Original Article

Experimental evaluation of anti convulsant and analgesic activity of a pyrimidine derivative 4CPTP

Prem Kumar Verma[1], Garima Bhutani[2*], Rahul Saini[3], Seema Rani[4]

[1]Professor, [2,3]Assistant Professor, [4]Associate Professor, Department of Pharmacology, BPS GMC for women, Khanpur Kalan, Sonepat.
Email: garimaahuja2010@yahoo.com

Abstract

Aim: To study the anti convulsant and analgesic activity of pyrimidine derivative 4CPTP. **Material and methods:** The anticonvulsant activity of 4CPTP (40, 60, 80 and 100 mg/kg) was assessed using maximum electroshock seizure (MES) test and metrazol test using albino mice. The tail flick test was used to assess the analgesic activity in rats. **Results:** 4CPTP produced dose dependent increase in the mean percentage of mice protected from electroshock and metrazol induced convulsions. The significant analgesic action of the drug was seen only at 100 mg/kg dose. **Conclusions:** 4 CPTP possesses anti convulsant and analgesic action in experimental animals.
Keywords: maximum electroshock seizure test, metrazol, pyrimidines, phenobarbitone.

[*]**Address for Correspondence:**
Dr. Garima Bhutani, Assistant Professor, Department of Pharmacology, BPS GMC for women, Khanpur Kalan, Sonepat, Haryana, INDIA.
Email: garimaahuja2010@yahoo.com
Received Date: 01/09/2015 Revised Date: 22/09/2015 Accepted Date: 12/10/2015

Access this article online	
Quick Response Code:	Website: www.medpulse.in
	DOI: 14 October 2015

INTRODUCTION

Epilepsy is one of the most common central nervous system disorders. Its medical importance is magnified by the fact that it tends to last for the life time, is seldom completely cured and may have profound psychopathological consequences. The aim of giving anti epileptic drugs is to control and totally prevent all seizure activity at an acceptable level of side effects. With the currently available drugs, this can be achieved in about half of the patients. Another 20-30% attain partial control, while the rest remain refractory.[1] Hence there is a continuous search for an ideal anti epileptic drug which suppresses all seizures without causing any untoward effect.

Phenobarbitone, which is a pyrimidine derivative, was the first efficacious anti epileptic drug introduced in 1912. It is still considered as one of the cheapest and least toxic anti epileptics. It has broad spectrum efficacy against generalised tonic clonic, simple partial and complex partial seizures. However its major drawbacks are sedation and behaviour abnormalities.[2]

Pyrimidine is a six membered cyclic compound that contains four carbon and two nitrogen atoms at position one and three and is known as diazine. Its derivatives and many other compounds containing pyrimidine ring in their basic structure are widely used in clinical practice and have shown varied types of activity and therapeutic applications. Apart from anti convulsants like phenobarbitone, some anti cancer agents like 5 fluorouracil, floxuridine and cytarabine, anti malarial agent like pyrimethamine, anti fungals like fluocytosine, anti bacterial agents like trimethoprim, anti virals like idoxuridine and anti thyroid agents like thiouracil are pyrimidine derivatives.[3]

Structural modification of the existing compound is nowdays one of the main line of approach of new drug development. The compound under this study has also been obtained through structural modification of phenobarbitone and hence belongs to thiopyrimidine series. The chemical formula of the compound is 1(4-carboxy phenyl-4:4:6-trimethyl-1H,4H, pyrimidine- 2

How to site this article: Prem Kumar Verma, Garima Bhutani, Rahul Saini, Seema Rani. Experimental evaluation of anti convulsant and analgesic activity of a pyrimidine derivative 4CPTP. *MedPulse – International Medical Journal* October 2015; 2(10): 652-655. http://www.medpulse.in (accessed 16 October 2015).

thiol). Due to its structural resemblance to phenobarbitone, this compound was tested for its anti convulsant property. Apart from anti convulsant activity, analgesic activity of this compound was also studied.

MATERIAL AND METHODS

Drugs

Test compound (4CPTP) was dissolved in alkaline medium (sodium hydroxide) in 276: 40 ratio of the compound and sodium hydroxide respectively. Effect of sodium hydroxide in the above used ratio was separately studied and it was confirmed that it did not possess any anti convulsant activity at this concentration. Further dilutions of the compound were made in distilled water. The drug was used in doses of 40, 60, 80 and 100 mg/kg body weight of the animal. Phenobarbitone (in doses of 25 and 50 mg/kg body weight) was used as a standard anticonvulsant drug. For assessing the analgesic activity of the test compound, morphine (2.5 and 5 mg/kg body weight) and phenobarbitone (25 and 50 mg/kg) were used as standard comparators. Control group animals received distilled water.

