Effect of promethazine on seizure activity and its interactions with antiepileptic drugs diazepam and phenytoin in Rats

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ABSTRACT

Background: Current antiepileptic drugs (AEDs) are effective in controlling seizures in about 70% patients but use is often limited by adverse effects. Promethazine, H1 receptor antagonist, has a controversial status in patients of epilepsy. Both pro and antiepileptic effect has been documented in various animal studies. Hence, this study was designed to see the effect of promethazine, an H1 antihistaminic drug and its interactions with antiepileptic drugs in rats.

Methods: The effect of promethazine (10mg/kg) and its interactions with antiepileptic drugs diazepam and phenytoin was assessed by using maximal electroshock seizures (MES) and chemoshock (PTZ) method.

Results: Promethazine along with diazepam in subtherapeutic doses exerted significant protection against MES induced seizures whereas no such protection was observed with PTZ method rather the seizure threshold was reduced.

Conclusions: Subtherapeutic doses of Promethazine alone and in combination with diazepam showed protection against seizures in MES method. However, proconvulsant effect was seen with PTZ method suggesting histamine plays a protective role in development of seizures. This shows dual behavior of promethazine on MES and PTZ induced seizures.

Keywords: Diazepam, MES, Pentylenetetrazol, Phenytoin, Promethazine

INTRODUCTION

Epilepsy is a chronic neurological disorder that affects people of all ages. Around 39 million people worldwide and 7 million in India have epilepsy.¹,² Epilepsy is more common in older people.³,⁴ In the developed world, onset of new cases occurs most frequently in babies and the elderly.⁵ In the developing world, onset is more common in older children and young adults, due to differences in the frequency of the underlying causes.⁶ Current antiepileptic drugs are effective in controlling seizures in about 70% of patients but their use is often limited by adverse effects. Antiepileptic drugs are among the most common classes of drugs responsible for either isolated cutaneous reactions. "Antiepileptic Hypersensitivity Syndrome" is a severe dose independent, idiosyncratic reactions to aromatic anticonvulsants like phenytoin and carbamazepine that may result in end organ damage.⁷,⁸

Histamine plays important role in cutaneous adverse drug reactions caused due to drug hypersensitivity.⁹,¹⁰ Also histamine has stimulatory effects upon neurons. It also has suppressive ones that protect against the susceptibility to
convulsions, drug sensitization, denervation, supersensitivity, ischemic lesions and stress.\textsuperscript{11} Antihistaminics may play a crucial role in management of cutaneous ADR caused due to antiepileptic drugs. Promethazine, H\textsubscript{2} receptor antagonist is an antiallergic and antiemetic drug having an additional centrally acting anticholinergic property. But, there is controversy regarding use of promethazine as an antihistaminic agent in patients of epilepsy. Both pro and antiepileptic effect of promethazine has been documented in various animal studies.\textsuperscript{12-17} Hence the present study was designed to study the effect of promethazine on seizure activity and its interactions with antiepileptic drugs diazepam and phenytoin in rats using subtherapeutic doses.

**METHODS**

The study protocol was approved by Institutional Animal Ethics Committee (IAEC). All the pharmacological experiments were conducted using albino rats (n = 10), weighing between 150 and 200g. The animals were maintained under controlled environmental conditions such as temperature (21±2°C), relative humidity (30-70\%), and photoperiod of 12/12 h period. They were provided with standard commercial pelleted diet and Aquaguard drinking water ad libitum. They were acclimatized for at least 7 days before the start of experiments. Convulsive tests were carried out between 12.00-15.00 hrs.

