OPIOID ANALGESICS POTENTIATE THE ANTI-INFLAMMATORY AND INHIBIT ULCEROGENIC ACTIVITY OF ASPIRIN.

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Summary

The common recommendation of opioids to treat non inflammatory pains may not be rational in view of reported literature regarding anti inflammatory activity of morphine. In the present study morphine (a mixed agonist), pethidine (predominantly μ agonist) and pentazocine (powerful k agonist) have been investigated for their possible anti-inflammatory and ulcerogenic activity in normal as well as in aspirin treated albino rats for any interactions. Aspirin in the dose of 200 mg/kg and 54 mg/kg p.o., Morphine in the dose of 4.5 mg/kg and 1.5 mg/kg i.m., Pethidine in the dose of 45 mg/kg and 15 mg/kg i.m., Pentazocine in the dose of 13.5 mg/kg and 4.5 mg/kg i.m., were administered in different groups of albino rats to study their effect on inflammation induced by carrageenan or a foreign body. Gastric mucosal studies were also carried out. Therapeutic equivalent doses of these opioids produced significant anti inflammatory activity, almost comparable to that of Aspirin (200 mg/kg).The sub anti-inflammatory dose of opioids potentiated the anti-inflammatory activity of aspirin. The similar dose of these opioids when combined with 200mg/kg and 54 mg/kg of aspirin produced significant gastric ulceration as compared to control, but reduced significantly the ulcerogenic effect of aspirin. Keeping in mind the dependence potential of these opioids, further clinical trials are necessary to establish the efficacy of such combination in the treatment of inflammation.

Key words: Aspirin, Inflammation, Morphine, Pentazocine, Pethidine, Ulcer protection.

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Introduction

The differentiation of opioid analgesics from aspirin like agents on the basis of innate anti-inflammatory activity of the latter ones appears to be incorrect in view of reported literature about morphine. Morphine has been reported to inhibit prostaglandin responses in intestinal smooth muscle [1], and to inhibit the carrageenan induced oedema, probably by antagonising bradykinin [2].There are several reports suggesting that morphine stimulates prostaglandin biosynthesis in different animals [3,4] and protects gastric mucosa against damage, induced by different agents like indomethacin [5], ethanol [6], platelet activating factor [7], 0.6 N HCl and 0.2 N NaOH [8] and stress [9]. There appears to be a controversy regarding the effect of morphine on gastric mucosa, since morphine has been reported to potentiate the drug induced gastric damage[10].Such damage has been hypothesized, probably due to enhanced leukotriene C4, decreased prostaglandin activities and inhibition of the protective neuropeptides released in the gastric mucosa [10].

In fact, commercial preparations of dextro-propoxyphene hydrochloride (Butaproxyvon) in combination with non steroidal anti-inflammatory drugs (NSAIDs) like diclofenac sodium or acetaminophen have been recommended to treat severe pains that are not relieved by NSAIDs[11] alone. In fact additive analgesic effect of preparations containing a NSAID and an opioid has been observed in patients [12].

However, the efficacy of such combinations involving various opioids and NSAIDs in the treatment of inflammatory disorders is not clearly documented. Though morphine has been investigated for its anti-inflammatory, gastroprotective and ulcerogenic activity, similar information regarding other commonly used opioids viz., pethidine and pentazocine appears to be scanty. Therefore in the present study morphine (a mixed agonist),pethidine (predominantly μ agonist) and pentazocine (a powerful k agonist), have been investigated for their effect on acute and subacute inflammation as well as gastric mucosa and their interaction with aspirin a commonly used NSAID in male Wistar rats.

Materials and methods

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

Drugs used and their doses: 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 immediately 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 54,1.5,15,4.5 for aspirin, morphine, pethidine hydrochloride and pentazocine hydrochloride respectively.

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. Aspirin was administered orally while opioids were injected in gluteal muscles.

Acute inflammation: Overnight fasted (with water ad lib) animals were subdivided in to a control and 7 treatment groups to receive the dose (mg/kg) of (i) aspirin 200, (ii) morphine 4.5, (iii) pethidine 45, (iv) pentazocine 13.5. Remaining three groups received morphine 1.5 or pethidine 15 or pentazocine 4.5 in addition to 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 [13] 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 and two sterile grass piths (25x 3 mm) 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 experiment. The animals were divided in to 8 groups (n=6 in each), two groups received vehicle (control) or aspirin 200 mg/kg (standard).Remaining 6 groups received aspirin dose 54 mg/kg (3 groups) or 200 mg/kg (3 groups).One each from these treatment groups also received morphine 1.5 mg/kg or pethidine 15 mg/kg or pentazocine 4.5 mg/kg. The treatments were started on the day of implantation and was repeated every twenty four hours, regularly for ten days.

