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EVALUATION OF ANALGESIC, ANTIPYRETIC AND ANTI-INFLAMMATORY ACTIVITY OF SPIROBARBITUNYLPHENOTHIAZINES IN RODENTS

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Abstract : Analgesic, antipyretic and anti-inflammatory activities of newly synthesized spirobarbitunylphenothiazines viz 10-[7,11-Di (4-4' dimethoxphenyl)-3-oxo-9-methylaminoimino-2, 4-diazaspiro [5.5] undecane 1, 5 dione] acetylphenothiazine (test drug A) and 10-[7, 11-Di (N.N - dimethylaminophenyl)-3-oxo-9-methylaminoimino-2, 4-diazaspiro [5, 5] undecane-1, 5 dione] acetylphenothiazine (test drug B) have been screened in Swiss mice and Wistar rats. The peripheral analgesic activity of test drugs A and B was investigated by acetic acid induced writhing test in Swiss mice while the central analgesic action was assessed by hot-wire (tail flick test) of the analgesiometer and tail-clip test in Wistar rats. Antipyretic activity was assessed on Brewer's yeast induced pyrexic model while antiinflammatory activity was seen on carrageenan induced hind paw oedema. Analgesic activity was found to be only of peripheral type as there was reduction of 66% in writhing responses by test drugs A and B in dose of 80 mg/kg in mice. No change in the tail flick responses was observed on analgesiometer or by tail clip by both the test drugs. Reduction of 1.5 to 2.0°C in the rectal temperature was observed in pyretic rats by test drugs A and B in dose of 80 mg/kg. 80% reduction in paw volume was noted in 80 mg/kg dose of both the test drugs which was comparable to the anti-inflammatory activity of 300 mg/kg, p.o. of phenylbutazone.

Key words : spirobarbitunylphenothiazines analgesic antipyretic anti-inflammatory

INTRODUCTION

Substituted phenothiazines have been reported to possess anti-inflammatory activity (1, 2, 3). Inflammation is an agonising reaction in the body and causes a lot of stress. Barbiturates are CNS depressant agents and produce sedation thereby suppressing the stress caused by agony of inflammation. Incorporating barbiturate moiety in the phenothiazine compound may therefore be thought to benefit the patients of inflammatory disorder. Furthermore it has been observed that subsitution at 2 and 10 position of phenothiazine by different alkyl, aryl and heterocyclic moieties may modulate the anti-inflammatory activities (4, 5, 6, 7).

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Such spirobarbitunylphenothiazines viz 10-[7,11-Di (4-4' dimethoxyphenyl)-3-oxo-9-methylaminoimino-2, 4-diazaspiro [5.5] undecance 1, 5 dione] acetylphenothiazine (test drug A) and 10-[7,11-Di(N,N-dimethylaminophenyl)-3-oxo-9-methylaminoimino 2,4,-diazspiro [5.5] undecance-1,5dione] acetylphenothiazine (test drug B) were therefore synthesized in the Medicinal Chemistry Division of our Department and were screened for analgesic, antipyretic and anti-inflammatory activities.

METHODS

Experimental animals: The experiments were conducted on Swiss mice (20–25 gm) and Wistar rats (100–150 gm) of either sex. The animals were kept in polypropylene cages and maintained on balanced ration with free access to clean drinking water. Animals were divided into eight groups of six mice/rats each.

Group 1: Treated with propylene glycol (2.0 ml/kg), i.p. and served as control group.

Group 2: Fed with known standard drug and

Groups 3 to 8: Treated with different doses of test drugs (20, 40, and 80 mg/kg, i.p.) A and B respectively.

The test drugs A and B were dissolved in propylene glycol (2.0 ml). The experiments were conducted at an ambient temperature of 25–27°C.

Determination of LD_{50}: The LD_{50} of these compounds was determined in Swiss mice and Wistar rats by the method of Paget and Barnes (8).

Analgesic activity: The peripheral analgesic activity of test drugs A and B was investigated by the acetic acid induced writhing test in Swiss mice (9, 10, 11). The movements were observed for the number and total duration after i.p. injection of 0.1 ml of a 0.6% solution of acetic acid. Aspirin suspended in 1% carboxymethylcellulose (200 mg/kg, p.o.) was used as standard drug for comparing analgesic effect at peripheral level. The central analgesic action of test drugs A and B was assessed by hot-wire of the analgesiometer (12) and tailclip test (13) in Wistar rats. Morphine sulphate injection (30 mg/kg, s.c) was used as standard drug for central analgesic effect. Test drugs A and B and aspirin were given 15 min and morphine 5 min before the noxious stimulus.

Antipyretic activity: The antipyretic activity of test drugs A and B was evaluated using Brewer's yeast induced pyrexia in Wistar rats (14). Fever was induced by injecting 2.0 ml/kg, (s.c) of 20% aqueous suspension of Brewer's yeast in normal saline below the nape of neck and rectal temperature was recorded by clinical thermometer. Prior to the experiment, the rats were maintained in separate cages for 7 days and the animals with approximately constant rectal temperature (38.5–39.1°C) were selected for the study. Aspirin suspended in 1% carboxymethylcelluose (200 mg/kg, p.o.) was used as standard drug for comparing the antipyretic action of test drugs A and B.

