THE INFLUENCE OF SOME ANTIDEPRESSANTS ON INFLAMMATION IN ALBINO RATS

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Summary

The Objective of the present study was to evaluate the anti-inflammatory activity of chemically unrelated antidepressants with different mechanism of action viz. amitriptyline, fluoxetine, maprotiline and mianserin. Amitriptyline, fluoxetine, maprotiline and mianserin in their clinical equivalent doses and aspirin in the dose 200 mg/kg were administered orally in different groups of Wistar rats weighing 175±25g to study their effect on acute inflammation induced by carrageenan and subacute inflammation induced by subcutaneous implantation of foreign bodies in the axillae/groin. The animals were administered a single dose of various drugs in acute studies and the treatment was repeated once daily for 10 days in subacute studies. On 11th day granulomas were dissected out for noting their dry weight and histopathological studies. Amitriptyline and mianserin treated groups decreased significantly ($P<0.05$) pedal edema, granuloma dry weight and granuloma formation indicating significant anti-inflammatory activity. Conversely the fluoxetine group showed increased paw edema and no effect on granuloma formation while, maprotoline produced no significant anti-inflammatory activity in both the models of inflammation.

Key words: Amitriptyline, Aspirin, Fluoxetine, Inflammation, Maprotoline, Mianserin.

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Introduction

Inflammation, a spontaneous biological reaction to variety of noxious stimuli of varied nature including local injury, involves a complex interaction of various chemical mediators like histamine, serotonin, chemotactic factors etc. [1] Therefore variety of drugs that antagonizes the chemical mediators could be effective to alleviate the signs of inflammation. The list of anti-inflammatory agents appears to be endless like non steroidal anti-inflammatory drugs, glucocorticoids etc. It has been reported that endogenous catecholamine epinephrine possess anti-inflammatory activity [2],[3],[4],[5] and beta blockers potentiate the anti-inflammatory activity of adrenaline.[6] Therefore, it is obvious that drug modifying sympathetic activity could influence the process of inflammation. In support of this view, catechol-o-methyl transferase (COMT) inhibitors like pyrogallol and monoamine oxidase (MAO) inhibitors like nialamide have been reported to possess anti-inflammatory activity.[2]

Most of the antidepressants used in clinical practice are well known to influence adrenergic and serotonergic reuptake mechanisms and mianserin like drugs may not influence these. Amitriptyline inhibits norepinephrine (NE) as well as serotonin reuptake equally,[1] while clomipramine [1], [7] fluoxetine [7], [8] inhibits serotonin reuptake selectively and maprotiline, [9] desipramine [1] selectively inhibit NE reuptake. The interference of these antidepressants with reuptake of NE, serotonin or both has been extensively studied on central nervous systems [11],[12],[13],[14] and a study indicates similar effects of these antidepressants in the periphery also.[9]

Due to such interactions with the neurotransmitter reuptake in the periphery, they could be expected to influence the process of inflammation. Literature survey in this regard indicates that antidepressants like desipramine, nortripytline, amitriptyline and imipramine inhibit inflammatory oedema in rats.[15] Moreover, there appears to be a controversy regarding anti-inflammatory activity of beta blockers. A study reported that beta blocking agents potentiate the anti-inflammatory activity of adrenaline on carrageenan oedema,[6] while propranolol has been shown to prevent the anti-inflammatory activity of isoprenaline in similar study.[4] Surprisingly, sympatholytics like reserpine, guanethidine, alpha methyl dopa and propranolol also have been reported to possess anti-inflammatory activity in carrageenan inflammation and beta blockers reverse the anti-inflammatory activity of adrenaline in dextran induced inflammation. [16]

In view of above reports it is obvious that the role of catecholamine in modulating the inflammatory process does not appear to be clearly understood. Moreover various antidepressants used in clinical practice possess varying effect on NE reuptake.