On the eleventh day the rats were sacrificed with an overdose of anaesthesia to remove cotton pellets, grass piths and 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. The grass piths were preserved in 10% formalin for histopathological studies.

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 [14] 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 <0.05 was considered as significant.

Results

Table I: Effect of various treatments on carrageenan induced rat paw oedema.

Groups (n=6 in each)	Drugs and Dose (mg/kg)	Paw volume in ml (Mean±S.E)		
		1 Hr	3Hr	6Hr
1	Control (2% gum acacia)	0.141±0.027	0.330±0.042	0.483±0.030
2	Aspirin 200	0.070±0.013*	0.150±0.033*	0.266±0.033*
3	Morphine 4.5	0.050±0.018*	0.116±0.035*	0.175±0.049*
4	Pethidine 45	0.108±0.045*	0.183±0.061*	0.233±0.060*
5	Pentazocine13.5	0.133±0.033	0.166±0.042*	0.158±0.041*
6	Morphine 1.5 +Aspirin 54	0.075±0.024*	0.050±0.017*	0.041±0.015*
7	Pethidine 15 +Aspirin 54	0.050±0.018*	0.033±0.010*	0.016±0.019*
8	Pentazocine 4.5 +Aspirin 54	0.025±0.013*	0.050±0.009*	0.050±0.009*

ANOVA followed by Dunnet's test, *p<0.001.*Significant anti-inflammatory activity in all the treated groups.

Table II: Effect of various treatments on foreign body induced granulomas and ulcer index.

Groups (n=6 in each)	Drugs and Dose (mg/kg)	Mean granuloma dry weight(mg/100 gB.W) Mean± S.E.	Ulcer Index Mean± S.E.
1	Control (2% gum acacia)	28.330±0.812	11.660±0.693
2	Aspirin 200	10.160±0.584*	53.550±0.926
3	Morphine 1.5 +Aspirin 54	9.160±0.584*	20.000±1.155*
4	Pethidine 15 +Aspirin 54	9.150±0.612*	16.660±0.626*
5	Pentazocine 4.5 +Aspirin 54	8.300±0.612*	21.660±0.693*
6	Morphine 1.5 +Aspirin 200	8.150±0.629*	30.000±0.516*
7	Pethidine 15 +Aspirin 200	7.430±0.624*	18.330±0.589*
8	Pentazocine 4.5 +Aspirin 200	5.630±0.402*	30.000±0.516*

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

All the combination groups significantly increased the ulcer index when compared with control but significantly decreased the ulcer index when compared with that of aspirin alone treated group.

Carrageenan induced acute inflammation:

Effects of therapeutic equivalent dose of aspirin, morphine, pethidine and pentazocine individually, as well as, combination of SAI doses of these opioids with SAI dose of aspirin on acute inflammation are shown in Table I. Except pentazocine (13.5 mg/kg) at first hour, all the treated groups showed significant ($p<0.001$) reduction in the paw volume when compared with that of control at 1, 3 and 6 hours.

Sub acute inflammation (foreign body induced granulomas):

Effects of therapeutic equivalent dose of aspirin, combination of SAI doses of morphine, pethidine as well as pentazocine with that of aspirin, and SAI dose of these Opioids with anti inflammatory dose of Aspirin on mean granuloma dry weight (mg/100 g. B.W) are shown in Table II. All the treatment groups showed significant ($p<0.001$) reduction in mean granuloma dry weight.

Histopathological studies:

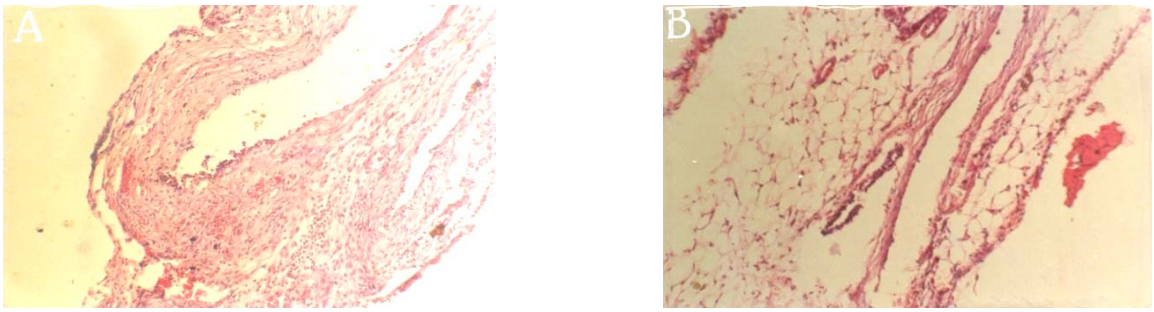
The grass piths of all the eight groups used in the sub acute inflammation studies were subjected to histopathological studies (Fig I). The histological study of the granulomas revealed a decrease in the thickness of granulation tissue, vascularity, collagen content and the fibroblast number in all the treated groups when compared with that of control group, confirming their anti inflammatory property.