Drugs used were injections of promethazine, diazepam and phenytoin. Solutions of these drugs were prepared freshly in desired strength in water. Drugs were injected intraperitoneally (i.p.). All drugs were given in subtherapeutic doses, which were decided by trial and error method. Experimental design for the study was:\textsuperscript{18}

- **Group I:** Control: 0.1ml/100 gms
- **Group II:** Promethazine alone
- **Group III:** Antiepileptic drug alone (Diazepam or Phenytoin)
- **Group IV:** Promethazine + Antiepileptic drugs (Diazepam or Phenytoin)

Methods of convulsive tests selected:

**Supramaximal Electroshock Seizures (MES)\textsuperscript{19}**

Rats were tested for tonic hind limb extensor phase (TEP) of electroshock seizure with a convulsimeter using current strength of 150mA for 0.2 seconds through the ear electrode.\textsuperscript{20} During screening rats not showing typical extensor phase were discarded. For observing interaction of promethazine with antiepileptic drugs 10 rats were pretreated with subtherapeutic dose of promethazine, 10 rats with diazepam or phenytoin and another 10 rats with combination of promethazine and antiepileptic drugs (diazepam or phenytoin)

**Chemically induced seizure (PTZ)\textsuperscript{21}**

Pentylenetetrazol (PTZ) was given intraperitoneally (ip.) in a dose of about 70 mg/kg producing seizures in 100% rats without any mortality. Observations were made for 30 minutes for convulsions to occur after injection of PTZ. Rats were divided in group of 10.\textsuperscript{22} Potentiation of PTZ convulsions by promethazine was elucidated further by taking subtherapeutic dose of PTZ with subtherapeutic dose of promethazine. The effect of promethazine in combination with antiepileptic drugs diazepam and phenytoin was also compared using PTZ method.

**Statistical analysis**

All values are expressed as percentage of animals showing protective effect. Comparison of percentage protection in promethazine, diazepam, phenytoin, and promethazine + diazepam or phenytoin with control was done by proportion test.\textsuperscript{18} Data was analyzed on STATA statistical software. P values <0.05 was considered as statistically significant and p<0.01 as highly significant.

**RESULTS**

Effect of promethazine, and antiepileptic drugs diazepam and phenytoin alone and in combination against MES are shown in Table 1 and 2. It shows that there was 20%, 30% and 20% protection with promethazine, diazepam and phenytoin alone. While promethazine in combination with diazepam and phenytoin showed 80% and 20% protection of which initial was highly significant (p<0.01).

| **Table 1:** Effect of promethazine and diazepam alone and in combination using electroshock (MES) method in rats. |
|---|---|---|---|---|
| **Drugs** | **Dose (mg/kg)** | **Number of animals** | **Percentage of animals protected showing abolition of extensor phase** | **p-value** |
| Control | 10 | 00 | | 0.1360 |
| Promethazine | 10 | 10 | 20 | 0.0603 |
| Diazepam | 2 | 10 | 30 | |
| Promethazine+diazepam | 10+2 | 10 | 80 | 0.008** |

\[ p \text{ value of Promethazine, Diazepam and Prometazine + Diazepam is compared with Control} \]
\[ \text{□} \text{ p value of Promethazine + Diazepam is compared with addition of Promethazine and Diazepam} \]
\[ * \text{ p value <0.05 is significant} \]
\[ ** \text{ p value <0.01 is highly significant} \]
Table 2: Effect of promethazine and phenytoin alone and in combination using electroshock (MES) method in rats.

| Drugs          | Dose (mg/kg) | Number of animals | Percentage of animals protected showing abolition of extensor phase | p-value |
|----------------|--------------|-------------------|---------------------------------------------------------------|---------|
| Control        | 10           | 10                | 00                                                             |         |
| Promethazine   | 10           | 10                | 20                                                             | 0.1360  |
| Phenytoin      | 5            | 10                | 20                                                             | 0.1360  |
| Promethazine+Phenytoin | 10+5   | 10                | 20                                                             | 0.1360  |

* p value of Promethazine, Phenoytoin and Prometazine + Phenytoin is compared with Control; □ p value of Promethazine + Phenytoin is compared with addition of Promethazine and Phenoytoin; * p value <0.05 is significant; ** p value <0.01 is highly significant.

