# ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF DOPAMINE ANTAGONISTS AND THEIR INTERACTION WITH ASPIRIN IN WISTAR RATS.

**Ravi Kumar, [1]P.A. Patil*, [1]Suneel.I.Majagi**

KLE College of Pharmacy and [1]Department of Pharmacology, J.N. Medical College,

Nehru Nagar, Belgaum-590010, Karnataka- India.

**Summary**

In the present study, analgesic and anti inflammatory activity of three antipsychotics viz, chlorpromazine, thioridazine and haloperidol as well as a non antipsychotic dopaminergic antagonist domperidone is studied in both acute and sub-acute model of inflammation in rats. The study also aimed to elicit the possible interaction of these dopamine antagonists with aspirin, in acute and sub-acute models of inflammation. All the dopamine antagonists in their therapeutic equivalent dose exerted significant anti-inflammatory activity.The sub anti-inflammatory (SAI) doses of chlorpromazine (2.25mg/kg), thioridazine (2.25mg/kg) and haloperidol (0.18mg/kg) domperidone (2.25mg/kg) when coadministered with SAI dose of aspirin (54mg/kg) showed significant anti-inflammatory activity in both acute and subacute model of inflammation. All the dopamine antagonists individually in their therapeutic equivalent dose and their SAI dose when coadministered with that of aspirin showed significant analgesic activity except haloperidol at 1st hour. Except thioridazine the combinations of chlorpromazine, haloperidol and domperidone with aspirin in their SAI dose did not produce significant gastric ulceration. Such an interaction of dopamine antagonists with NSAIDs like aspirin is worth exploiting clinically, if the present findings could be extrapolated to humans.

**Key words: Analgesia, Aspirin, Chlorpromazine, Domperidone, Haloperidol, Inflammation, Interaction, Thioridazine.**

***Corresponding author**: Dr. P.A.Patil, Professor,

Department of Pharmacology, J.N. Medical College,

Nehru Nagar, Belgaum-590010.

Karnataka- India. E-mail: drpapatil@yahoo.co.in

Phone: 0831-24091828,Fax: 08312470759.

## Introduction

Several drug combinations have been tried for treatment of pain and inflammation to ensure better effect with least toxicity. Long back, caffeine like stimulants were combined with aspirin to potentiate analgesic activity of the latter and still, such a combination appears to be a superior analgesic as compared to aspirin alone [1].Similarly phenobarbitone a CNS depressant is also well known to potentiate analgesic activity of aspirin [2] and therefore is used along with aspirin like drugs to treat severe pain. Other CNS depressants like diazepam could also be expected to exert such synergistic activity with aspirin like drugs. Morever, in contrast to phenobarbitone, diazepam has been reported to possess analgesic activity [3].It is not clear whether sedative activity contributes for analgesic activity.

Other CNS depressant like chlorpromazine which was used earlier as tranquillizer and sedative in place of diazepam could also be expected to potentiate the analgesic activity of aspirin like drugs if, CNS depressant activity was mainly responsible for potentiating analgesic effect.

Largactil, a popular brand name of chlorpromazine probably suggest a large number of its pharmacological actions. It has been shown not only to possess antipsychotic and other CNS activity (like sedative, antiemetic etc) but also some autonomic effects viz., adrenergic-alpha blocking, anticholinergic, anti 5-HT and dopaminergic blocking activity [4]. Chlorpromazine has been reported to possess week anti-inflammatory activity [5] and analgesic activity [6].While, some phenothiazine (8-trifluoromethyl derivative) have been reported to possess almost a dose dependent and anti-inflammatory activity [5].

It has been also reported that dopamine agonists like pergolide and bromocriptine possess anti-inflammatory activity [7].These reports about phenothiazines and dopamine agonists apparently appears to be paradoxical, since the phenothiazines block dopamine receptors. Literature survey in this regard indicates that there is paucity of information regarding anti-inflammatory and analgesic activity of various phenothiazines. The role of dopaminergic receptors in the pathogenesis of pain and inflammation is also not well established.

In the present study, therefore, three major tranquillizers known to block dopamine receptors viz., chlorpromazine and thioridazine, which are phenothiazine derivatives, haloperidol, a butyrophenone derivative which is chemically unrelated to earlier drugs and domperidone a dopamine antagonist without antipsychotic activity, have been investigated for their possible analgesic and anti-inflammatory activity. Chlorpromazine and thioridazine were selected on the basis of their differential action on the autonomic nervous system viz., chlorpromazine has potent alpha-adrenergic blocking action while thioridazine possesses antimuscarinic action. All these drugs were used in therapeutic equivalent dose, either alone or in combination with sub anti-inflammatory dose of aspirin in acute and sub acute model of inflammation in Wistar rats.

