insanlarda etkinliğini belirlemek için klinik çalışmalar gereklidir.

INTRODUCTION

Status epilepticus is a severe condition of shared epilepsies¹. It is a life intimidating neurological medical crisis² which may lead to loss of life in many instances if not cured suitably. Lorazepam and Diazepam (DZ) are the first choice drugs for short period of time to control status epilepticus³. However lorazepam has been superior to diazepam⁴. Valproate (VPT), phenobarbitone and phenytoin are endorsed for the long period management of status epilepticus⁵. The choice of first line drugs is limited for management of status epilepticus and the drugs suggested have serious side effects and drug-drug interactions⁶⁻⁷. Nimodipine (NMD) is a voltage gated calcium channel inhibitor permitted by FDA for hypertension since it inhibits the L type calcium channels in blood vessels⁸.

NMD inhibit and modify voltage gated calcium channels in central nervous system⁹ (Zapater *et al.*, 1998). Low and high voltage gated calcium channels have crucial role in neuronal functions in major parts of the CNS embracing excitability¹⁰⁻¹¹ (Khosravani and Zamponi 2006; Iftinca and Zamponi 2009). Hence it seems appropriate for the CCBs to be employed for treatment of epilepsy¹²⁻¹³. NMD in several animal studies has also showed increasing antiepileptic effects over other anti-convulsion drugs e.g. carbamazepine, valproate and lamotrigine in the management of acute seizures¹⁴⁻¹⁵. Pregabalin (PGB) is well tolerated, efficient and FDA permitted its use in patients of epilepsy¹⁶⁻¹⁷. Its antiepileptic action is related to bind alpha-2-delta voltage-gated calcium channels including many other pharmacodynamic anti-seizure actions in the CNS¹⁸. The objective of this study was to assess the cumulative anti-epileptic effects of PGB and NMD in acute seizure model of epilepsy in mice.

MATERIALS AND METHODS

Animals

Consent was acquired from Board of Advanced Studies and Research to conduct the current study at Department of Pharmacology, University of Karachi. Mice used in the study were handled according to the guidelines by National Institute of Health¹⁹. The ethical approval for the use of animals was granted by the departmental research committee. The experiments were performed on male albino mice whose weight ranged from 20-25 g. The animals were housed in a controlled environment with regular provision of food and water. The temperature was maintained at 21 ± 2°C with 12-hour light/dark cycle. The animals were exposed to the location

for 2-3 days before the experiments could be performed. The tests were done from 9:00 am to 12:30 pm.

Drug Treatment

Acute attacks were induced by PTZ. Meanwhile, PGB and NMD were used as test drugs. Diazepam and valproate were employed to equate the antiepileptic effects of the test drugs. Normal saline was used for animals in control group. HEJ Research Center, University of Karachi provided PTZ. Diazepam and Valproate injections were acquired from local market manufactured by Roche Pakistan Ltd. and Abbot Laboratories, Pakistan Ltd, Karachi respectively. PGB capsules manufactured by Getz Pharma Pvt. Limited, Karachi were obtained from local market as well. Novartis Pharma, Pakistan Ltd, Karachi provided NMD injections. PGB, NMD and PTZ were liquefied in sterile normal saline. Aluminum foil was used to cover PTZ solution in order to avoid disintegration. The solutions were composed regularly and used within an hour of their inception. Pregabalin capsule contents were dissolved in to sterile water and administer SC, while other drugs were given by intraperitoneal route.

Procedure

Convulsions in all mice were induced via PTZ at the dose of 90 mg/kg through subcutaneous administration²⁰. PGB and NMD were given separately as well as in combination in six different doses. All drugs including test substance and standard drugs diazepam and valproate were given 40 minutes earlier to PTZ by intraperitoneal route. The mice were kept alone following administration of the PTZ, and witnessed; mortality protection was recorded in percentage. The outcomes of mice remained without seizures up to cutoff time were stated as 0. The number of mice survived (mortality protection) and death rate were recorded in percentage. The anticonvulsant effect, seizure patterns and mortality protection recorded by the combine regimen (PGB+NMD+PTZ) in six doses of the test drugs were equated with PTZ, individual effects of PGB+PTZ and NMD+PTZ and lastly with reference drugs (DZ+PTZ and VPT+PTZ). Period of 60 minutes was taken as cut off time for seizure defense after giving PTZ with or minus test drugs²¹⁻²⁵. The anti-epileptic effects of PGB and NMD were assessed by observing seizure protection in percentage.