Table 3 shows seizure producing effect of combination of promethazine (fixed dose) with pentylenetetrazol (PTZ). It shows that graded doses of PTZ alone at a dose of 30, 40 and 50 mg/kg do not produced convulsions in rats. Convulsions were observed in 40% of animals at a dose of 60mg/kg and in 100% of animals at a dose of 70mg/kg of PTZ without any mortality. Promethazine in a fixed subtherapeutic dose of 10mg/kg in combination with graded dose of PTZ showed 70% convulsions at 30mg/kg and 100% convulsions at a dose of 40, 50, 60, 70mg/kg dose of PTZ with decreasing order of average time of onset of convulsion and increase in mortality. Table 4 and 5 shows effect of promethazine, diazepam and phenytoin alone and in combination using Chemoshock seizure induced by pentylenetetrazol. Results shows that there was 00%, 30% and 20% of animals were protected with promethazine, diazepam and phenytoin respectively. With combination of promethazine this protection was 30% and 00% in diazepam and phenytoin group resp. which was not significant (p>0.05).

Table 3: Seizure producing effect of combination of promethazine (fixed dose) with pentylenetetrazol (PTZ).

| Promethazine (mg/kg) | PTZ (mg/kg) | Animals convulsing (%) | Average time of onset of convulsion (min) | Mortality |
|----------------------|-------------|-------------------------|------------------------------------------|-----------|
| --                   | 30          | 00                      | 00                                       |           |
| --                   | 40          | 00                      | 00                                       |           |
| --                   | 50          | 00                      | 00                                       |           |
| 10                   | 70          | 100                     | 11                                       | 00        |
| 10                   | 30          | 70                      | 13.5                                     | 00        |
| 10                   | 40          | 100                     | 08                                       | 00        |
| 10                   | 50          | 100                     | 8.5                                      | 02        |
| 10                   | 60          | 100                     | 5                                        | 02        |
| 10                   | 70          | 100                     | 3.1                                      | 03        |

Table 4: Effect of promethazine and diazepam alone and in combination using chemoshock (PTZ) method in rats.

| Drugs         | Dose (mg/kg) | Number of animals | Percentage of animals protected showing abolition of tonic clonic phase | p-value |
|---------------|--------------|-------------------|------------------------------------------------------------------------|---------|
| Control       | 10           | 10                | 00                                                                      |         |
| Promethazine  | 10           | 10                | 00                                                                      |         |
| Diazepam      | 0.5          | 10                | 30                                                                      | 0.0603  |
| Promethazine +diazepam | 10+0.5 | 10                    | 30                                                                      | 0.0603  |

* p value of Promethazine, Diazepam and Prometazine + Diazepam is compared with Control; □ p value of Promethazine + Diazepam is compared with addition of Promethazine and Diazepam; * p value <0.05 is significant; ** p value <0.01 is highly significant.

Table 5: Effect of promethazine and phenytoin alone and in combination using chemoshock (PTZ) method in rats.

| Drugs           | Dose (mg/kg) | Number of animals | Percentage of animals protected showing abolition of tonic clonic phase | p-value |
|-----------------|--------------|-------------------|------------------------------------------------------------------------|---------|
| Control         | 10           | 10                | 00                                                                      |         |
| Promethazine    | 10           | 10                | 00                                                                      |         |
| Phenytoin       | 5            | 10                | 20                                                                      | 0.1360  |
| Promethazine+phenytoin | 10+5  | 10                 | 00                                                                      | 0.1287  |

* p value of Promethazine, Phenoytoin and Prometazine + Phenytoin is compared with Control; □ p value of Promethazine + Phenytoin is compared with addition of Promethazine and Phenoytoin; * p value <0.05 is significant; ** p value <0.01 is highly significant.
DISCUSSION

The present work investigated the effect of Promethazine, a H1 receptor antagonist, on seizure activity and its interactions with antiepileptic drugs diazepam and phenytoin in rats. The results of the present work provided evidence that subtherapeutic doses of promethazine alone and in combination with diazepam showed significant protection against tonic hind limb extensor phase (TEP) of electroshock seizures and this combination may have beneficial results in grandmal seizures.

