

Study of potential drug-drug interactions between benzodiazepines and four commonly used antiepileptic drugs in mice

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ABSTRACT

Background: Benzodiazepines (BZD) is one of the commonly used drug groups for certain neurological diseases. As sometimes, the anti-epileptic drugs (AEDs) may be used concomitantly with BZD there is a potential for drug-drug interactions. Study aimed to study potential drug-drug interactions between four commonly used AEDs (phenytoin, carbamazepine (CBZ), phenobarbitone, sodium valproate) and BZD (diazepam, clonazepam) in mice using maximal electroshock seizure (MES) method and pentylenetetrazole (PTZ) method.

Methods: Adult male albino mice were divided into four different groups of six animals each and anti-epileptic activity was assessed using MES method and PTZ method. Group I acted as a control, Group II received any one of the four AEDs (phenytoin, CBZ, phenobarbitone or sodium valproate) in sub-effective doses, Group III received diazepam or clonazepam alone, Group IV received a combination of diazepam or clonazepam with any one of the AEDs.

Results: In MES method, the groups receiving combination of diazepam with phenytoin and CBZ showed significant protection compared to the control group ($p < 0.01$ and $p < 0.02$), respectively. However, diazepam in combination with sodium valproate and phenobarbitone did not show any significant protection compared to the control group and individual antiepileptic group. All the four antiepileptic showed significant protection against MES seizure in combination with clonazepam when compared to control group. In PTZ method, combination of sodium valproate with clonazepam showed significant protection compared to control group ($p < 0.02$). However, this was not observed with diazepam-valproate combination.

Conclusion: Clonazepam potentiates the action of all the four anti-epileptics while diazepam potentiates only phenytoin and CBZ against MES seizures. Clonazepam but not diazepam potentiates the action of sodium valproate against PTZ seizures.

Keywords: Antiepileptic drugs, Benzodiazepine, Drug-drug interaction, Maximal electroshock seizure method, Pentylenetetrazole method

INTRODUCTION

Epilepsy is one of the most common neurological disorders in any country, including India. In India, studies have reported prevalence rates of average 500 cases per 100,000 population.¹ Around one-fifth of this epilepsy, patients are subjected to more than one anti-epileptic drugs (AEDs).²⁻⁴ Polytherapy increases risk of interactions with commonly prescribed drugs like benzodiazepine (BZD) group.⁵ BZD like diazepam and clonazepam is prescribed for the treatment of anxiety, panic attacks, insomnia, seizures, muscle spasm, withdrawal of BZD, alcohol and opiate, restless leg syndrome etc.^{6,7} Combination of commonly used drugs like BZD with AEDs may produce antagonism, additive, and supra-additive anticonvulsant effects. The drug

combination providing the supra-additive effects seems to be clinical significance. However, when the supra-additive anticonvulsant efficacy is also associated by a distinct increase in toxicity, the protective index may not be affected or even lowered. Synergistic interactions have been shown for the combinations of valproate-phenytoin/ethosuximide, topiramate carbamazepine (CBZ)/phenobarbital.⁸

It is, therefore, important to identify their potential interactions (with each other and other substances) in order to avoid altering their efficacy or potentiating their side effects. Older AEDs such as phenytoin, CBZ, phenobarbital, and valproic acid can significantly interfere not only with each other and other AEDs, but also with other treatments. Although newer AEDs have a more favorable

pharmacokinetic profile, they are not entirely exempt from interactions and they are also commonly administered in combination with older AEDs.⁹ Concomitant administration of AEDs (phenytoin, phenobarbitone, sodium valproate, CBZ) and diazepam or clonazepam have produced variety of drug interactions in clinical as well in vivo and in vitro animal studies.¹⁰⁻¹³

As a few such studies have been reported, the present experimental study was undertaken with four conventional AEDs, phenytoin, CBZ, sodium valproate, and phenobarbitone, combined with two BZD diazepam or clonazepam, to find out whether diazepam or clonazepam modify the effect of these AEDs when they are co-administered. i.e., whether any significant drug interaction is possible between diazepam, clonazepam, and AEDs in a single dose.

Objectives

To study drug-drug interactions between BZD and conventional AEDs in mice using maximal electroshock seizure (MES) and pentylenetetrazole (PTZ) methods.

METHODS

The study was carried out after obtaining approval from Institutional Animal Ethics Committee. Swiss Albino male mice weighing 25-30 g were used. Animals were housed under standard laboratory conditions in plastic cages at an ambient temperature of 25±2°C and 45-55% relative humidity with a 12 hr light/dark cycle. They were fed standard animal feed (Pranav Agro) and allowed water *ad libitum*.

Each set of experiment consisted of following four groups (n=6).

Group-I (Control group): 0.4 ml of 0.3% tween 80 i.p. (i.p) in distilled water.