All animals used in study were distributed into three portions i.e. A, B and C, each portion having ten groups and each group includes 12 mice. Group I in each portion worked as control and was administered sterile normal saline, group II mice were given PTZ and animals in groups III to VIII were given diverse doses of test drugs. Mice in groups IX and X were given diazepam and valproate. Diazepam was given in the dose of 7.5 mg/kg and valproate 100mg/kg, forty minutes prior to the administration of PTZ. Six groups of portion-A were given pregabalin doses ranging from 30 to 55 mg/kg with a difference of 5mg/kg in each group, animals of portion-B were given NMD in the doses of 2.5, 5, 7.5, 10, 12.5 and 15 mg/kg. Animals in portion- C were given six different combinations of PGB +NMD i.e. 30 + 2.5, 35 + 5, 40 + 7.5, 45 + 10, 50 + 12.5 and 55 + 15 mg/kg respectively, forty minutes prior to administration of pentylenetetrazole.

Statistical Interpretation

The standard statistical procedure was not carried out in current study since results were exhibited in percentage and considered as marked when the response of the tested drugs was equal to ED₅₀ of PGB and considered as highly substantial when the response was more than ED₅₀ for PGB.

RESULTS

Table 1 summaries the results in all animals groups in three divisions, while **figure 1** shows the results of animals only in section-A. PGB as a single agent in its six doses from 30 to 55 mg/kg

protected mortality from 42% to 67%. Both the reference drugs VPT and DZ provided 100% mortality protection. The antiepileptic effects of PGB when matched to reference drug valproate and diazepam were comparatively inferior in all epileptic attacks.

Table 1 summaries results of all animals groups in three divisions, while **figure 2** shows the results of animals only in section-B. It was observed that NMD as a single agent in its first three doses i.e. 2.5, 5 and 7.5 mg/kg exhibited 100% mortality; however at the dose of 10 mg/kg mortality protection was 8%, whereas at doses of 12.5mg/kg and 15mg/kg the mortality protection was 17%. VPT and DZ protected 100% animals from mortality. The antiepileptic effect of NMD when matched to standard drugs valproate and diazepam were highly inferior in all seizure patterns.

Table 1 summaries the results in all animals groups in three sections, however **figure 3** shows the results of animals in section-C. The animals treated in combination dose of 30 +2.5 mg/kg PGB and NMD exhibited 50% mortality protection, which was substantial as compared to PGB40mg/kg, while animals treated with combination doses of 35+5 and 40+7.5 mg/kg PGB and NMD respectively showed 58% mortality protection. The animals given the combination of PGB and NMD in the doses of 45+10 mg/kg revealed 67% mortality protection. However maximum mortality protection of 83% was observed by the combination doses of 50+12.5mg/kg and 55+15mg/kg of doses PGB and NMD.

Table 2 and figure 4 shows the difference in mortality protection among PGB, NMD, combination of PGB + NMD and valproate. The difference in mortality protection among NMD in first three dose and valproate was -100; while NMD demonstrated 92% less mortality protection compared to valproate at 10mg/kg and 83% less mortality protection at dose of 12.5 and 15mg/kg. PGB demonstrated 58% less mortality protection at 30 and 35mg/kg doses which was reduced to 50% at the dose of 40mg/kg. While at the dose of 45mg/kg, 42% less mortality protection was noted, however at the doses of 50 and 55mg/kg PGB demonstrated 33% less mortality protections compared to valproate. The combination of PGB and NMD showed better results in reducing mortality. The difference in mortality protection at the doses of 30+2.5mg/kg and 35+5mg/kg as compared to valproate was only 50% which was improved to 42% at the dose combination of 40+7.5mg/kg. The difference in mortality protection was further improved to 33% at doses of 45+10 mg/kg, which was further reduced to 17% at the combination doses of 50+12.5mg/kg and 55+15mg/kg as compared to valproate.

Table 3 shows comparison of mortality protection for PGB and combinations of PGB and NMD. The PGB and NMD combination in the dose of 30+2.5 mg/kg demonstrated 8% higher mortality protection, while the combination dose of 35+5mg/kg revealed 16% higher mortality protection. However the combination doses of 40+7.5 and 45+10 for PGB and NMD demonstrated only 8% and 9% mortality protection respectively. The rate of mortality protection was again consistently higher up to 16% at the doses combination of 50+12 mg/kg and 55+15mg/kg of PGB and NMD as compared to PGB alone.

