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Original Research Article

Assessment of efficacy of promethazine on seizure activity and its interactions with antiepileptic drugs lorazepam and sodium valproate in rats

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ABSTRACT

Background: Presently available antiepileptic drugs are effective in controlling seizures in more than half of patients of all epilepsy but use is often limited by adverse effects. H1 receptor antagonists, have 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 lorazepam and sodium valproate in rats.

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

Results: Promethazine along with lorazepam and sodium valproate 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 lorazepam and sodium valproate showed protection against seizures in MES method. However, proconvulsant effect was seen with PTZ method. This shows dual behavior of promethazine on MES and PTZ induced seizures.

Keywords: Lorazepam, Maximal electroshock seizures, Pentylenetetrazol, Sodium valproate, Promethazine

INTRODUCTION

Epilepsy is the disorder is characterised by seizures but not all seizures are due to epilepsy-febrile seizures or drug induced seizures, for example. The world health organization estimates that around 39 million people worldwide and 7 million in India have epilepsy. Epilepsy is more common in older people. In the developed world, onset of new cases occurs most frequently in babies and the elderly. In the developing

world, onset is more common in older children and young adults, due to differences in the frequency of the underlying causes.⁷ 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

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aromatic anticonvulsants like phenytoin and carbamazepine that may result in end organ damage.^{8,9}

Inflammatory agent histamine plays important role in cutanous adverse drug reactions caused due to drug hypersensitivity. 10,11 Also histamine has stimulatory effects upon neurons. It also has suppressive ones that protect against the susceptibility to convulsions, drug sensitization, denervation, supersensitivity, ischaemic lesions and stress. 12 Antihistaminics may play a crucial role in management of cutaneous ADR caused due to antiepileptic drugs. Promethazine, H₁ 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. 13-18 Hence the present study was designed to study the effect of promethazine on seizure activity and its interactions with antiepileptic drugs lorazepam and sodium valproate in rats using subtherapeutic doses.

METHODS

The study protocol was approved by Institutional Animal Ethics Committee (IAEC approval no: 01/2009/CPCSEA). The study was conducted between January 2010 to May 2012. 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, lorazepam and sodium valproate. Drugs were given in the dose of 0.2 ml/100 gm body weight. Total volume did not exceed more than 1.2 ml. Drugs were given by intraperitoneal route. Inj. promethazine (Phenargan 25 mg/ml, 2 ml amp), inj. sodium valproate (Encorate 100 mg/ml, 5 ml vial) and inj. lorazepam (Lopez 2 mg/ml, 2 ml amp) were used for the study. All drugs except pentylenetetrazol (PTZ) are available in injectable form. PTZ was available in powder form. Dilutions of PTZ were done freshly before experiment. All drugs were given in subtherapeutic doses, which were decided by trial and error method. Experimental design for the study was:¹⁹

Group I: Control: 0.1 ml/100 gms,

Group II: Promethazine alone,

Group III: Antiepileptic drug alone (lorazepam or sodium valproate),

Group IV: Promethazine + Antiepileptic drugs (lorazepam or sodium valproate).

Methods of convulsive tests selected:

Supramaximal electroshock seizures²⁰

Rats were tested for tonic hind limb extensor phase (TEP) of electroshock seizure with a convulsiometer using current strength of 150 mA for 0.2 seconds through the ear electrode. 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 lorazepam or sodium valproate and another 10 rats with combination of promethazine and antiepileptic drugs (lorazepam or sodium valproate).

Chemically induced seizure²²

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 10.²³ 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 lorazepam and sodium valproate 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, lorazepam, sodium valproate, and promethazine + lorazepam or sodium valproate with control was done by proportion test. ¹⁹ Data was analyzed on STATA statistical software. P<0.05 was considered as statistically significant and p<0.01 as highly significant.

RESULTS

Effect of promethazine, and antiepileptic drugs lorazepam and sodium valproate alone and in combination against supramaximal electroshock seizures (MES) are shown in Table 1 and 2. It shows that there was 20% protection with promethazine, lorazepam and sodium valproate alone. While promethazine in combination with lorazepam and sodium valproate showed 90% and 60% protection of which initial was highly significant (p<0.01).

Table 3 shows seizure producing effect of combination of promethazine (fixed dose) with 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 60 mg/kg and in 100% of animals at a dose of 70 mg/kg of PTZ without any mortality. Promethazine in a fixed subtherapeutic dose of 10 mg/kg in combination with graded dose of PTZ showed 70% convulsions at 30 mg/kg and 100% convulsions at a dose of 40, 50, 60, 70 mg/kg dose of PTZ

with decreasing order of average time of onset of convulsion and increase in mortality.

Table 1: Effect of promethazine and lorazepam alone and in combination using electroshock MES method in rats.

