ABSTRACT

Objective: To explore the antinociceptive effects of combination of ketorolac with different opioids in central and visceral nociception. Methods: Tail flick method and writhing method were used as animal models of central and visceral nociception, respectively. Results: Coadministration of subeffective doses of combination of ketorolac (10mg/kg) with different opioids (morphine 1.5mg/kg, pethidine 10mg/kg, fentanyl 20µg/kg, buprenorphine 0.05mg/kg, and tramadol 10mg/kg) increased pain threshold (percentage analgesia) in tail flick method and decreased the number of writhes (increased percentage inhibition) in writhing method significantly. Keterolac fentanyl combination in subeffective doses was more effective in enhancing the pain threshold, producing highly significant antinociceptive effect (93.33% analgesia) in tail flick method whereas the combination of subeffective doses of ketorolac and tramadol produced highly significant effect (88.99% inhibition) in writhing method. Conclusion: The present study suggests that coadministration of ketorolac with fentanyl produces the maximum analgesia in central pain model whereas ketorolac tramadol combination is more effective in visceral pain. In clinical practice this would allow use of combination for effective analgesia according to the type of pain.

Keywords: ketorolac, opioid, tail flick, writhing

INTRODUCTION

Ketorolac is a nonsteroidal anti inflammatory drug with potent analgesic and modest anti inflammatory activity. Ketorolac in common with other NSAIDs is an inhibitor of prostaglandin synthesis. The pharmacological target of ketorolac is cyclo oxygenase enzyme (COX) which is responsible for synthesis of prostaglandins. Two isoforms of COX enzyme i.e.COX-1 and COX-2 are known. Ketorolac is a nonselective COX enzyme inhibitor. The widespread use is limited due to possibility of gastrointestinal and renal bleeding. Opioid analgesics are thought to produce analgesia through their actions in the CNS. Improved knowledge about opioid pharmacology has provided better analgesia and greater patient satisfaction. However the problems of adverse opioid effects remain. Administration of opioid in the perioperative setting may contribute to early or delayed respiratory depression, confusion, nausea and vomiting, decreased gastrointestinal motility and pruritus. In response to this issue, ketorolac is commonly administered in the perioperative setting. There is evidence in humans that the analgesia provided by 30 mg parenteral ketorolac may be as effective as 12 mg parenteral morphine in the post operative period. Nevertheless, if pain persists or breaks through the initial dose of NSAID, extended use of parenteral NSAID increases the risk of hepatic or renal impairment, bleeding complications, gastrointestinal erosions...
and ulcerations.\textsuperscript{(7,12,13)} Many, if not all, of these adverse effects are dose dependent. Therefore using combinations of medications that offer synergy should allow reduction in required dosage and decrease the incidence of adverse effects.

Recently, combined use of NSAID and opioid is advocated in clinical practice to reduce the dose related side effects of both drugs.\textsuperscript{(14,15,16)} Further some clinical studies also suggest that NSAIDs potentiate the analgesic effects of opioid.\textsuperscript{(17)} Present study was mainly aimed to explore the interaction between ketorolac and different opioids in animal models of central and visceral nociception.

MATERIALS AND METHODS

**Animals:** Albino rats (150-250 gms) of either sex were used. Animals were housed under standard laboratory conditions with free access to food and water ad libitum. The rats were fasted from 8 am on the day of experimentation. The experimental protocol was approved by institutional animal ethics committee.

**Drugs:** Following drugs were used; ketorolac (Dr Reddy’s Lab), morphine (Troikaa Pharmaceuticals), pethidine (Verve Health Care Ltd), fentanyl (Troikaa Pharmaceuticals), buprenorphine (Neon Laboratories), tramadol (Pyramal Health Care) and 4% NaCl (prepared freshly).