Table-1

Antiepileptic effect of Pregabalin, Nimodipine and their combination in acute model of epilepsy

Groups	Doses mg/kg	Mortality Protection %
Normal Control	10ml/kg	100
PTZ	90	00
PGB	30	42
PGB	35	42
PGB	40	50*
PGB	45	58**
PGB	50	67**
PGB	55	67**
NMD	2.5	00
NMD	5.0	00
NMD	7.5	00
NMD	10	8
NMD	12.5	17
NMD	15	17
PGB:NMD	30 : 2.5	50*
PGB :NMD	35 :5	58**
PGB :NMD	40 : 7.5	58**
PGB :NMD	45 : 10	67**
PGB :NMD	50 : 12.5	83**
PGB :NMD	55 : 15	83**
VPT	100	100
DZ	7.5	100

n=12; PTZ= Pentylenetetrazole; PGB= Pregabalin
NMD= Nimodipine; VPT= Valproate; DZ=Diazepam

*Marked as compared to 40mg/kg PGB (ED50)

** Highly substantial as compared to 40mg/kg PGB (ED50)

TABLE 2

Nimodipine, pregabalin and PGB+NMD in comparison to valproate in mortality protection in acute model of epilepsy

NMD v/s VPT dose mg/kg	Difference in mortality protection	PGB v/s VPT	Difference in mortality protection	PGB:NMD v/s VPT Dose mg/kg	Difference in mortality protection
NMD v/s VPT 2.5 100	-100	PGB v/s VPT 30:100	58	PGB:NMD v/s VPT 30 :2.5/100	50
NMD v/s VPT 5 100	-100	PGB v/s VPT 35:100	58	PGB:NMD v/s VPT 35 : 5/100	42
NMD v/s VPT 7.5 100	-100	PGB v/s VPT 40:100	50	PGB:NMD v/s VPT 40 :7.5/100	42
NMD v/s VPT 10 100	92	PGB v/s VPT 45:100	42	PGB:NMD v/s VPT 45 :10/100	33
NMD v/s VPT 12.5 100	83	PGB v/s VPT 50:100	33	PGB: NMD v/s VPT 50:12.5/100	17
NMD v/s VPT 15 100	83	PGB v/s VPT 55:100	33	PGB: NMD v/s VPT 55:15/100	17

NMD=Nimodipine; PGB=Pregabalin; VPT=Valproate; Difference in Mortality protection (%)

TABLE 3
Comparison of mortality protection pregabalin and PGB+NMD in acute model of epilepsy

Treatment Groups	Mortality Protection	Treatment Groups with Doses	Mortality Protection	Difference in Mortality Protection
PGB:30	42	PGB : NMD 30 :2.5	50*	8
PGB:35	42	PGB :NMD 35 : 5	58**	16
PGB:40	50	PGB :NMD 40 :7.5	58**	8

PGB:45	58	PGB :NMD 45 :10	67**	9
PGB:50	67	PGB: NMD 50:12.5	83**	16
PGB:55	67	PGB: NMD 55:15	83**	16

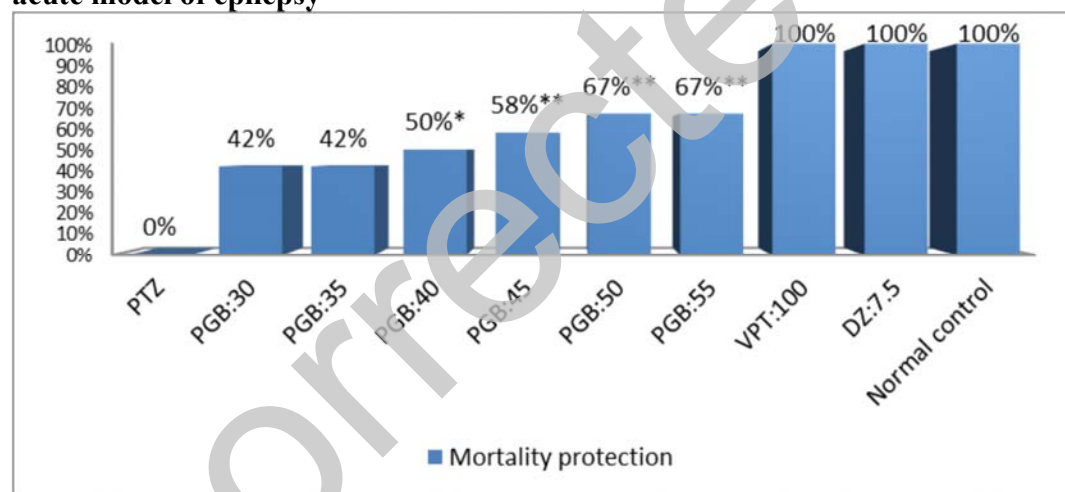
PGB=Pregabalin; NMD=Nimodipine; n= 12; Doses (mg/kg)