Anti-inflammatory activity: Animals were divided into eight groups comprising six animals in each group. In all groups, acute inflammation was produced by subplantar injection of 0.1 ml of freshly prepared 1% suspension of carrageenan in normal saline in right hind paw of the rats and paw volume was measured plethysmometrically at 0 and 3 h after carrageenan injection (15). Animals were premedicated either with propylene glycol (2.0 ml/kg, i.p) or test drugs A and B (20, 40 and 80 mg/kg, i.p.) or phenylbutazone (300 mg/kg, p.o) 30 min before carrageenan injection. Mean increase in paw volume was measured and percentage inhibition from control was calculated.

Statistical analysis: The results are presented as mean ± SEM. Statistical analysis of data was performed using student's 't' test to study the differences amongst the means (16).

RESULTS

LD_{50}: The approximate i.p. LD_{50} in Swiss mice and Wistar rats have been found to be 300 mg/kg and 800 mg/kg respectively.

Analgesic effect: In acetic acid induced writhing test, both the drugs A and B showed significant suppression in 40 and 80 mg/kg doses. Onset of writhing was delayed and was comparable to the suppression observed with 200 mg/kg, p.o. of aspirin (Table I). The duration of writhing was also shortened significantly but here only the 80 mg/kg dose of test drugs A and B was comparable to aspirin. The total number of writhing responses was also reduced from 48.42 ± 2.06 in control to 16.42 ± 2.16 and 15.12 ± 2.36 in the 80 mg/kg dose of test drug A and B respectively. The reaction time to noxious stimulus in analgesiometer was not significantly

TABLE I: Effect of spirobarbitunylphenothiazines (test drugs A and B) on acetic acid induced writing in Swiss mice (n = 6).

Drug	*Dose mg/kg, i.p.*	*Writhing*		
		Minutes		
		Onset	*Duration*	*Number*
Propylene glycol	2.0 ml	5.24±1.42	240.01±12.85	48.42±2.06
Aspirin	200, p.o.	15.10±1.34***	70.12±11.87***	12.24±2.18***
Test drug A	20	6.24±1.42	210.32±11.44	44.12±2.08
Test drug A	40	10.11±1.34*	180.12±11.72**	30.12±2.18**
Test drug A	80	12.62±1.18**	70±9.07***	16.42±2.16***
Test drug B	20	9.10±1.28*	190.12±11.72*	39.56±2.16*
Test drug B	40	11.23±1.24**	165.32±11.07**	35.22±2.18**
Test drug B	80	15.12±1.10***	75.34±10.98***	15.12±2.36***

Test drug A – 4, Methoxyphenylspirobarbitunylphenothiazine
Test drug B-N, N – Dimethylaminophenylspirobarbitunylphenothiazine.

*P<0.05, **P<0.01, ***P<0.001 in comparison with propylene glycol treated control group.

increased in rats by test drugs A and B in doses of 40 and 80 mg/kg. In tail clip test, test drugs A and B also failed to alter the reaction time significantly even at the dose 80 mg/kg.

Antipyretic effect: The experimental pyrexic rats showed a mean increase of 2°C in rectal temperature, 20 h after Brewer's yeast injection. The fall in rectal temperature was observed after 1 h in 40 mg/kg dose, while in 80 mg/kg dose, antipyretic activity was seen at 30 min also. The antipyretic activity of 80 mg/kg dose was comparable to that seen with aspirin 200 mg/kg, p.o. (Fig. 1).

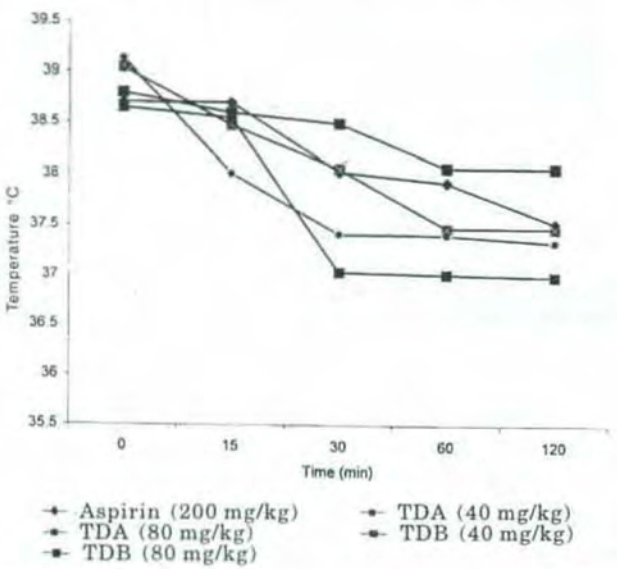

Fig. 1: Effect of spirobarbitunylphenothiazines (test drugs A and B) on Brewer's yeast indueed Pyrexia in Wistar Rals (n = 6).