Animals

For assessment of anti convulsant activity, adult albino mice of either sex weighing between 15-35 gms were used in the present study. They were randomised into 7 groups of 10 mice each. Control group was injected with distilled water intra peritoneally. Standard groups received phenobarbitone (25 and 50 mg/kg) and test groups received 4 CPTP (40, 60, 80 and 100 mg/kg) by intra peritoneal route.

For assessment of analgesic activity, rats of either sex, weighing between 75-130 gms were taken and randomly divided into 9 groups, receiving intra peritoneal injection of distilled water, phenobarbitone (25 and 50 mg/kg), morphine (2.5 and 5 mg/ kg) and 4CPTP (40, 60, 80 and 100 mg/kg) respectively.

Assessment of anti convulsant activity

Maximal electroshock method

30 minutes after giving the drug, a drop of normal saline was instilled into the eyes of each animal. The corneal electrodes were placed over the cornea of mice and electroshock was given using electric current of 30 mA for 0.2 seconds with the help of techno convulsiometer. The abolition of hind limb tonic extension component was defined as protection against MES test.[4]

Chemoshock or metrazol seizure threshold test

After 30 minutes of drug administration, metrazol (pentylenetetrazole) was given subcutaneously in all the groups in dose of 85 mg/kg body weight and mice were subsequently observed for 60 minutes for occurrence of seizures. Only those convulsions consisting of an episode of tonic spasm of at least 5 seconds were considered. Abolition of such convulsions was considered as protection from metrazol induced convulsions. [5]

The mean percentage of mice protected from MES and metrazol induced convulsions were calculated and compared between all the groups. Statistical analysis was done using Chi-square test and Fischer Exact test.

Assessment of analgesic activity

Analgesic activity was tested by radiant heat method using the hot nichrome wire analgesiometer. The tail of the animal was exposed to heat using current strength of 8 mA. The reaction time ie the time in which the animal withdrew its tail was noted. To avoid the risk of damage to the tail, maximum exposure was kept as 15 seconds. Baseline readings were taken and then repeat readings were taken 30 minutes after injection of drugs. Animals with a reaction time of more than 10 seconds were taken as protected. Statistical analysis of mean reaction time before and after giving drug was done using paired t test.

RESULTS

Anti convulsant activity

As depicted in table 1, the mean percentage of mice protected against maximal electroshock induced convulsions were 50%, 70%, 80% and 90% at 40 mg, 60 mg, 80 mg and 100 mg doses of 4 CPTP respectively. In the case of animals receiving phenobarbitone, the percentage was 50% and 100% for 25 and 50 mg dose respectively.

Similarly, 40%, 60%, 60% and 100% of animals were protected by 4CPTP when given at doses of 40 mg, 60 mg, 80 mg and 100 mg respectively during metrazol seizure induced test. Phenobarbitone treated animals showed percentage protection of 90 and 100% at doses of 25 and 50 mg respectively.

No animal in the control group (receiving distilled water) was found to be protected from convulsions.

As shown in table 2, statistical analysis reveals that 60, 80 and 100 mg/kg dose of 4CPTP showed significantly higher anti convulsant action than 25 mg/kg of phenobarbitone in MES induced convulsions. In metrazol induced convulsions, 4CPTP performed better than 25 mg/kg of phenobarbitone only when given at 100 mg/kg dose. Whereas in higher (50 mg/kg) dose, phenobarbitone possesses significantly higher anti convulsant action than all doses of 4CPTP in both MES and metrazol induced convulsions.

Table 1: Mean percentage of animals protected from MES and metrazol induced convulsions

S. no.	Drug	Dose (mg/kg body weight)	Mean percentage protection against convulsions	
			MES induced convulsions	Metrazol induced convulsions
1	4CPTP	40	50	40
2	4CPTP	60	70	60
3	4CPTP	80	80	60
4	4CPTP	100	90	100
5	Phenobarbitone	25	50	90
6	Phenobarbitone	50	100	100
7	Distilled water	0.5 ml	0	0

Table 2: Comparison of p values for different doses of 4CPTP and phenobarbitone for MES and metrazol induced convulsions

		MES induced convulsions		Metrazol induced convulsions	
		Phenobarbitone			
		25 mg/kg	50 mg/kg	25 mg/kg	50 mg/kg
4CPTP	40 mg/kg	>0.05	<0.05	<0.05	<0.05
	60 mg/kg	<0.05	<0.05	>0.05	<0.05
	80 mg/kg	<0.05	<0.05	>0.05	<0.05
	100 mg/kg	<0.05	<0.05	<0.05	-

Analgesic activity

As seen in table 3, morphine in doses of 5 mg and 10 mg produced statistically significant increase in mean reaction time of 86.21% and 161.06 % respectively. 25 mg of phenobarbitone significantly increased the reaction time by 6.61% whereas 50 mg of phenobarbitone on the contrary decreased the reaction time by 4.04 %. Although, 4 CPTP produced an increase in reaction time by 0.82%, 1.2 %, 1.56 % and 6.93% in graded doses of 40, 60, 80 and 100 mg respectively, but the results were statistically significant only at 100 mg/kg dose.