*Marked as compared to 40mg/kg PGB (ED50)

**Highly substantial as compared to 40mg/kg PGB (ED50)

FIGURE-1

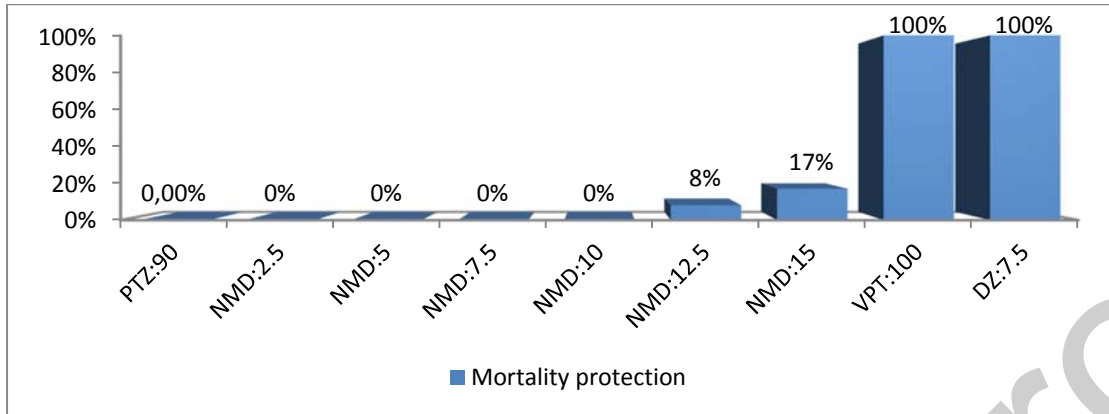
Comparative effects of Pregabalin, Valproate and Diazepam on mortality protection in acute model of epilepsy



PTZ=Pentylenetetrazole; PGB=Pregabalin; VPT=Valproate; DZ= Diazepam

FIGURE-2

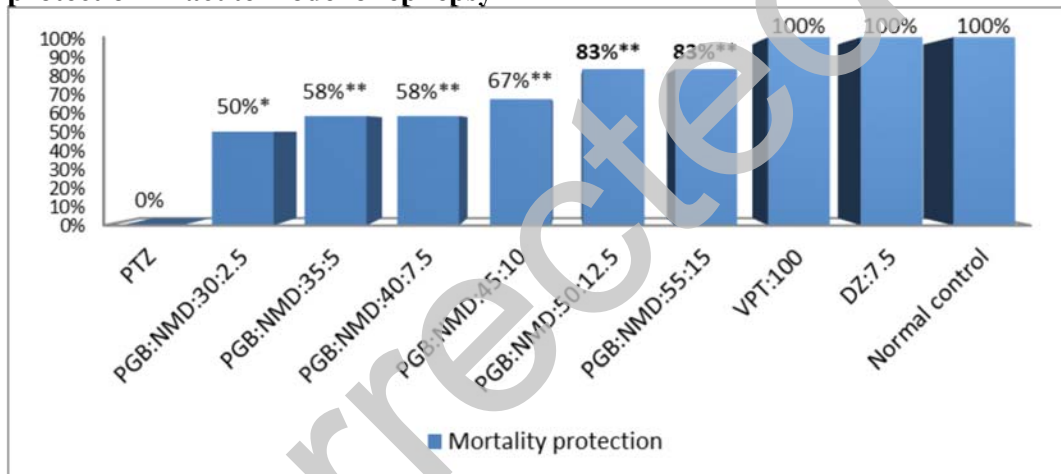
Comparative effects of Nimodipine, Valproate and Diazepam on mortality protection in acute model of epilepsy



PTZ=Pentylenetetrazole; NMD= Nimodipine; VPT=Valproate; DZ= Diazepam

FIGURE- 3

Comparative effects of Pregabalin: Nimodipine, Valproate and Diazepam on mortality protection in acute model of epilepsy