Group-II: Any one of the following conventional AEDs alone, that is phenytoin, CBZ, sodium valproate or phenobarbitone i.p.

Group-III: Any one of the two BZD, that is, diazepam or clonazepam i.p.

Group-IV: Received combination of any one of the conventional AEDs used in Group-II with either of the drugs used in Group-III e.g. phenytoin with diazepam, CBZ with clonazepam.

Study comprised of total 15 groups evaluated by MES method¹⁴ and 9 groups evaluated by PTZ method.¹⁵

Hence, sub-effective doses of all AEDs were used that is a dose, which is substantially, lower than the dose

producing significant activity. According to weight and group, each mouse was pretreated with the tween 80 or any of anticonvulsant drug, diazepam and clonazepam were given 45 mins, 30 mins and 60 mins before giving electroshock or injection. Of PTZ, respectively. The doses used were-phenytoin sodium (15 mg/kg in both methods), CBZ (35 mg/kg in both methods), sodium valproate (150 mg/kg, in PTZ method - 50 mg/kg), phenobarbitone (20 mg/kg in both the methods), diazepam (5 mg/kg, in PTZ method - 0.5 mg/kg), clonazepam (1 mg/kg in both methods). MES method-seizures were produced by giving electroshocks by electroconvulsimeter (Techno Lab, Lucknow) using ear electrodes. For giving electroshocks 60 (15±4) mA current was used for the duration of 0.2 sec using ear electrodes. Mice were observed for the presence or absence of tonic hind limb extension as the endpoint in MES method.

PTZ method

According to the weight and group, each mouse was pretreated with the anticonvulsant drugs.

After 30 mins of administering anticonvulsant drug, seizures were produced by i.p. PTZ (60 mg/kg). The mice were observed for 30 mins for the presence or absence of clonic convulsions. The control mice developed a characteristic sequence of seizures with or without death within 30 mins.¹⁶ Protection was considered when the given drug abolished clonic convulsive phase of seizures.

All the experiments were carried out at same time each day that is between 10:00 a.m. and 1.00 p.m.

When two drugs administered concomitantly, they will either show additive or antagonistic effect, which will be seen as increase or decrease in protection effect.

Statistical analysis

Chi-square test was used to calculate the difference between two treatment groups. p<0.05 was considered significant.

RESULTS

MES method

Diazepam with antiepileptic drugs

Phenytoin (Group-II) and diazepam (Group-III) showed protection in 50% which was statistically not significant (p>0.1 vs. control group). Combination of phenytoin with diazepam (Group-IV) interacted additively and showed protection in six mice (100%, p<0.01) when compared with the control group. When compared with phenytoin treated Group (II) and diazepam treated Group (III), the result was

not significant ($p > 0.1$). CBZ also showed results similar to that of phenytoin. Sodium valproate and diazepam alone did not show significant protection as compared to the control group ($p > 0.5$ and $p > 0.1$ vs. control group, respectively). The combination of diazepam with sodium valproate also failed to produce any significant difference compared to all the three groups. Phenobarbitone combined with diazepam also failed to produce any significant difference compared to all the three groups (Figure 1).

Clonazepam with antiepileptic drugs

Phenytoin and clonazepam alone did not produce significant protection compared to the control group ($p > 0.1$ vs. control group). Combination of phenytoin with clonazepam administered in same doses showed significant protection when compared with the control group ($p < 0.005$).

Combination of CBZ with clonazepam administered in same doses showed significant protection when compared with the control group ($p > 0.1$, respectively). Sodium valproate and phenobarbitone when combined with clonazepam also showed significant protection ($p < 0.01$ and $p < 0.02$, respectively) (Figure 2).

PTZ method

Sodium valproate and diazepam did not show significant protection when compared with the control group ($p > 0.5$ and $p > 0.1$ vs. control group, respectively). Combination of sodium valproate with diazepam did not show significant protection when compared with the control group or groups treated with individual drugs alone ($p > 0.05$). Sodium valproate and clonazepam alone did not show significant protection when compared with the control group ($p > 0.5$ and $p > 0.1$ vs. control group, respectively). Combination of sodium valproate with clonazepam (Group-IV) showed protection in 83.33% which was significant compared with the control group ($p < 0.02$), but not significant when compared with individual drugs alone ($p > 0.05$) (Figure 3).

DISCUSSION

Study was carried out with an aim to find out the possible drug-drug interactions of diazepam and clonazepam with four most commonly used AEDs, i.e., phenytoin, CBZ, sodium valproate, and phenobarbitone. All the four antiepileptic drugs, phenytoin, CBZ, sodium valproate, and phenobarbitone that have been used in the present study are reported to be active against generalized tonic-clonic seizures produced by MES method. These drugs when co-administered with one another, they produce drug interactions. When two drugs administered concomitantly from different groups, they showed an additive effect in our study, which was seen as an increase in the percentage protection. In our study, we came across only additive drug interactions between AEDs and BZDs.