## Materials and methods

**Animals:** The complete course of experiments were carried out using healthy male rats of Wistar strain, weighing between 100-150 grams. The animals were acclimatized to normal laboratory conditions with 12-hr natural light-dark cycle and were maintained on standard laboratory diet with free access to water.

**Drugs used and their doses:** The adult clinical doses of the drugs were converted into rat equivalent doses with the help of converting table [8]. The drugs (with their adult therapeutic daily dose in parenthesis) used were chlorpromazine 4.5 mg/kg (50 mg), thioridazine 4.5mg/kg (50 mg), haloperidol 0.36 mg/kg (4 mg), domperidone 4.5 mg/kg (50 mg),aspirin 200 mg/kg (2 g). After confirming the anti-inflammatory activity with their therapeutic equivalent dose in carrageenan (acute) induced inflammation, a series of experiments were conducted to elicit their dose immediate next to effective dose that just failed to show anti-inflammatory activity and was taken as sub-antiinflammatory (SAI) dose. The SAI dose(mg/kg) were found to be 2.25 for chlorpromazine, thioridazine, domperidone,0.18 for haloperidol and 54 for aspirin. In acute studies all the treatments were administered to different groups of animals (n=6 in each) in a single dose, thirty minutes prior to subplantar injection of carrageenan while in sub acute studies the treatment was started after implanting the sterile foreign bodies and continued every 24 hours for 10 days. Control animals received equivalent volume of gum acacia suspension. All the drugs were administerd orally as a suspension with 1% gum acacia.

**Acute inflammation:** Overnight fasted (with water ad lib) animals were subdivided in to a control and 9 treatment groups to receive the dose (mg/kg) of, (i)aspirin 200,(ii)chlorpromazine 4.5,(iii)thioridazine 4.5,(iv)haloperidol 0.36,(v)domperidone 4.5.Remaining four groups received chlorpromazine 2.25 or thioridazine 2.25 or haloperidol 0.18 or domperidone 2.25 with aspirin 54. Acute inflammation was produced by subplantar injection of 0.05 ml of 1% carrageenan (from Sigma Co. St Louis) in left hind paw. A mark was put on the leg at the malleolus to facilitate uniform dipping at subsequent readings. The paw volume was measured with the help of plethysmograph by mercury displacement method at zero hour (immediately after injecting carrageenan). The same procedure was repeated at 1,3 and 6 hour. The difference between 0 hour and subsequent reading was taken as actual oedema volume.

**Subacute inflammation:** Subacute inflammation was produced by method D'Arcy et.al [9] with some modification. In overnight starved(with water ad lib) rats after clipping the hair in axillae and groin, under light halothane anaesthesia, two sterile cotton pellets weighing 10 mg were implanted subcutaneously, through a small incision. Wounds were then sutured and animals were caged individually after recovery from anaesthesia. Aseptic precautions were taken throughout the procedure. The animals were subdivided in to a control (vehicle) and a standard group (n=6 in each) to receive the dose (mg/kg) of aspirin 200 alone. Remaining groups received chlorpromazine 2.25 or thioridazine 2.25 or haloperidol 0.18 or domperidone 2.25 with aspirin 54. The treatments were started after implantation and were repeated every twenty four hours, regularly for ten days. On eleventh day the rats were sacrificed with an overdose of anaesthesia to remove cotton pellets, stomachs. The pellets, free from extraneous tissue were dried overnight at 60° C to note their dry weight. Net granuloma formation was calculated by substracting initial weights of cotton pellet (10mg) from the weights noted. Mean granuloma dry weight for various groups was calculated and expressed as mg/100 gm of body weight.

**Analgesic activity:** Janssen's caudal immersion test as described by Turner [10] was adapted for assessing analgesic activity. The test was carried out in animals subjected for carrageenan induced inflammation. The reaction time as indicated by complete withdrawal of tail, was noted in various treated groups at an interval of 1, 2 and 3 hour after drug administration to calculate the mean reaction time.

**Ulcer index:** Stomachs were cut open along the greater curvature and gently washed with normal saline. Gastric mucosa was examined for the presence of erosions, haemorrhagic spots, ulcer and perforation if any, with the help of magnifying lens. To determine the severity of the ulcer, an arbitrary scoring system as described earlier [11] was followed. Ulcer index was calculated as mean score of ulcer severity in all the treated groups and was compared with that of control. All the procedures were performed in accordance with the CPCSEA guidelines and the study was approved by IAEC.

**Statistical Analysis**: Data were expressed as Mean ± SEM and analysed by ANOVA followed by Dunnet's test and 'p' value equal to or $<0.05$ was considered as significant.