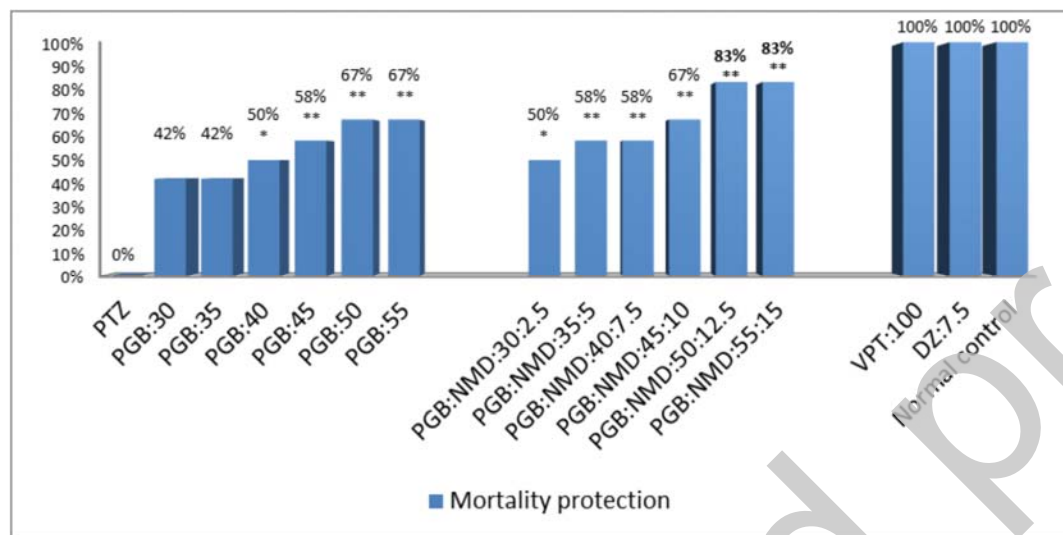


*Marked as compared to 40mg/kg PGB (ED50)

**Highly substantial as compared to 40mg/kg PGB (ED50)

FIGURE- 4

Comparative effects of Pregabalin: Nimodipine, Valproate and Diazepam on mortality protection in acute model of epilepsy



PTZ=Pentylentetrazole; PGB=Pregabalin; NMD=Nimodipine; VPT=Valproate; DZ= Diazepam;
 *Marked as compared to 40mg/kg PGB (ED50)
 **Highly substantial as compared to 40mg/kg PGB (ED50)

DISCUSSION

The discovery of new anti-epileptic drugs (AEDs) though have widened the selection of drugs to treat epilepsies on long term basis but none of the newer AEDs have been permitted for the treatment of status epilepticus. Even likelihood and usefulness of these new AEDs is still scanty and no AED has been found to retain best properties²⁶. Exploration of drug combinations that may have potential to terminate and prevent status epilepticus is the main object of our study. Thus newer AEDs with better effectiveness and unique mechanisms are now needed to deliver active combinations to treat patients with epilepsy²⁷. Animal models may only be employed to forecast drugs combinations that are effective in clinical setting²⁴.

Antiepileptic drugs may have inhibitory or additive effects, however drugs possessing additive effects appears to be of medical importance^{21,28}. Therefore in present investigation PGB in combination with NMD had been evaluated for synergistic antiepileptic effects in mice acute seizure model of epilepsy. It was assumed that combination of AEDs may potentiate anticonvulsant effects; this hypothesis was supported by the additive actions of tiagabine and gabapentin. Outcomes of present investigation evidently demonstrated that the activation of the neurotransmitters may have produced additive effect²⁹. The anticonvulsant effects of oxcarbazepine and gabapentin in another investigation were found to be a mixture of both drugs at the fixed ratios exerting additive effects against electro-convulsions³⁰.

PGB and NMD showed 50% mortality protection when given in the combination dose of 30+2.5mg/kg; however when PGB and NMD were given separately in the doses of 30mg/kg and 2.5 mg/kg the mortality protection was 42% and 0% respectively. Hence there was 8% increase in mortality protection as compared to PGB and 50% increase as compared to NMD alone (table 1). Similarly PGB and NMD showed 58% mortality protection in the combination dose of

35+5mg/kg, however when these drugs were given separately in the doses of 35mg/kg and 5mg/kg the mortality protection was 42% and 0% respectively. Hence there was 16% increase in mortality protection as compared to PGB and 58 % increase in response as compared to NMD in PTZ-induced acute seizures (table 1).