Ulcer Index:

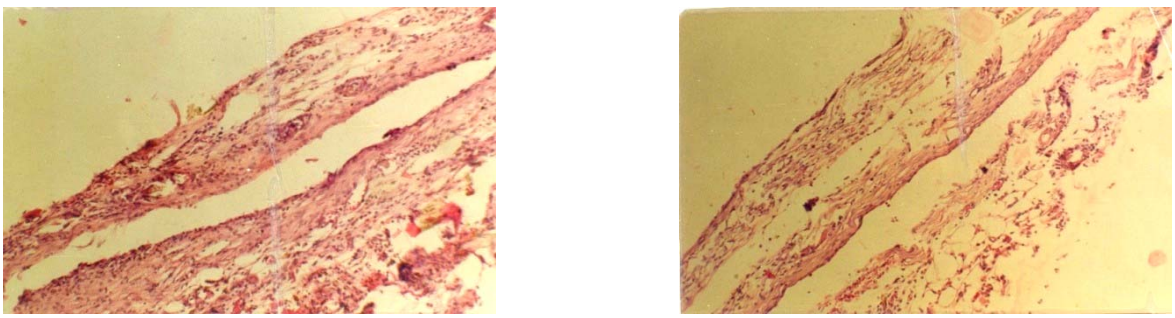
Similarly effects of anti inflammatory dose of aspirin, combination of SAI doses of morphine, pethidine and pentazocine with that of aspirin, as well as with anti inflammatory dose of aspirin on ulcer index are shown in Table II. All the combination groups showed significant ($p<0.001$) increase in the ulcer index when compared with control but showed significant ($p<0.001$) decrease in the ulcer index when compared with that of aspirin alone treated group.

Figure 1 . Microphotographs of granulation tissues stained with H&E (100X)

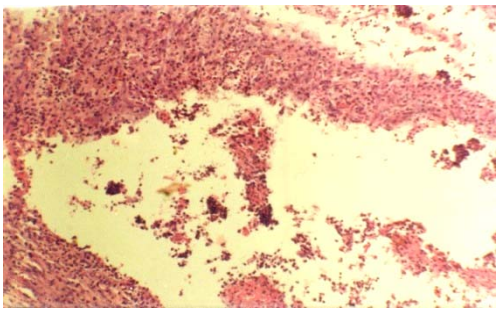
a.Control (vehicle) b.Aspirin (200 mg/kg)

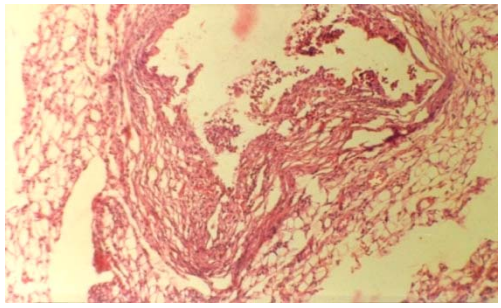


c. Morphine (1.5mg/kg) with Aspirin(54 mg/kg) d.Pethidine (15mg/kg) with Asprin (54 mg/kg)

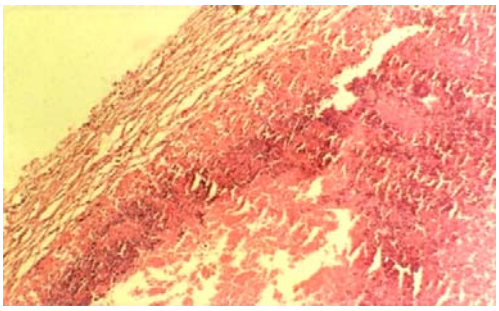


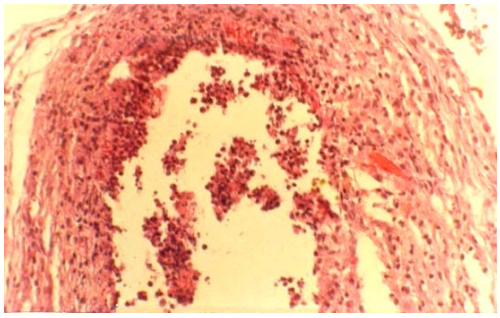
e. Pentazocine (4.5mg/kg) with Aspirin(54mg/kg) f.Morphine(1.5mg/kg) with Aspirin(200mg/kg)





g. Pethidine(15mg/kg) with Aspirin(200mg/kg) h. Pentazocine(4.5mg/kg) with Aspirin(200mg/kg)