## Results

### Carrageenan induced acute inflammation:

Therapeutic equivalent dose of aspirin, chlorpromazine, thioridazine, haloperidol, domperidone as well as combination of SAI dose of these dopamine antagonists with that of aspirin significantly inhibited paw edema and increased mean reaction time (except haloperidol at 1 hour) to thermal stimulus (Table I).

### Sub acute inflammation (foreign body induced granulomas):

Therapeutic equivalent dose of aspirin and coadministration of SAI dose of chlorpromazine, thioridazine, haloperidol and domperidone individually with that of aspirin decreased mean granuloma dry weight significantly when compared with that of control (Table II).

### Ulcer Index:

Therapeutic equivalent dose of aspirin and combination of SAI dose of thioridazine with that of aspirin showed significant ($p<0.05$,$p<0.01$) increase in ulcer index, where as in other groups no significant change was observed (Table II).

**Table I: Effect of various treatments on carrageenan induced rat paw oedema and thermal pain (caudal immersion test).**

| Groups (n=6) | Drugs and Dose mg/kg | Paw volume in ml (Mean ± S.E) | | | Mean value in seconds (Mean ± S.E) | | |
|---|---|---|---|---|---|---|---|
| | | 1 Hr | 3 Hr | 6 Hr | 1 Hr | 2 Hr | 3 Hr |
| 1 | Control | 0.168 ±0.012 | 0.300 ±0.026 | 0.442 ±0.015 | 1.670 ±0.094 | 1.680 ±0.088 | 1.680 ±0.054 |
| 2 | Aspirin 200 | 0.108** ±0.010 | 0.117 *** ±0.025 | 0.167*** ±0.021 | 4.601 *** ±0.079 | 4.740 *** ±0.085 | 4.820*** ±0.082 |
| 3 | Chlorpromazine 4.5 | 0.050** ±0.029 | 0.027 ** ±0.043 | 0.087*** ±0.034 | 4.410* ±0.270 | 5.120 ** ±0.306 | 4.620 *** ±0.133 |
| 4 | Thioridazine 4.5 | 0.100* ±0.026 | 0.150** ±0.02 | 0.133*** ±0.031 | 4.510*** ±0.115 | 4.940*** ±0.187 | 4.840*** ±0.474 |
| 5 | Haloperidol 0.36 | 0.108** ±0.024 | 0.162** ±0.031 | 0.162*** ±0.024 | 4.030 ±0.146 | 4.330* ±0.199 | 4.671 ** ±0.242 |
| 6 | Domperidone 4.5 | 0.087* ±0.024 | 0.150*** ±0.013 | 0.200*** ±0.016 | 4.101* ±0.134 | 4.401** ±0.132 | 4.450*** ±0.138 |
| 7 | Chlorpromazine 2.25 with Aspirin 54 | 0.069*** ±0.016 | 0.106 *** ±0.022 | 0.087*** ±0.020 | 4.120** ±0.094 | 4.490** ±0.172 | 4.630*** ±0.135 |
| 8 | Thioridazine 2.25 with Aspirin 54 | 0.092** ±0.015 | 0.142*** ±0.024 | 0.183*** ±0.038 | 4.710*** ±0.172 | 4.710*** ±0.086 | 4.950 *** ±0.14 |
| 9 | Haloperidol 0.18 with Aspirin 54 | 0.114* ±0.021 | 0.158*** ±0.020 | 0.117*** ±0.015 | 4.270* ±0.203 | 4.520** ±0.246 | 4.550** ±0.177 |
| 10 | Domperidone 2.25 with Aspirin 54 | 0.087*** ±0.012 | 0.106*** ±0.017 | 0.169*** ±0.016 | 4.480** ±0.154 | 4.721*** ±0.192 | 4.801*** ±0.179 |

ANOVA followed by Dunnet's test, $p<0.05$*, $p<0.01$** and $p<0.001$***.

**TableII: Effect of various treatments on foreign body induced granulomas, ulcer index.**

| Groups (n=6) | Drugs and Dose mg/kg | Granuloma dry weight (mg/100 g. B.W) Mean± S.E. | Ulcer Index Mean± S.E. |
|---|---|---|---|
| 1 | Control | 75.60 ± 6.74 | 11.67±4.01 |
| 2 | Aspirin 200 | 42.25±1.21*** | 31.67± 4.77** |
| 3 | Chlorpromazine 2.25 with Aspirin 54 | 54.04± 2.30* | 21.67±7.49 |
| 4 | Thioridazine 2.25 with Aspirin 54 | 35.37±1.22*** | 28.33±4.77* |
| 5 | Haloperidol 0.18 with Aspirin 54 | 46.13±4.66** | 10.00±4.47 |
| 6 | Domperidone 2.25 with Aspirin 54 | 59.27± 1.06* | 21.00± 5.43 |

ANOVA followed by Dunnet's test, $p<0.05$*, $p<0.01$** and $p<0.001$***.