Therefore, the anti-inflammatory activity reported for some of the antidepressants is not a common property to all of them. Hence the present study was planned to detect the anti-inflammatory activity if any, of chemically unrelated antidepressants that differ in their mechanism of action viz. amitriptyline, fluoxetine, maprotiline and mianserin.

Materials and methods

Animals: Adult male healthy Wistar rats weighing 175 ±25 g were obtained from the central animal house, J.N.Medical College Belgaum and were acclimatized to 12:12 h light - dark cycle for 10 days prior to the day of experimentation. They were maintained on standard rat chow pellet (Amrut Brand) and water *ad libitum.* The study was approved by the IAEC constituted as per the guidelines of CPCSEA, New Delhi.

Acute inflammation: Overnight starved rats with free access to water were divided into several groups (n=6 in each) to receive various treatments. Calculated clinical equivalent doses (in mg/kg) of 15.3 amitriptyline, 4.5 fluoxetine, 10 maprotiline, 5.4 mianserin and 200 of aspirin in 2% gum acacia suspension as vehicle were administered orally in a single dose while, the control group received 0.5ml of 2% gum acacia suspension orally.

Thirty minutes after vehicle and aspirin, 5 hours after amitriptyline and fluoxetine, 7 hours after maprotiline and one hour after mianserin administration, 0.05 ml of 1% carrageenan in normal saline was injected into the sub plantar region of the left hind paw, as per the technique of Winter et al.[17]
A mark was put at the malleolus to facilitate uniform dipping at subsequent readings. The paw edema was measured with the help of plethysmograph by mercury displacement at zero h (immediately after injecting carrageenan) and the procedure was repeated at 0.5,1,2,4 and 6 h.

The percentage inhibition of edema was calculated using formula $=1-\frac{Vt}{Vc} \times 100$

Vt and Vc were edema volume in drug treated and control groups respectively.

Analgesic activity: All the animals employed for carrageenan induced paw edema study were utilized to elicit the analgesic activity of different drugs. After restraining the animal, the tail upto the base was dipped in hot water bath maintained at 55±0.5o C. Time required for withdrawal of tail (reaction time) in seconds was noted at 1, 2, 4 and 6h after various treatments. Cut off time was 15 seconds. The mean reaction in various groups was then analyzed.

Subacute inflammation: Rats were divided into several groups of six in each. After clipping the hair in axillae and groin, under light halothane anesthesia, two sterile cotton pellets weighing 10 mg each and two sterile grass piths (25X2mm each) were implanted randomly, subcutaneously through a small incision. Wounds were sutured and animals were then caged individually after recovery from anesthesia. Aseptic precautions were taken throughout the experiment. The rats then received the antidepressants orally in

clinical equivalent dose as mentioned in acute studies. The treatment was started on the day of implantation and repeated every 24 hours for 10 days. On eleventh day, the rats were sacrificed with an overdose of anesthesia to remove the cotton pellets and grass piths. The pellets, free from extraneous tissue were dried overnight at 60ºC to note their dry weight. Net granuloma formation was calculated by subtracting the initial weight of cotton pellet from the weights noted. Mean granuloma dry weight for various groups were calculated and expressed in mg/100g body weight. The grass pith granulomas were preserved in 10% formalin for histopathological studies.

Statistical analysis: Data expressed as mean ± SEM was analyzed by one-way ANOVA followed by Dunnet's post hoc test and P values ≤ 0.05 was considered significant.

Results

Acute studies: As expected, aspirin significantly ($P<0.05$) reduced paw edema as compared to the controls throughout the observation period. Amitriptyline (15.3mg/kg) and mianserin (5.4mg/kg) significantly suppressed paw edema at 4^{th} and 6^{th} hour. Conversely fluoxetine (5.4mg/kg) increased while maprotoline (10mg/kg) failed to alter significantly the paw edema.(Table 1)

There was significant ($p<0.01$) delay in reaction to thermal stimulus in aspirin, amitriptyline, maprotiline and mianserine group.(Table 2)

Subacute studies: Mean granuloma dry weight (mg % body weight) in aspirin, amitriptyline and mianserin groups were significantly ($P<0.05$) lower than control indicating their anti-inflammatory activity. While maprotiline and fluoxetine did not show significant suppression of granuloma formation. (Table 1)

The granulation tissue sections stained with haematoxylin and eosin revealed a marked reduction in thickness of granulation tissue, collagen content and fibroblast number as compared to control in aspirin, amitriptyline and mianserin treated groups (Figure 1).