Note: Markedly decreased vascularity, fibroblast number and collagen content in all treated groups when compared with control.

Discussion

Results of the present study indicate that therapeutic equivalent doses of morphine, pethidine and pentazocine possess significant anti inflammatory activity. When coadministered with aspirin all of them potentiated anti-inflammatory activity of the latter. The present findings agree with the earlier studies, reporting dose dependent anti inflammatory activity of morphine [2] and significant anti inflammatory activity of pethidine in similar model of inflammation [15]. There is paucity of information about similar activity of pentazocine and interaction studies between different opioids and aspirin.

Physical quantification of 10 day old granuloma and their histopathological studies support the anti inflammatory activity of various treatments.The present finding that SAI doses of all the three opioids when coadministered with anti-inflammatory dose of aspirin, significantly reduced ulcerogenic potential of the latter. This finding is in agreement with earlier reports involving the studies with various ulcerogens like indomethacin [5], ethanol [6], NaOH [8]. However the present findings differ from the earlier one in which morphine has been reported enhance indomethacin [10] and paf induced gastric damage [7]. The discrepancy could be due to high and single dose of morphine (7.5 mg/kg) and i.v route of administration in earlier studies.

Though the present study does not aim to explore the mechanisms involved in observed anti-inflammatory activity of opioids, based on earlier reports several mechanisms could be proposed. In addition to supraspinal and spinal μ and k receptor involvement, morphine effect may be due to its inhibitory effect on bradykinin [2], and prostaglandin antagonism [1,16]. However prostaglandin antagonism by morphine appears to be least likely mechanism involved since

morphine has been reported to enhance prostaglandin synthesis [3,4] while its effect on polymorphonuclear cells as reported earlier [17] could be contributing. Vasodilation per se contributing for [18] or potentiating [19] anti-inflammatory activity as suggested in earlier reports may be contributing for morphine and pethidine activity but not for that of pentazocin which has sympathetic stimulant activity. Other kappa receptor mediated anti-inflammatory mechanisms such as reduced adhesion molecule expression, inhibition of cell trafficking, reduced TNF release and expression etc., have been reported [20].

The findings of the present study indicate that maximum anti-inflammatory effect was produced by pethidine in acute inflammation among the different opioids used. The probable explanation may be that pethidine has got additional anticholinergic action and releases less histamine. An anticholinergic R,R-glycopyrrolate has been reported to act synergistically with rolipram and budesonide in inhibiting inflammatory mediators like TNF-alpha [21].It is difficult to explain maximum anti-inflammatory effect pentazocin in sub acute studies and may probably due to its sympathetic stimulant action, since adrenalin and other beta agonists have been reported to suppress inflammation[22,23].

Decreased ulcer index and suppression of ulcerogenicity of aspirin by morphine in the present study could be explained by earlier report where in morphine has been shown to decrease gastric acid secretion and increased barrier mucus level [24].However, there is paucity of information about antiulcerogenic mechanisms of pethidine and pentazocin. In comparison to morphine better gastroprotective effect of pethidine as observed in the present study may be due to its lesser potential for histamine release.

Irrespective of mechanisms involved, all the three opioids tested in the present study showed significant anti-inflammatory activity. When coadministered with aspirin not only potentiated the anti-inflammatory activity but also significantly decreased the ulcerogenicity of the latter.

There is no clear information regarding the dose of these opioids leading to dependence development on chronic use. If the present experimental findings could be extrapolated to human beings, there appears to be a scope for combination of NSAIDs with pentazocine like agonist-antagonist opioid, for the short term treatment of inflammatory disorders. Opioids which are available for oral administration are worth-while investigating for their salutary effect in combination with small dose of aspirin like drugs, if not in all but, for the treatment of post operative inflammation, acute exacerbations of chronic inflammation etc. However, the efficacy and safety of such combinations need to be confirmed clinically.

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