Interactions seen between anti-epileptics with diazepam

With BZD also, these drugs produce drug interactions.¹⁷ Metabolism of diazepam has been reported to be enhanced as a result of induction of hepatic drug-metabolizing enzymes following phenytoin therapy as shown in an earlier study of Kutt and Harden (1999)¹⁸ and Dhillon and Richens (1981).¹⁰ However, in the present study, the combination of sub-effective doses of phenytoin and diazepam showed synergistic (additive) effect (combination showed 100% protection). This could be because, diazepam is metabolized by CYP2C19 isoform of cytochrome P450. Phenytoin is also metabolized by same isoform. Hence, concurrent administration of a single dose of diazepam may increase plasma concentration of phenytoin by decreasing its rate of metabolism.^{19,20} Action of phenytoin is mediated by slowing of the rate of recovery of voltage-activated Na^+ channels from inactivation and action of diazepam is mediated by facilitation of gamma-amino butyric acid (GABA) activity. Thus, these two drugs acting through distinct mechanisms may be the reason responsible for producing additive effect, i.e., a pharmacodynamic interaction. Our finding is supported by a study of Usha et al (2003); which showed BZD have good efficacy as add on treatment to phenytoin in refractory epileptic patients.²⁰ Similarly, additive effect was observed with a combination of CBZ and diazepam. CBZ has mechanism similar to that of phenytoin hence similar pharmacodynamic interaction could be responsible for this interaction. Combination of valproate and diazepam produced additive but statistically nonsignificant effect. Earlier study has shown that valproate is highly bound to plasma proteins and readily displaces other bound anticonvulsant drugs.²¹ Diazepam and valproate have been reported to have a synergistic effect.²² Number of mice protected with combination of phenobarbitone with diazepam (4) were less than the total of number of mice protected with phenobarbitone alone (2) and number of mice protected with diazepam alone (3), though the protective effect seem to be antagonistic (combination showed 66.66% protection), p value was not significant ($p > 0.05$) when compared with control group. Phenobarbitone increases metabolism of diazepam by inducing microsomal enzyme.^{19,23,24}

Interactions seen between anti-epileptics with clonazepam

Number of mice protected with the combination of phenytoin with clonazepam (6) were equal to the total of number of mice protected with phenytoin alone (3) and the number of mice protected with clonazepam alone (3). Thus, clonazepam showed synergistic (additive) effect with phenytoin.

Similarly, clonazepam showed potentiating effect with CBZ, sodium valproate, and phenobarbitone.

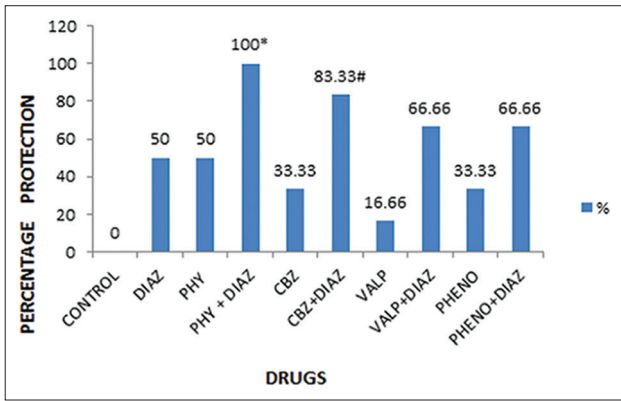


Figure 1: Comparison of percentage protection of antiepileptic drugs and their combination with diazepam using MES method in mice (n=6 for each group). *p<0.01 - Combination of phenytoin with diazepam showed significant protection when compared with control group. #p<0.002 - Combination of carbamazepine with diazepam showed protection when compared with control group.

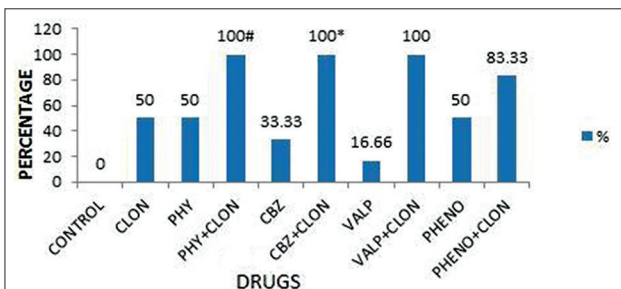


Figure 2: Comparison of percentage of antiepileptic drugs and their combination with clonazepam using MES method in mice (n=6 for each group). #p<0.005 - Combination of phenytoin with clonazepam showed significant protection when compared with control group. *p<0.005 - Combination of carbamazepine with clonazepam showed significant protection when compared with control group. @p<0.01 - Combination of sodium valproate with clonazepam potentiating effect was observed which was significant when compared with control group. \$p<0.02 - combination of phenobarbitone with clonazepam showed protection when compared with control group.