TABLE 1: EFFECT OF VARIOUS TREATMENTS ON ACUTE AND SUBACUTE INFLAMMATION

Treatment Group	Paw edema volume (ml)				Granuloma dry weight (mg % body weight)
	Mean ± SEM				
	1 hr	2 hr	4 hr	6 hr	
Vehicle	0.19±0.01**	0.24±0.02	0.36±0.01	0.42±0.03	43.91±1.33
Aspirin(200mg)	0.1±0.01*	0.11±0.01*	0.14±0.01*	0.16±0.02*	24.33±1.46*
Amitriptyline(15.3mg)	0.12±0.01	0.14±0.01	0.20±0.02*	0.22±0.02*	33.11±3.35*
Fluoxetine(4.5mg)	0.20±0.01	0.26±0.02	0.40±0.02	0.45±0.02	44.62±2.01
Maprotiline(10mg)	0.16±0.01	0.20±0.01	0.30±0.03	0.34±0.03	40.06±1.63
Mianserine(5.4mg)	0.13±0.01	0.15±0.01	0.22±0.02*	0.24±0.02*	33.25±3.01**

**P<0.05, *P<0.01 as compared to control

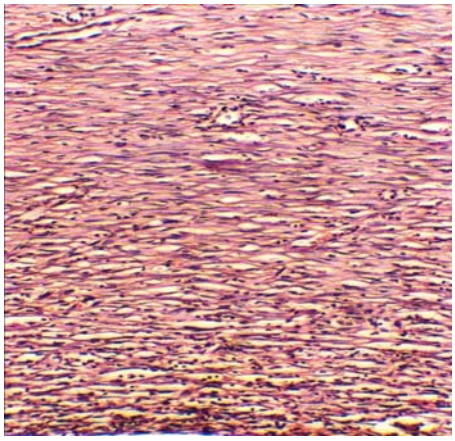
TABLE 2. EFFECT OF VARIOUS TREATMENTS ON REACTION TIME TO THERMAL PAIN (CAUDAL IMMERSION TEST)

Treatment Group	Reaction time in seconds (Mean ± SEM)			
	1 hr	2 hr	4 hr	6 hr
Vehicle	1.67±0.05	1.68±0.06	1.67±0.06	1.69±0.05
Aspirin(200mg)	2.81±0.05*	3.08±0.10*	3.01±0.14*	2.70±0.06*
Amitriptyline(15.3mg)	2.49±0.05*	2.65±0.08*	2.82±0.08*	3.20±0.13*
Fluoxetine(4.5mg)	1.70±0.13	1.80±0.12	1.71±0.01	1.70±0.05
Maprotiline(10mg)	2.40±0.10*	2.61±0.15*	2.79±0.20*	2.75±0.03*
Mianserine(5.4mg)	2.25±0.12*	2.33±0.07*	2.55±0.03*	2.72±0.10*

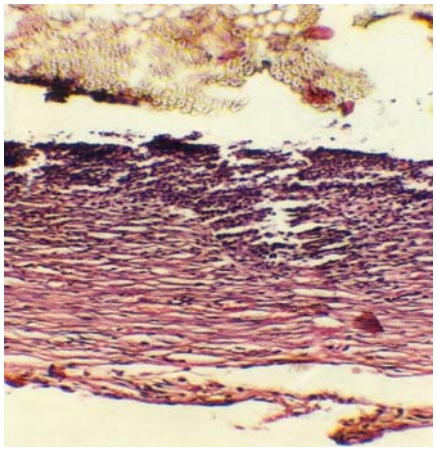
*P<0.01, as compared to control

FIGURE 1. PHOTOMICROGRAPHS OF GRANULATION TISSUE STAINED WITH H & E (100X)

