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Anti-Inflammatory Effects of Morphine and Gabapentin, Alone and in Combination, in Rats

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ABSTRACT

Background and Objective: Pain and inflammation are know to be important defense mechanisms response to the variable nociceptive to the body. The present study was aimed to assess the anti-inflammatory effects of morphine and gabapentin alone as combination of both by using formalin induced paw edema model. **Methodology:** Thirty adult sprague dawley rats (150-200 g) were selected and were divided into five groups (n = 6). Group I received normal saline intraperitoneally i.p. and used as control. Group II received diclofenac (10 mg kg^{-1} i.p.), Group III received morphine (15 mg kg^{-1} i.p), Group IV received gabapentin (300 mg kg^{-1} i.p.) and Group V received combination of both i.e., morphine (15 mg kg^{-1} i.p. and gabapentin 300 mg kg^{-1} i.p.). The rats paw edema was produced by injecting 2% formalin subcutaneously on the dorsal surface of right hind paw. Animals received the drug treatment 30 min before the injection of formalin and the paw volume was measured at 0, 30, 60, 120 and 240 min after formalin challenge with the help of mercury plethysmometer. Percentage inhibition of paw volume was also calculated at the same time intervals in all the groups. **Results:** Both morphine and gabapentin also as in combination caused a significant ($p<0.01$) reduction in paw volume at various time intervals as compared to saline treated groups. The effect of gabapentin was comparable to that of diclofenac while the effect of morphine on reduction in paw volume was inferior. But the combination of both gabapentin and morphine showed significant reduction in paw volume as compared to individual drug. Similar results were observed in percentage inhibition of paw volume in all the treatment groups. **Conclusion:** Gabapentin morphine alone and also in combination produce an anti-inflammatory effect. Gabapentin was superior to morphine and also the combination is helpful in reducing the paw volume in formalin induced rats paw edema.

Key words: Gabapentin, morphine, inflammation, diclofenac

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INTRODUCTION

Inflammation is body's response to disturbed homeostasis caused by infection, injury or trauma resulting in systemic and local effects[1]. Though inflammation is protective in some situations if untreated can lead to serious complications. Various drug treatment options are available for the treatment of inflammation. These are nonsteroidal anti-inflammatory (NSAIDs) agents such as naproxen, mefenamic acid, diclofenac etc. More severe inflammatory disease, such as arthritis are treated with steroidal hormones and glucocorticoids e.g., prednisolone, betamethasone sodium sulphate etc. Because many of the inflammatory agents are only short acting and often produce severe side effects, the need for new therapies continues.

Gabapentin, a structural analog of neurotransmitter gaba-aminobutyric acid (GABA), is a well tolerated anticonvulsant drug, effective in various animal seizure models. Now a days, it is used both as an add-on and monotherapy for prevention and treatment of convulsions[2]. Experimental models of neuropathic pain and inflammatory hyperalgesia have shown that gabapentin has antinociceptive effects which are hypothesized to be mediated by modulation of glutamate and GABA receptors and substance P neurotransmission[3,4]. It appears to be effective in reducing allodynia and hyperalgesia induced by inflammatory responses or nerve injury[5].

Morphine, an opioid receptor agonist, is an anti-nociceptive by actions in the central nervous system. The anti-inflammatory effects of morphine are not clearly demonstrated and have been the subject of several studies in the recent past. When administered systemically, it attenuates the inflammation and progress of the disease[6] and can reduce inflammation-induced extravasation[7] and edema[8] induced by several stimuli like carrageenan, yeast and capsaicin. When administered

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directly into the inflamed site, variable pro and anti-inflammatory effects of morphine have been reported which are dose dependent[9].

Despite extensive investigations on analgesic mechanisms of gabapentin and morphine, not much has been studied about their anti-inflammatory role. The present research work was therefore, aimed to investigate the anti-inflammatory effect of gabapentin and morphine in an appropriate model of inflammation.