PGB and NMD showed 67% mortality protection in the combination dose of 45+10mg/kg, however when PGB and NMD were given separately in the doses of 45mg/kg and 10mg/kg the mortality protection was 58% and 8% respectively. Hence there was 9% increase in mortality protection as compared to PGB and 59% increase in mortality protection as compared to NMD (table 1). The animal groups received the combination doses of 50+12.5 mg/kg and 55+15mg/kg PGB and NMD exhibited maximum anticonvulsant effects with 83% mortality protection, which was 17% and 67% more than individual response of PGB and NMD respectively. Hence synergistic effects have been observed at all the combination doses of PGB and NMD. Thus the combined regimen decreased the ED₅₀ of the PGB from 40mg/kg to 30 mg/kg. The decrease in ED₅₀ was 10mg/kg which was 25% decrease in the ED₅₀ of the PGB in the treatment of acute seizures in mice (table-1). This shows that addition of calcium channel blocker NMD with PGB exerted remarkable synergistic anti-seizure effects by increasing the mortality protection though did not abolished the seizures completely.

Pharmacokinetic and pharmacodynamic profile of PGB reveals it to be a well-tolerated drug^{17, 31-32} and has been recognized by the FDA for the treatment of epilepsy³³⁻³⁴. PGB in several investigations have validated its value for the treatment of partial and generalized seizures. PGB have also shown its effectiveness in refractory epilepsy, thus combination treatment may have broader range to manage many forms of convulsions¹⁶. Hence in this study we anticipated that anti-epileptic actions of PGB can be amplified or altered when given in combination with NMD. Calcium channel antagonists have shown to retain anticonvulsant effects in experimental models and enhance the guarding activity of some AEDs³⁵. NMD is a dihydropyridine calcium channel antagonists that blocks N-and P/Q-type calcium channels, having greater affinity for both of these channels³⁶⁻³⁷. Numerous investigations have shown substantial augmented anticonvulsants actions of amlodipine and nimodipine on topiramate, valproate, phenobarbitone and other AEDs^{15,30,38}. Thus there are convincing reasons to show that existing investigation have important medical prospective.

PGB as monotherapy in acute studies showed marginal to moderate effects on mortality protection. The effects were inferior as compared to the efficacy of VPT and DZ in all test doses. None of the doses of PGB as monotherapy in acute studies demonstrated 100% mortality protection against PTZ; moreover the mortality protection of PGB monotherapy in present study was dose dependent. PGB demonstrated minimum mortality protection of 42% at 30 and 35mg/kg which was 58% less than both reference drugs. The mortality protection was enhanced to 50% at 40mg/kg, which was however 50% less than VPT and DZ. The maximum mortality protection 67% by PGB was observed at the doses of 50 and 55 mg/kg in acute seizures but still it was 33% less than both reference drugs. The efficacy of PGB in acute seizures was 42% to 67% compared to the efficacy of VPT (Table 1 and Figure 4).

The low voltage-gated calcium channels are widely distributed throughout the central nervous system and have shown their contribution in major processes like neuronal excitability and synaptic transmission³⁹. Among dihydropyridines NMD is thought to block both L and T type of calcium channels⁴⁰. Thus results of the present study suggest that calcium channel blocker NMD had some involvement in reducing seizures and death rate probably by inhibiting T-type low voltage calcium channels hence combination of PGB and NMD revealed greater anti-epileptic activity in comparison to their individual effects at same doses.

CONCLUSION

The anti-epileptic combination employed in the current study demonstrated over all marked seizure protection, however PGB and NMD in combination dose of 50+12.5mg/kg showed maximum mortality protection, which was 83%. Therefore it may be concluded that the calcium channel blocker, nimodipine exhibited anticonvulsant effects by potentiating the anti-epileptic effects of pregabalin in combination. The main clinical importance of the combination therapies would be reduction in doses of pregabalin along with several time efficacious effects than individual effects.

Hence the results of the present study gives clear indication to assume that the combination of PGB and NMD may provide better option for the use of these regimens to treat status epilepticus as well as for typical and atypical epilepsies. The combination of PGB and NMD may have substantial prospective to treat diverse types of epilepsies comprising resistance epilepsies due to channel modifying effects. The major advantage of combination therapy would be low doses of PGB with better anti-seizure effects. The most important point of this study is the use of combination regimens of PGB/NMD in short and long term control of status epilepticus, however such a use may requires further animals as well as clinical studies.

Conflict of Interest

Authors have no conflict of interest to declare.

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