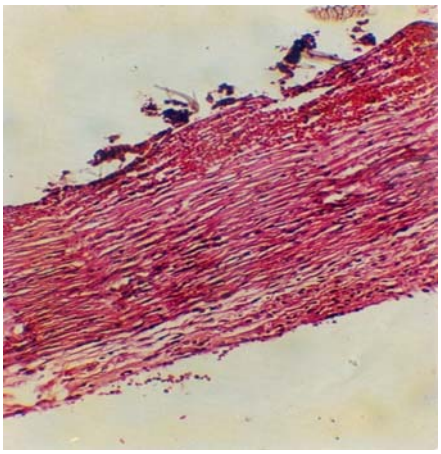
Control



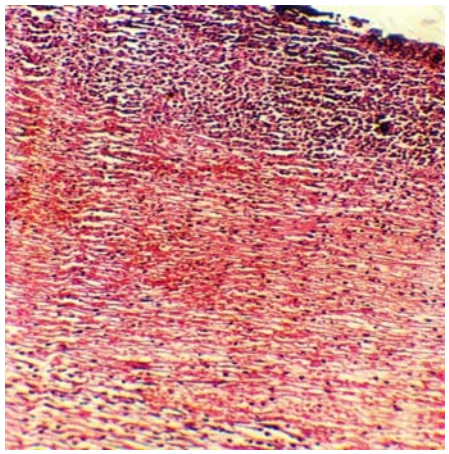
Aspirin (200mg/Kg b.w)



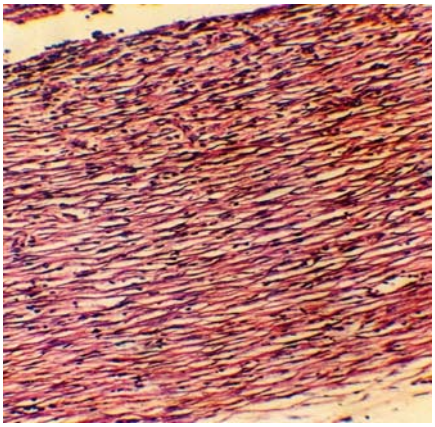
Amitriptyline (15.3mg/Kg b.w)



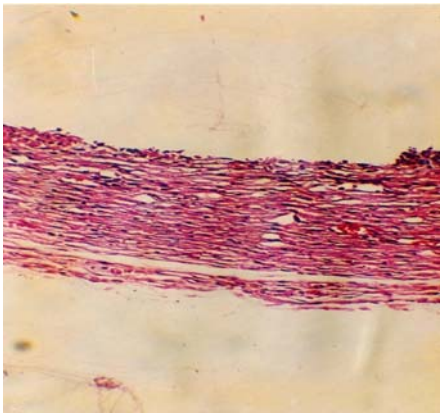
Fluoxetine (4.5mg/Kg b.w)



Maprotiline (10mg/Kg b.w)



Mianserin (4.5mg/Kg b.w)



As compared to control decreased granulation tissue, collagen content and fibroblast number in aspirin, amitriptyline and mianserine treated groups.

Discussion

Therapeutic equivalent doses of four commonly used antidepressants having different mechanism of action were investigated for their possible anti-inflammatory and analgesic activity. Amitriptyline and mianserin showed significant anti-inflammatory as well as analgesic activity. Maprotiline showed significant analgesic activity whereas fluoxetine showed neither anti-inflammatory nor analgesic activity.

