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RESEARCH ARTICLE

Anticonvulsant activity of gabapentin in mice - An experimental study

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ABSTRACT

Background: Gabapentin (GBP) is used as an adjuvant drug in the treatment of partial seizures. However, there exist limited data demonstrating its antiepileptic activity in generalized seizures. **Aims and Objectives:** This study aims to evaluate the anticonvulsant activity of GBP using experimental models in mice. **Materials and Methods:** The study was conducted after the Institutional Animal Ethics Committee approval. Anticonvulsant activity of GBP in mice was evaluated against maximal electroshock (MES) and pentylenetetrazol (PTZ)-induced convulsions. MES is a standard model used to evaluate drugs which are effective in grand mal epilepsy, whereas PTZ is used to evaluate drugs effective in petit mal epilepsy. A total of 12 mice were assessed in each model, six mice in each group for control and test. The control group was administered 0.2 ml normal saline single dose per day i.p and test group was administered GBP (0.468 mg/g body wt. of mice) single dose per day i.p for 5 consecutive days. On the 5th day, the anticonvulsant activities were evaluated using both models. **Results:** In MES model, we observed 83.34% protection against tonic hind limb extension (THE) in GBP group while there was 0% in control group. However, in PTZ model, GBP was ineffective in preventing seizures but was effective in reducing the severity of seizures and mortality (16.67%) compared to control (100%). **Conclusion:** The results obtained showed that GBP significantly inhibited generalized seizures (protection against THE) induced through MES. However, GBP has partial protective effect on PTZ-induced seizures.

KEY WORDS: Gabapentin; Antiepileptic; Generalized Seizures; Maximal Electroshock-induced Convulsions; Pentylenetetrazol-induced Convulsions; Experimental Model

INTRODUCTION

Epilepsy is the most common neurological diseases causing significant morbidity burden. It is characterized by recurrent, usually unprovoked seizures, as well as by cognitive and psychosocial consequences.[1]

Drug therapy is the standard of care for epilepsy.[2] Antiepileptic drugs (AEDs) have been the mainstay for the management of epilepsy, in which better control of seizures is seen in about 70% of the patients. While around 30% of patients remain uncontrolled and require polytherapy, which itself has the potential risk of adverse reactions and drug interactions.[3]

The quest of finding newer pharmacological agents led to the introduction of gabapentin (GBP). It is multimodal drug, which not only has an antiepileptic effect but also further research has shown its effectiveness in pain management.[4] Studies have proven its effectiveness in the management of focal epilepsy, as an adjuvant as well as monotherapy drug.[5-7] Its mechanism of action is still not fully understood. The effectiveness of GBP in focal seizure with/without secondary generalization[8] has led to our hypothesis to assess the anticonvulsant activity of GBP in generalized seizure. Therefore, the present experimental study was undertaken to evaluate the anticonvulsant activity of GBP in mice using standard models of epilepsy.

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MATERIALS AND METHODS

The present study was conducted after the approval of the Institutional Animal Ethics Committee (Approval no 2016/07/11, dated 20/8/2016) and was conducted in accordance with the recommended guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA), India.

Experimental Animal

The study included naïve Swiss albino mice weighing 20 ± 5 g of either sex. The animals were procured from Bombay College of Veterinary, Mumbai. The animals were housed 6 per cage, kept at room temperature (maintained at 22 ± 0.5°C) with alternating 12-h light/dark cycle. Food and water were provided *ad libitum*. The animals were acclimatized for at least 7 days in animal house and then for 2 h in laboratory before the experiment. All experiments were performed during the light phase of cycle.

Chemicals Used

1. Pentylenetetrazol (PTZ)
2. GBP
3. Normal saline and surgical spirit.

Equipment's Used

1. Digital weighing machine
2. Surgical hand gloves, tuberculin syringe, and needles
3. Test tube, beakers and flask, stirrer
4. Ear electrodes (mice)
5. Electroconvulsiometer.

Experimental Models

Anticonvulsant activity was evaluated by the following standardized models:[9-11]

1. Maximal electroshock seizure (MES): This is a standard model primarily used to evaluate compounds which are effective in grand mal epilepsy was used
2. PTZ seizure model: PTZ model in mice is primarily used to evaluate compounds which are effective in petit mal epilepsy was used.