In contrast to electroshock method when promethazine (10mg/kg) was tested alone and in combination with antiepileptic drugs in chemoshock method, it did not show any protection rather a proconvulsant action was seen, suggesting histamine plays a protective role in the development of convulsions. Hence its use in petitmal or absence seizures cannot be recommended.

The exact underlying mechanisms of such dual behavior of promethazine on MES and PTZ induced seizures are unclear. The reason may be the essential difference between the mechanism of tonic extension and that of clonus. When promethazine was tested by PTZ induced seizures for experimental activity, it significantly reduced threshold for seizures. The possible explanations for proconvulsant activity of promethazine could be:

- Blockade of histamine induced opening of homomultimeric GABA_A receptors.  
- Blockade of H1 receptor mediated reduction of a background K⁺ current in central neuron.
- Selective inhibition of brain NaK-ATPase.

Promethazine also has centrally acting anticholinergic properties. It is postulated that acetylcholine plays a role in proconvulsant action of the muscarinic agonist pilocarpine used in experimental models of human epilepsy. It is observed that stimulation of brain muscarinic receptors cause persistent tonic clonic convulsions suggesting enhancement of muscarinic neurotransmission as a mechanism of induction of seizure activity by agents that inhibit neural acetylcholinesterase (e.g. organophosphate inhibitors).

It is well known that GABAergic and glutaminergic mechanisms are directly associated with the seizure activity. When histamine diffuses away from its synapse to a glutamate synapse containing NMDA receptors, it can act at an allosteric modulatory site called the polyamine site, to alter the actions of glutamate at NMDA receptors. The role of histamine and function of this action are not well clarified.

Further, H1 receptor activation causes excitation in most brain regions (brainstem, thalamus, hypothalamus, cortex, amygdala, striatum) through Gq11 protein and a direct block of a leak potassium conductance or phospholipase inositol triphosphate (IP3) and diacylglycerol (DAG) mediation. IP3 releases calcium ions from internal stores and activates a number of calcium dependent processes, including opening of a cation channel (TRPC) or the stimulation of Na⁺- Ca⁺⁺ exchanger (NCX). Centrally acting anticholinergics like promethazine by blocking H1 receptors can interfere with functions in these important brain regions.

The results of present study are in consistent with the previous study conducted by Tanaka et al who reported anticonvulsant activity of some local anaesthetics, some antihistaminics, spasmylotics, analgesics and some other miscellaneous drugs. Majority of these drugs caused excitation and convulsions in toxic doses. The toxic convulsion was always type of clonic seizure and tonic extension never occurred. Hence, they coined a new term ‘antextensors’ in order to avoid contradictory explanation such as ‘anticonvulsant property’ of convulsant drugs.

In the present study it was found that promethazine was capable of preventing tonic extension in MES seizures but when promethazine was tested by PTZ induced seizures for its experimental activity, it significantly reduced threshold for seizures. In combination with antiepileptic drugs, the anticonvulsant activity of promethazine is limited to electroshock seizure and no protection was afforded by these compounds against PTZ convulsions. Rather, it tends to facilitate the clonic seizures of PTZ.

Limitations

- Results of animal study cannot be fully extrapolated to human epilepsy and seizures. It has to be concluded by a clinical study.
- Promethazine has a strong sedative action and hence concurrent use with benzodiazepines is again a limiting factor for clinical use of promethazine in the management of epilepsy.
- Problem of convulsive phenomena creates a degree of background concern about its use.
- More needs to be known about the difference in the mechanism of action of promethazine on MES and PTZ induced seizures which will help to decide the safety of use of promethazine in grandmal and petitmal epilepsy.

CONCLUSION

Authors can extrapolate finding of present study that promethazine reduces seizure threshold in PTZ induced seizures to conclude that the use of promethazine in combination with antiepileptic drugs in petitmal seizures cannot be recommended.

Combination of promethazine with diazepam showed highly significant protection against MES induced seizures. Promethazine in combination with phenytoin did not show any protection against MES induced seizures. So, this combination is not likely to have any clinical significance.
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