Amitriptyline is a well known inhibitor of neuronal reuptake of NE and 5HT in CNS [1] and this might be true for peripheral neurons also. In fact, the enteric neuronal system in the periphery has been shown to have close resemblance with central neurons in their 5HT reuptake in the presence of fluoxitene like drugs [7] therefore, amitriptyline could be expected to influence amine reuptake in the periphery as it does in the CNS. Its anti-inflammatory activity could be explained on the basis of increased sympathetic activity since catecholamine like epinephrine and nor-epinephrine and isopenaline have been reported to possess anti-inflammatory activity.[4] Atropine like activity of amitriptyline might also contribute for its anti-inflammatory activity since atropine has been shown to antagonize the prostaglandin E induced vascular permeability,[18] a crucial event in the pathogenesis of inflammation. Moreover methanetheline, – an atropine like drug has been shown to be a strong anti-inflammatory agent against chemical inflammation in rats. [19] The antiinflammatory activity of amitriptyline could also be due to inhibition of PG synthesis, as reported earlier.[20] Decrease in the uptake of 5HT by neurons due to amitriptyline could be expected to augment the inflammatory process since 5HT is a known mediator of inflammation. The observed anti-inflammatory activity of amitriptyline in the present study could be due to relatively lower density of 5HT neurons in the periphery. Moreover the exogenously administered 10mg/kg of 5HT has been reported to exert anti-inflammatory activity.[21] It also has been reported to possess the H_1 receptor antagonistic activity in rats brain [8] if this is true in the periphery also then it clearly explains the anti-inflammatory activity of amitriptyline. Its analgesic activity appears to involve dual mechanism- peripheral PG antagonism as well as PG synthesis inhibition and increased 5HT activity in CNS which has been implicated with the analgesic activity.[22]

Failure of fluoxetine to exert analgesic and anti-inflammatory activity in the present study could be explained on the basis of its selective effect on the neuronal uptake of serotonin. Its poor antimuscarinic activity might indicate its negligible antiprostaglandin activity and since it is not a tricyclic, it may not affect PG synthesis like amitriptyline. Ligand binding study has clearly shown that fluoxetine is a weaker H_1 antagonist[8] in contrast to amitritpyline thus explains its absence of anti-inflammatory activity. The role of 5HT receptors in modulating analgesic activity appears to be controversial, thus increased brain 5HT concentrations inhibits morphine analgesiain mice and this inhibition was reversed with 5HT antagonist methysergide [23] while intraventricular injection of 5HT in mice significantly increased antinociception action of number of narcotic analgesic.[24]

Failure of maprotiline to exert significant anti-inflammatory activity is difficult to explain despite its selective inhibitory effect on the neuronal NE uptake should have produced significant anti-inflammatory activity in contrast to the observed effect in the present study. Therefore it appears that NE uptake inhibition may not be a main contributing factor for anti-inflammatory activity.

It has been reported earlier that endogenous catecholamines are not involved in the anti-inflammatory activity of desipramine ,a selective NE uptake inhibitor having significant anti-inflammatory activity.[4] The discrepancy in the anti-inflammatory activity of desipramine and maprotiline could be explained by tricyclic structure of desirpamine due to which the drug inhibits PG synthesis.[20] Its weak anticholinergic activity as compared to that of amitriptyline might also explain its negligible anti-inflammatory activity. Analgesic activity of maprotiline in absence of its anti-inflammatory activity is difficult to explain. Whether its α_2 adrenoceptor blocking property [11] or the unbalance between NE and 5HT turn over weight caused by it are responsible for its observed analgesic activity is a mere speculation. Endogenous opioids have been suggested to be involved antinociceptive activity of tricyclic antidepressants.[26] Such possibility with maprotiline might explain its analgesic activity.

Mianserin which does not affect significantly NE and 5HT uptake has produced significant analgesic and anti-inflammatory activity. It appears to be more reasonable to assign its anti-inflammatory activity to its reported antihistaminic property. [27] In view of paucity of reports regarding its analgesic activity, it is very difficult to hypothesize the mechanism underlying.

The analgesic activity of some of the antidepressants observed [16],[28],[29],[30] in clinical practice appears to be unrelated to their antidepressants activity according to the present study. The present finding therefore indicate the analgesic and anti-inflammatory use of some of the antidepressants in clinical practice, provided the present study could be extrapolated to the human beings. Clinical studies in this regard are worthwhile.

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