Table 3: Mean reaction time of animals using tail flick method before and after giving drug

S. no.	Drug	Dose (mg/kg)	Mean reaction time (in seconds)		Mean increase in percentage
			Before drug ± S.E.	After drug ± S.E	
1	Morphine	5	5.45± 0.28	10.15±0.312	86.21 (p<0.001)
2	Morphine	10	5.65±0.27	14.75±0.46	161.06 (p<0.001)
3	Phenobarbitone	25	5.90±0.38	6.29±0.26	6.61 (p<0.05)
4	Phenobarbitone	50	5.93±0.35	5.69±0.26	4.04 (p>0.05)
5	4CPTP	40	4.82±0.27	4.86±0.20	0.82 (p>0.05)
6	4CPTP	60	4.20±0.11	4.25±0.20	1.20 (p>0.05)
7	4CPTP	80	4.16±0.20	4.22±0.11	1.56 (p>0.05)
8	4CPTP	100	4.82±0.25	5.16±0.34	6.93 (p<0.05)
9	Distilled water	2 ml	5.54±0.06	5.59±0.08	0.72 (p>0.05)

DISCUSSION

In the present study, test compound 4CPTP was found to exhibit anticonvulsant activity in MES and metrazol induced seizures in mice in a dose dependent manner. The drugs which are effective against tonic hind limb extension induced by electroshock usually have been proven to be effective against partial and tonic clonic seizures in humans. Metrazol test, on the other hand, is most useful for identifying drugs that are effective against myoclonic seizures in humans.[2] Hence it can be predicted from this study that 4CPTP may be efficacious against generalised tonic clonic seizures, cortical focal seizures and myoclonic seizures in human patients. The test compound when given in 60-100 mg/kg dose, was found to have better anti convulsant action than 25 mg/kg of phenobarbitone. However when compared to higher doses (50 mg/kg) of phenobarbitone the test compound was found to be less efficacious.

Since the test compound is chemically similar to phenobarbitone and showed anti convulsant activity against both the MES and metrazol induced seizures, it can be assumed that these drugs may be acting by enhancing the GABAergic activity and opening the chloride channel.

The test compound was seen to produce statistically significant analgesic action only at 100 mg/kg dose. On the contrary, phenobarbitone shows hyperalgesic action at higher doses.

To the best of our knowledge, no other similar study could be found which assesses the anti convulsant and

analgesic actions of 4CPTP in experimental animals. Hence more number of animal studies are required to confirm these findings.

CONCLUSION

The test compound, 4 CPTP which has structural resemblance to phenobarbitone is seen to possess anti convulsant activity in experimental animals. It also exhibited analgesic activity which is contrary to hyperalgesic action of phenobarbitone.

REFERENCES

1. Tripathi KD. Anti epileptic drugs. 7th edition. New delhi: Jaypee brothers medical publishers; 2013. p. 258-81.
2. McNamara JO. Pharmacotherapies of the epilepsies. In: Brunton LL, Chabner BA, Knollmann BC, editors. Goodman and Gilman's The pharmaceutical basis of therapeutics. 12 th edition. New York: Tata Mc Graw Hill; 2011. p. 583-608.
3. Gupta AK, Sanjay, Kayath HP, Singh A, Sharma G, Mishra KC. Anticonvulsant activity of pyrimidine thiols. Indian Journal of Pharmacology 1994; 26: 227-8.
4. Castel-Branco MM, Alves GL, Figueiredo IV, Falcão AC, Caramona MM. The maximal electroshock seizure (mes) model in the preclinical assessment of potential new antiepileptic drugs. Methods Find Exp Clin Pharmacol 2009;31(2): 101-6.
5. Mandhane SN, Aavula K, Rajamannar T. Timed pentylenetetrazol infusion test: A comparative analysis with s.c. PTZ and MES models of anticonvulsant screening in mice. Seizure (2007) 16, 636-44.

Source of Support: None Declared
Conflict of Interest: None Declared