S. no.	Drugs	Dose (mg/kg)	Number of animals	Percentage of animals protected showing abolition of extensor phase	P value
1	Control		10	0	
2	Promethazine	10	10	20	■ 0.1360
3	Lorazepam	2	10	20	■ 0.1360
4	Promethazine +Lorazepam	10+2	10	90	■0.001** □0.0092**

[■]P value of promethazine, lorazepam and prometazine + lorazepam is compared with Control, □ p value of promethazine + lorazepam is compared with addition of promethazine and lorazepam; *p<0.05 is significant; **p<0.01 is highly significant.

Table 2: Effect of promethazine and sodium valproate alone and in combination using electroshock MES method in rats.

S. no.	Drugs	Dose (mg/kg)	Number of animals	Percentage of animals protected showing abolition of extensor phase	P value
1	Control		10	0	
2	Promethazine	10	10	20	■0.1360
3	Sodium Valproate	75	10	20	■0.1360
4	Promethazine +Sodium Valproate	10+75	10	60	■0.0034** □0.3006

[■]P value of promethazine, sodium valproate and prometazine + sodium valproate is compared with control; □ p value of promethazine + sodium valproate is compared with addition of promethazine and sodium valproate; * p<0.05 is significant; **p<0.01 is highly significant

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

S. no.	Promethazine (mg/kg)	PTZ (mg/kg)	Animals convulsing (%)	Average time of onset of convusion (min)	Mortality
1		30	0		00
2		40	0		00
3		50	0		00
4		60	40	14	00
5	10	70	100	11	00
6	10	30	70	13.5	00
7	10	40	100	08	00
8	10	50	100	8.5	02
9	10	60	100	5	02
10	10	70	100	3.1	03

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

S. no.	Drugs	Dose (mg/kg)	Number of animals	Percentage of animals protected showing abolition of tonic clonic phase	P value
1	Control		10	0	
2	Promethazine	10	10	0	-
3	Lorazepam	0.5	10	30	■ 0.0603
4	Promethazine +Lorazepam	10+0.5	10	30	■0.0603 □1.00

[■]P value of promethazine, lorazepam and prometazine + lorazepam is compared with control; \Box p value of promethazine + lorazepam is compared with addition of promethazine and lorazepam; *p<0.05 is significant;*** p<0.01 is highly significant.

Table 5: Effect of promethazine and sodium valproate alone and in combination using chemoshock method in rats.

S. no.	Drugs	Dose (mg/kg)	Number of animals	Percentage of animals protected showing abolition of tonic clonic phase	P value
1	Control		10	0	
2	Promethazine	10	10	0	-
3	Sodium valproate	50	10	20	■0.1360
4	Promethazine	10+50	10	20	■ 0.1360
	+Sodium valproate				□0.1000

[■]P value of promethazine, sodium valproate and prometazine + sodium valproate is compared with control; \Box p value of promethazine + sodium valproate is compared with addition of promethazine and sodium valproate; *p<0.05 is significant; **p<0.01 is highly significant.

Table 4 and 5 shows effect of promethazine, lorazepam and sodium valproate alone and in combination by using chemoshock seizure induced by PTZ. Results show that there was 00%, 30% and 20% of animals were protected with promethazine, lorazepam and sodium valproate respectively. With combination of promethazine this protection was 30% and 20% in lorazepam and sodium valproate group resp. which was not significant (p>0.05).

DISCUSSION

The present work investigated the effect of promethazine, a H1 receptor antagonist, on seizure activity and its interactions with antiepileptic drugs lorazepam and sodium valproate in rats. The results of the present work provided evidence that subtherapeutic doses promethazine alone and in combination with lorazepam showed significant protection against TEP of electroshock seizures and this combination may have beneficial results in grandmal seizures. In contrast to electroshock method when promethazine (10 mg/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 explainations for proconvulsant activity of promethazine could be (a) Blockade of histamine induced opening homomultimeric GABA_A recepors, (b) blockade of H₁ receptor mediated reduction of a background K+current in cenral neuron, (c) selective inhibition of brain NaK-ATPase.24-26

Promethazine also has centrally acting antcholinergic 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).²⁷

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. ²⁹

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).³⁰ Centrally acting anticholinergics like promethazine by blocking H1 receptors can interfere with functions in these important brain regions.

In our previous study we have also compared subtherapeutic doses of promethazine alone and in combination with diazepam and phenytoin. In that study it was demonstrated that subtherapeutic doses of promethazine alone and in combination with diazepam showed significant protection against TEP of electroshock seizures and this combination may have beneficial results in grandmal seizures³¹

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, spasmolytics, analgesics and some other miscellaneous drugs. ¹⁸ 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 'antiextensors' 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.

CONCLUSION

We 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 lorazepam showed highly significant protection against MES induced seizures. Promethazine in combination with sodium valproate also showed significant protection against MES induced seizures. So these combination are likely to have clinical significance.

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.

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