Anti-inflammatory effect: The inhibition of carrageenan induced hind paw oedema was highly significant with phenylbutazone (300 mg/kg, p.o.) after 3 h of injection. The rats fed with test drugs A and B (20, 40 mg/kg) had no significant reduction in paw volume, while in the dose of 80 mg/kg test drugs A and B had significant reduction in hind paw oedema after 3 h of carrageenan injection (Fig. 2).

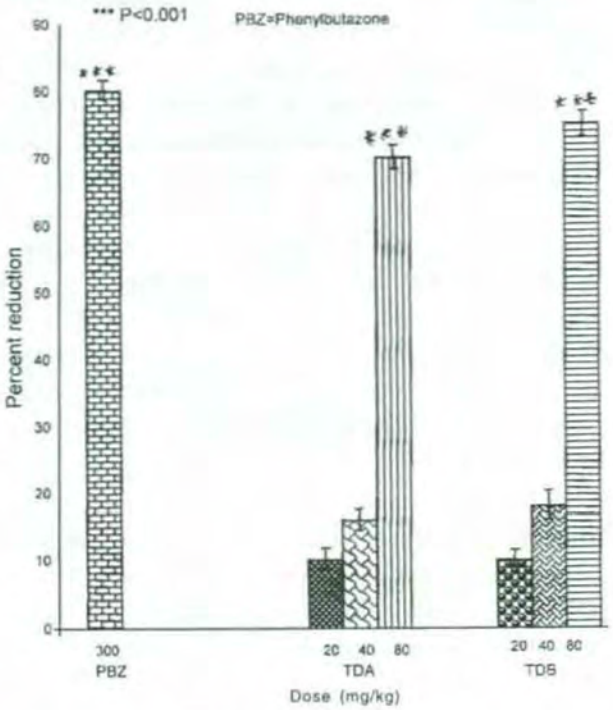

Fig. 2: Reduction of carrageenan induced hind paw oedema by spirobarbitunylphenothiazines (test drugs A & B) in Wistar rats (n = 6).

DISCUSSION

While neither the phenothiazines nor the barbiturates are known to possess analgesic and anti-inflammatory properties, our compounds (test drug A and B) showed both these activities in the experimental models employed. Both the drugs produced significant reduction in acetic acid induced writhing only in 80 mg/kg (Table I). However, there was no significant change in the reaction time either by tail flick or tail clip method, signifying that these are not the opioid type of analgesic. Since

inhibition of acetic acid writhing response is a pointer to the NSAIDs type of analgesic activity, our compounds (test drugs A and B) appear to have this type of analgesic activity. Furthermore the compounds (test drugs A and B) have been found to possess anti-inflammatory activity too. Both of them showed protective effect in carrageenan induced rat paw oedema in 3 h in 80 mg/kg dose. (Fig. 2). This further supports that the compounds are not of opioid type but NSAIDs type. On reviewing the structure of these compounds, it is noteworthy that they possess a carbonyl group at position 2 of the barbiturate ring. This carbonyl group is placed between the two electronegative amido nitrogens which favours lactam (Keto)-lactim (enol) tautomerization thus giving it an acidic character (17). The pKa shows close relationship with the biological activities of anti-inflammatory compounds such as salicylates, pyrazolones and indoles (18, 19). It is conjectured that acid character imparts the compound easy accessibility to the site of inflammation (20). Since our compounds (test drugs A and B) by tautomerization induce acidic character, they are bound to reach in sufficient concentration at the site of inflammation in the rat paw.

Phenothiazines possess antihistaminic and antiserotonic activities (21). Since histamine and serotonin are known mediators of inflammation (22), it can be thought that our compounds which possess phenothiazine moiety may be inhibiting oedema development by blocking the action of these mediators. However, this seems unlikely as inhibition of oedema by our compounds was not observed in the early phase of carrageenan induced oedema in which these mediators are known to act (22). Our compounds showed reduction in oedema at 3 h which corresponds to 2nd phase of development of oedema by carrageenan which is mediated by prostaglandins (23, 24). It therefore, appears that our compounds may be involved either in blocking the synthesis or action of prostaglandins. Direct evidence, however, is needed to establish the exact mechanism.

Both the compounds test drugs A and B exhibited antipyretic activity on pryrexia induced by Brewer's yeast in rats (Fig. 1). The onset of this antipyretic activity was 30 min and duration 2 h and coincided with those of analgesic activity. Since the onset and duration of antipyretic activity paralleled that of analgesic activity, the antipyrectic activity could not be due to phenothiazine moiety, which does not show analgesic activity. The antipyrectic activity thus appears to have similar mechanism as do the NSAIDs which have all the three components-analgesic, anti-inflammatory and antipyretic.

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