Modified Racine's scale (RS) was used to assess the intensity of a seizure in this model.[9]

Dose Calculation for GBP

In this study, we used human absolute dose of GBP which is 3600 mg/day. We calculated animal equivalent dose (AED) for mice using the formula (for 20 g mice).

Formula = Human absolute dose × conversion factor (0.0026).[12,13]

Using above-mentioned formula, AED was 9.36 mg/20 g (0.468 mg/g body wt.) of mice.

Experimental Groups

- Group A(Control): Normal saline 0.2 ml intraperitoneally.
- Group B (test drug): GBP 0.468 mg/g body wt. of mice intraperitoneally.

Methodology

MES seizure model: A total of 12 mice were assessed in this model, six mice in each group for control and test. The control group was administered 0.2 ml normal saline single dose per day and test group was administered GBP (0.468 mg/g body wt. of mice) single dose per day intraperitoneally at 10 am for 5 consecutive days. On the 5th day, 60 min after the last dosing, each mouse was given electroshock using fixed current - 50 mA, pulse frequency - 50 Hz, and duration - 0.2 s through ear electrode using electroconvulsiometer. Animals were observed for 1 h. Protection against tonic hind limb extension (THE) was considered as positive endpoint.

PTZ model

A total of 12 mice were assessed in this model, six mice in each group for control and test. The control group was administered 0.2 ml normal saline single dose per day and test group was administered GBP (0.468 mg/g body wt. of mice) single dose per day intraperitoneally at 10 am, respectively, for 5 consecutive days. On the 5th day, 60 min after the last dosing, the mice were administered PTZ 80 mg/kg intraperitoneally. Animals were observed for seizure activity for 1 h. Following endpoints were observed: Onset of seizure, duration of the first convulsion, seizure score, and occurrence of death.

Statistical Analysis

Data were entered into Microsoft Excel (version 2007) and statistically analyzed using Statistical Package for the Social Sciences IBM software version 20. Continuous data were analyzed using Student's – *t*-test. $P < 0.05$ was considered statistically significant.

RESULTS

MES Model

In the control group, all the six animals exhibited THE. Thus, no protection was observed in them, whereas in GBP group, one of six animals exhibited THE. Thus, GBP showed protection against THE in 83.34% (5/6) animals. On comparing GBP and control group in MES model, we found GBP exerted higher (83.34%) protection in comparison to control group (0%) [Figure 1].

PTZ Model

In the control group, the mean time for onset of seizure was 49.50 ± 11.14 s and the mean duration of seizure was 81.1667 ± 46.3 s. The mean seizure score observed in the control group was 5. In the control group, death was observed in all the six animals; therefore, the percent mortality in the control group was 100%, whereas in GBP group, the mean time for onset of seizure was 228.6 ± 224.1 s and the mean duration of seizure was 59.6 ± 64.05 s. The mean seizure score observed was 4. In GBP group, death was observed in one animal; therefore, the percent mortality was 16.67% [Table 1].

Comparison between GBP and Control Group

On comparing GBP and control group in PTZ model, we observed 100% mortality in the control group while 16.67% mortality in GBP group. However, the mean time for onset of seizure in control group was 49.50 ± 11.14 s while in GBP group, it was 228.6 ± 224.1 s. The difference observed in the mean between the two groups was not statistically significant [Table 2].

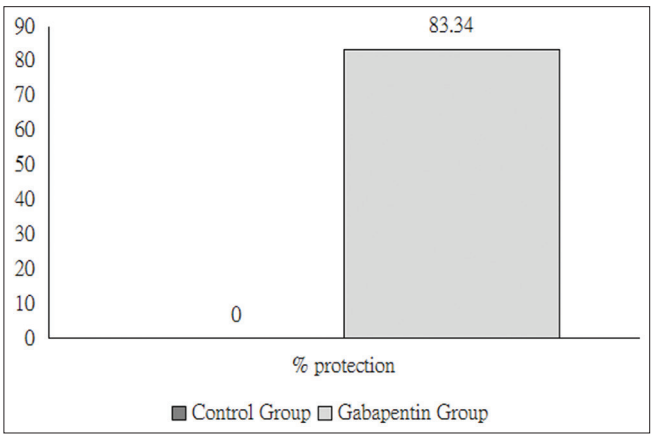

Figure 1: Gabapentin versus control - percent protection in maximal electroshock

Similarly, the mean duration of seizure in PTZ model in control group was 81.1667 ± 46.3 s while in GBP group, it was 59.6 ± 64.05 s. The difference observed in the mean between the two groups was not statistically significant [Table 3].