MATERIALS AND METHODS

The experimental study was conducted in the Department of Pharmacology, Pt. B D Sharma Post Graduate Institute of Medical Sciences, Rohtak, after approval from the Institutional Animal Ethical Committee (IAEC).

Drugs and dilutions: The following drugs were used: Gabapentin (Sun pharma, Mumbai, India), Morphine (Neon Laboratories, Mumbai, India), Diclofenac (Novartis India Ltd., Mumbai, India) and Formalin (Ranbaxy, Ahmedabad, India), all of which were purchased from local market. Gabapentin was dissolved in saline. All drugs were administered intraperitoneally (i.p.).

Animals: The study was performed on healthy adult Sprague-Dawley albino rats (150-200 g) of either sex. The animals were caged individually in a temperature controlled room with a 12 h light/dark cycle and were allowed free access to food and water. The care and maintenance of the animals were done as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) in India.

Grouping of animals: Thirty animals were divided into five groups of six each. Group I served as control and received comparable amount of normal saline. Group II served as standard and received diclofenac (10 mg kg^{-1})[10]. Group III, IV and V received the test compounds i.e., gabapentin (300 mg kg^{-1}), morphine (15 mg kg^{-1})[1], gabapentin (300 mg kg^{-1})+morphine (15 mg kg^{-1}), respectively.

Anti-inflammatory activity: Thirty minutes after the i.p. injection of different test compounds, edema was produced in all the groups by injecting 0.2 mL of 2% v/v formalin subcutaneously on the dorsal surface of right hind paw of the rats[11]. The paw of each rat was marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. For the assessment of anti-inflammatory activity, the paw volume was measured plethysmographically at 0, 30, 60, 120 and 240 min. The 0th min reading was considered as the initial paw size of the rats. The change in paw volume in test groups was compared with that of untreated control group. Two parameters were recorded: (1) reduction in paw volume (mL) with the help of mercury plethysmometer and (2) percentage inhibition of paw edema which was calculated by using the following formula[12]:

$$\text{Inhibition of edema (\%)} = 1 - \frac{\text{Mean increase in paw volume in drug treated group}}{\text{Mean increase in paw volume in saline treated group}} \times 100$$

Statistical analysis: Paw volume (mL) was calculated as (Mean±SEM). To compare different groups with saline group, one-way Analysis of Variance (ANOVA) was done followed by Dunnet's test, $p<0.05$ was considered significant.

RESULTS

Effect of various drugs on formalin-induced paw edema in rats: Results of different drug groups are shown in Fig. 1. All drug treatment groups suppressed