In PTZ method

With the combination of sodium valproate with diazepam effect was additive but non-significant compared with the control group. However, with the combination of sodium valproate with clonazepam potentiating effect was observed which was significant (p<0.02) when compared with the control group.

Polytherapy with AEDs has been studied in animal models. Bourgeois et al. (1990) studied the possible advantages of many AEDs combinations in mice.²⁵ Synergism may be due to enzyme inhibition (decrease metabolism) of AED by

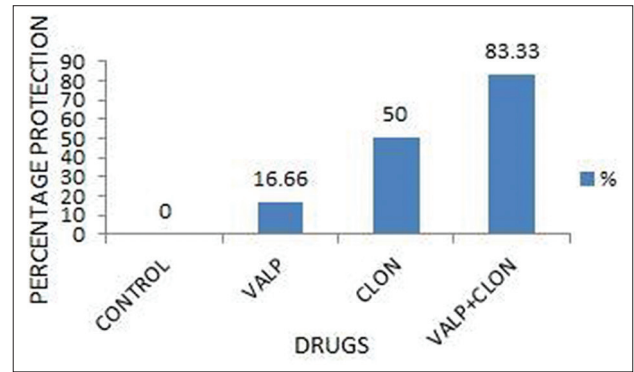


Figure 3: Comparison of percentage protection of sodium valproate and clonazepam using PTZ method in ice (n=6 for each group). *p<0.02 - Combination of sodium valproate with clonazepam showed significant protection when compared with control group.

BZD or BZD may displace anti-epileptics at their protein binding site and thus increasing free drug concentration of anti-epileptics. Synergism leads to decrease in amount or frequency of doses given of antiepileptics and hence, also decrease occurrence of adverse effects and increase patient compliance BZD are metabolized by CYP2C19 isoform of cytochrome P450. Phenytoin and phenobarbitone also metabolized by same isoform so concurrent administration of BZD, may increase plasma concentration of phenytoin or phenobarbitone by decreasing its rate of metabolism.¹⁰ Similarly, BZD are also metabolized by CYP3A4 and CBZ is also metabolized by CYP3A4. Hence, similar drug interaction may be occurring between these drugs. However, whether single doses can lead to such effects is questionable. As BZD and phenobarbitone have a similar mechanism of action that both facilitate binding of GABA on GABA_A-Cl⁻ channel complex, the additive effect may not be seen. Drug absorption depends on a number of factors including formulation, pKa, and lipid solubility which should be considered contiguously with splanchnic blood flow and intestinal pH, motility, bacterial flora, and the metabolic capacity of the gastrointestinal tract. A drug may alter the rate of absorption or the degree of absorption of other drugs in the gastrointestinal tract. These variables make prediction of drug absorption interactions very difficult.²⁶ However, while protein binding displacement has been implicated as a causative mechanism in many drug interactions; its importance has generally been overstated, being based largely on in vitro data. Since displacement makes more unbound (free) drug available for metabolism or glomerular filtration and the displaced drug can normally distribute out of the plasma compartment, increased unbound drug concentrations are usually only transient and, therefore, do not commonly give rise to altered pharmacological effects in the patient. Induction of drug metabolism is a complex, dose-related phenomenon. It requires the inducer to reach a critical concentration at an intra-nuclear receptor or regulation point from which up-regulation of messenger RNA occurs with consequent increases in enzyme protein production.²⁷ It is

unrealistic to expect clinicians to be familiar with all known interactions. However, particular care is needed with patients receiving drugs like anti-epileptics.

There are a limited number of studies reported from India on drug-drug interaction of AEDs with BZDs, our study focused on this grey area. We evaluated possible drug interactions between most commonly used conventional AEDs and two commonly used BZD.

Not all drug interactions are predictable, and the results of studies in experimental animals cannot be extrapolated directly to humans because of species' differences. Epilepsy is a chronic disease. In our study, we used only a single dose so this it can be further extended using multiple doses. Further study with BZD antagonist, flumazenil, can confirm the pharmacodynamic potentiation by diazepam of AEDs in MES method.

CONCLUSION

BZDs are used with conventional AEDs that can result in to drug-drug interactions. In MES method, phenytoin with diazepam and CBZ with diazepam, showed statistically significant protection compared to control group, whereas in the case of clonazepam all the four AEDs showed significant protection as compared to control group. In PTZ method, sodium valproate with clonazepam showed significant protection as compared to control group. Hence, clonazepam showed higher percentage protection as compared to diazepam. The clinical importance of a particular interaction has to be assessed in controlled clinical investigations.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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