DISCUSSION

The key findings of this study signify that GBP prevented seizures in MES model and reduced the severity of seizures and mortality in animal in PTZ model. GBP is a newer AED which has fewer adverse effects and drug-drug interaction.[14]

In our study, we observed that in GBP, there was protection among 83.34% of animals (mice) in MES model. This implies that THE stage was prevented in 83.34% of test animals when compared with control group. While in PTZ model, in our study, we observed that GBP was ineffective in preventing seizures. However, GBP was effective in reducing the severity of seizures which we graded using modified Racine score, that is, Grade 4 as compared to Grade 5 in control group. In GBP group, mortality was observed in only 16.67% while in control group, it was 100%. Our result signifies the effectiveness of GBP in reducing the severity of seizures when used as monotherapy in the management of petit mal epilepsy also. However, there exists paucity of data in literature on the usage of GBP as monotherapy in generalized epilepsy/absence seizures in either humans or animals models and, hence, were not able to compare our findings. A study conducted by Gautam *et al*. showed protective effects of graded doses of GBP on aminophylline-induced experimental status epilepticus in mice. They demonstrated that single doses of GBP showed incomplete protective effects against AMPH-induced convulsions, indicating partial protective effects of GBP. Data on mortality demonstrated that mice pre-treated with GBP at doses of 100, 200, and 500 mg/kg showed reduction in mortality to 20%, 22.2%, and 30%, respectively,

Table 1: Mean onset of seizures (in seconds), mean duration of seizure (in seconds), seizure score, and percentage of mortality

PTZ-induced convulsions	Total mice	Mean onset of seizure (sec)	Mean duration of seizure (sec)	Seizure score (Racine)	% mortality
Control	6	49.50±11.14	81.16 ±46.3	5	100
GBP	6	228.6±224.1	59.6±64.05	4	16.67

GBP: Gabapentin, PTZ: Pentylenetetrazol

Table 2: Comparison of the mean onset of seizure between control and gabapentin in PTZ model

Group	Total mice	Mean onset of seizure (sec)	*t* value	*P* value
PTZ-induced convulsions				
Control	6	49.50±11.14	1.956	0.079
GBP	6	228.6±224.1		

GBP: Gabapentin, PTZ: Pentylenetetrazol

Table 3: Comparison of the mean duration of seizure between control and gabapentin in PTZ model

Group	Total mice	Mean duration of seizure (sec)	*t* value	*P* value
PTZ-induced convulsions				
Control	6	81.16±46.3	0.666	0.520
GBP	6	59.6±64.05		

GBP: Gabapentin, PTZ: Pentylenetetrazol

in 120 min, and 80%, 77.8%, and 40%, respectively, in 24 h.[15] An isobolographic analysis study done by Borowicz *et al.* on the effect of GBP on the anticonvulsant activity of AEDs against electroconvulsions in mice revealed that combinations of GBP with other AEDs generally result in synergistic (supraadditive) interactions.[16] The antiepileptic mechanism of the action of GBP has remained poorly understood. According to Hendrich *et al.*, GBP binds to an exofacial epitope of the α2δ-1 and α2δ-2 auxiliary subunits of voltage-gated calcium channels, but acute inhibition of calcium currents by GBP is either very minor or absent. GBP inhibits calcium currents, only when applied chronically, but not acutely. GBP may act chronically by displacing an endogenous ligand that is normally a positive modulator of α2δ subunit function, thereby impairing the trafficking function of the α2δ subunits to which it binds.[17]

Strength of the study is that it is the first kind of a study which provides preclinical evidence of GBP (monotherapy) having potential anticonvulsive benefits in generalized seizures and absence/petit mal seizures. The study had few limitations. It was an animal study and can be influenced by biological variation. The sample size was small and was restricted by CPCSEA guidelines.

CONCLUSION

We have observed that GBP significantly inhibited generalized seizures (protection against THE) induced through MES. However, GBP has partial protective effect on PTZ-induced seizures. Further, prospective clinical studies and randomized, multicenter trials are warranted to optimize the evidence.

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