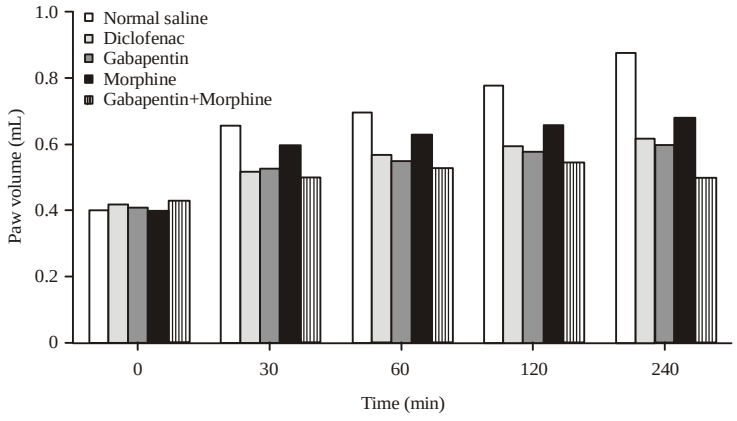

Fig. 1: Effect of various drug on paw edema

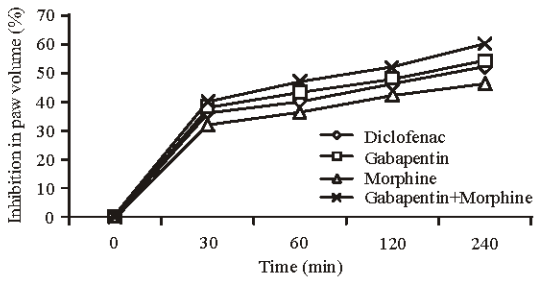

Fig. 2: Inhibition (%) in paw volume in different treatment groups

pedal edema throughout the observation period. Gabapentin, morphine and diclofenac alone as well as in combination group showed a significant ($p<0.01$) reduction in paw volume at various time intervals as compared to saline treated group. The reduction in paw edema with gabapentin and diclofenac was more in comparison to morphine. Combination of gabapentin and morphine showed significant inhibition at 240 min as compared to individual drugs.

Percentage inhibition of formalin induced paw edema: The results are shown in Fig. 2. Percentage inhibition of paw volume was more in gabapentin and diclofenac as compared to morphine. Combination of gabapentin and morphine showed enhanced reduction in paw edema when compared to the gabapentin, morphine and diclofenac alone.

DISCUSSION

The present study was aimed to evaluate the anti-inflammatory effect of an antiepileptic drug-gabapentin, an opioid agonist i.e., morphine and combination of both these drugs against formalin-induced rat paw edema. Intraplantar injection of formalin in rat provokes a localized inflammatory reaction which is a suitable method for evaluating the anti-inflammatory effects of the above mentioned drugs. All the three drugs i.e., gabapentin, morphine and diclofenac, when used individually, caused inhibition of paw inflammation. Effect of gabapentin was more compared to that produced by the other two drugs i.e., morphine and diclofenac.

In the present study, treatment with gabapentin demonstrated inhibition of paw inflammation and this is in conformity with another study where gabapentin showed anti-inflammatory effect in carrageenan induced rat paw edema[13]. It has been suggested that gabapentin affects calcium currents which might modulate neuronal excitability or release or synthesis of inflammatory mediators thereby, alleviating the inflammatory conditions[2].

Results of morphine are comparable to a study in which pretreatment with high doses (7 and 10 mg kg^{-1}) of intraperitoneal morphine exhibited anti-inflammatory effects in carrageenan induced paw edema in mice[14] whereas at low doses i.e., 1 mg kg^{-1}, intraperitoneally, it caused increased edema whereas no suppression of edema was observed at moderate doses (3 and 5 mg kg^{-1}). It has been suggested that the anti-inflammatory effects of morphine may be related to the presence of opioid binding sites on immune cells and to the opioid mediated modulation of several functions of these cells but the mechanism of morphine's immunomodulation is still not completely understood[14]. Some evidences suggest that supraspinal opioid pathways are involved in the immunosuppressive effects of morphine and these pathways may be distinct from those participating in opioid-induced analgesia[15].

Treatment with diclofenac significantly enhanced the anti-inflammatory effect and these results are similar to a study in which diclofenac reduced carrageenan induced paw edema in rats. Diclofenac, a COX-2 inhibitor, possesses anti-inflammatory effect by inhibiting COX-2 enzyme and thus the synthesis of prostaglandins, leukotrienes and thromboxanes is inhibited[10].

In the present study, co-administration of gabapentin and morphine significantly enhanced anti-inflammatory effect against formalin induced-paw edema in rats when compared to the individual drug and the results are comparable to those of other studies in which combination of both drugs had a greater anti-inflammatory effect[16,17]. Similarly, studies involving co-administration of gabapentin with other drugs such as tramadol[18], nimesulide[2] and diclofenac[18] have reported a therapeutic advantage over the individual drug for clinical treatment of pain and inflammation.

This study has demonstrated that co-administration of gabapentin with morphine has a significant synergistic anti-inflammatory effect, when compared to their effect individually on formalin induced hind paw edema. However, further studies are needed to substantiate these findings.

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