## Discussion

The results of the present study, clearly indicate that chlorpromazine, thioridazine, haloperidol and domperidone in their therapeutic equivalent dose significantly suppressed acute inflammation as well as showed significant analgesic effect in caudal immersion test. The analgesic and anti-inflammatory activities of chlorpromazine observed in the present study corroborates the findings of earlier reports [5,6].Earlier study on some phenothiazines indicate that 8-trifluoromethyl phenothiazine-1-carboxylic acid has dose dependent anti-inflammatory activity and chlorpromazine in high dose (20 mg/kg) also has moderate anti-inflammatory activity in UV induced erythema in guinea pigs[5]. A phenothiazine derivative like acylphenothiazine has also been reported to possess both anti-inflammatory and steroid like activity [12]. However, there is paucity of information regarding anti-inflammatory as well as analgesic activities of thioridazine, haloperidol and domperidone. Similarly all the dopamine antagonists suppressed subacute inflammation as did their SAI dose combination with that of aspirin. The synergistic interaction is obvious with single dose administration (carrageenan inflammation study) as well as with repeated administration (cotton pellet granulation study).

Chlorpromazine in the present study, either alone (4.5 mg/kg) or in combination with aspirin appears to be the most potent anti-inflammatory agent in acute inflammation, while thioridazine suppressed granuloma formation maximum (53.21%), as compared to others in subacute studies.

Apart from their therapeutic equivalent doses, SAI dose of chlorpromazine, thioridazine, haloperidol and domperidone when coadministered with that of aspirin showed significant analgesic effect in caudal immersion test. Experimental studies regarding analgesic activity of phenothiazines appear to be scanty. However, chlorpromazine has been reported to be useful in the treatment of cluster headache[6] and has been advocated as an adjunct to analgesics for selected refractory painful conditions [13,14]. Similarly analgesic efficacy of domperidone in the treatment of migraine [15] agrees with present finding. Based on the findings of the present study, it is rather difficult to comment on the mechanism of anti-inflammatory and analgesic action of the four dopaminergic antagonists used. These drugs could have more than one mechanism of action, either directly inhibiting the inflammatory mediators or by indirect mechanisms.

The anti-inflammatory effect of chlorpromazine and haloperidol has been attributed to modulation of monocytic as well as lymphocytic cytokines. Suppression of IL-2, IFN gamma, superoxide anion production and proinflammatory cytokines [16,17]. In addition to antihistaminic and adrenergic alpha blocking activity of chlorpromazine shared by haloperidol[18] and domperidone [19,20] could be responsible for their anti-inflammatory activity through suppression of prostaglandin synthesis, since α1 as well as α2 adrenergic receptor activation increases PG formation [21].Other proposed anti-inflammatory mechanisms of neuroleptics include prolactin release [22].

Though dopamine receptors have been suggested to exist in peripheral tissues like cholinergic neurons [23] in addition to vascular smooth muscle, their role in the pathogenesis of inflammation is not clearly understood. Morever their role appears to be controversial in pathogenesis of inflammation, since both dopamine agonists, pergolide [7] and dopamine antagonist chlorpromazine [5] have been reported to be anti-inflammatory. However in the present study contribution of dopamine blocking property for their anti-inflammatory activity can not be totally ruled out.

The nature of interaction appears to be pharmacodynamic rather than pharmacokinetic since no such kinetic interactions could be traced in the literature. Since serum levels of aspirin and various dopamine antagonists have not been monitored, pharmacokinetic interaction between these drugs can not be ruled out.

Combination treatment of SAI doses of chlorproamazine, haloperidol and domperidone with that of aspirin appears to be less ulcerogenic to the gastric mucosa as observed in the present study. But combination treatment of SAI dose of thioridazine with that of aspirin produced significant gastric ulcer which was almost comparable to that of aspirin (200mg/kg).Contrary to the expectation, present findings indicating non ulcerogenecity of domperidone and chlorpromazine agree with earlier studies [24,25] while ulcerogenic potential of thioridazine as observed in the present study differs from an earlier report [26].The discrepancy could be due to stress ulcer model used in the earlier study and aspirin coadministration in the present study. Probably stronger anticholinergic activity of thioridazine could have potentiated ulcerogenicity of aspirin by retaining the latter in stomach for longer time.

The findings of the present study clearly establish the analgesic and anti-inflammatory activity of all the four dopamine antagonists used in their moderate clinical doses and their synergistic activity with SAI dose of aspirin. The advantage with such combinations is insignificant gastric ulceration and such combination for the treatment of pain and inflammation could be safer in some selected individuals not tolerating or responding to the therapeutic doses of aspirin, provided the observations of the present study could be extrapolated to human beings. However, clinical studies are worth while to establish the efficacy of such combinations.

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