**Assessment of central nociception**

**Tail flick test (radiant heat induced nociception)**

The hyperalgesic response in the tail withdrawal test (analgesiometer) is generally attributed to central mechanism. Tail withdrawal latency from the radiant heat source was taken as endpoint. The intensity of the radiant heat was adjusted so that the baseline latency for tail withdrawal of rat was 4-5 seconds. A cut off time of 15 seconds was imposed to prevent any injury to tail. Analgesic response was expressed as percentage analgesia and was calculated as follows:

\[
\frac{\text{after drug} - \text{before drug}}{15 \text{(cut of time in sec)} - \text{before drug}} \times 100
\]

**Assessment of visceral nociception**

**Writhing method**

The rats were given 0.4ml/100gm of freshly prepared 4% NaCl solution by intraperitoneal route. Within few seconds the rats showed characteristics writhes which was the contraction of abdomen with extension of hind limbs. The number of writhes before and after drug administration was counted for a period of 10 minutes. Based on the number of writhes percentage inhibition was calculated by using the following formula:

\[
\frac{\text{writhes with NaCl} - \text{writhes with NaCl after giving drug}}{\text{writhes with NaCl}} \times 100
\]
**Drugs administration**

All the drugs were administered by intraperitoneal route (i.p.) and subsequent subeffective doses (producing 20-30% response) were determined. In tail flick method, combination of ketorolac with different opioids in subeffective doses was coadministered at one time and reaction time was observed before and 20-30 minutes after administration while in writhing method, number of writhes induced by 4% NaCl was calculated before and after injecting the drug combinations. All these drugs were diluted in distilled water to prepare solutions of desired strengths.

**STATISTICAL ANALYSIS**

Results were expressed as mean ± SEM. Paired t test was used to compare reaction time before and after injecting the drugs. Percentage analgesia and percentage inhibition in ketorolac group (group I), different opioids group (group II) and combinations of ketorolac with respective opioids (group III) were compared by one way analysis of variance (ANOVA). Bonferroni test was used for multiple comparisons whereas comparison of analgesia of addition of ketorolac and different opioids administered alone (group I and group II) with respective ketorolac opioid coadministration group (group III) was done by proportion test. The value of p<0.05 was considered as statistically significant and p<0.01 as highly significant.

**RESULTS**

**Effect of ketorolac on opioid antinociception in tail flick method in rats:**

Coadministration of subeffective doses of ketorolac (10mg/kg) with different opioids (morphine 1.5mg/kg, pethidine 10mg/kg, fentanyl 20µg/kg, buprenorphine 0.05mg/kg, and tramadol 10mg/kg) enhanced the antinociceptive effect which was maximally seen with ketorolac and fentanyl combination producing 93.33% analgesia which was highly significant as compared to per se effects of both drugs and was significant compared with addition of the per se of ketorolac and fentanyl.

**Effect of ketorolac on opioid antinociception in writhing method in rats:**

Coadministration of subeffective doses of ketorolac (10mg/kg) with different opioids (morphine 1.5mg/kg, pethidine 10mg/kg, fentanyl 20µg/kg, buprenorphine 0.05mg/kg, and tramadol 10mg/kg) enhanced the antinociceptive effect which was maximally seen with ketorolac and tramadol combination producing 88.99% inhibition which was highly significant as compared to per se effects of both drugs and was to the significant level when compared with addition of the per se of ketorolac and tramadol.

**DISCUSSION**

An important technique for decreasing side effects in pharmacology is the use of low doses of several agents that produce the same therapeutic effects. A potential advantage of using combination therapy is that analgesic effects can be maximized while the incidence of adverse effects is minimized. (18) Therefore using combinations of medications that offer analgesic synergism should allow a reduction in required dosage and decrease the incidence of adverse effects. (19)
Radiant heat method and 4% NaCl induced writhing method are recognized screening tests for potential antinociceptive properties of central and visceral nociception, respectively. Used together these two complimentary tests detect antinociceptive actions of all major analgesic drugs in clinical use. The type of interaction between drugs according to theory of drug interaction can be expressed as antagonism, addition or synergism when the combined effect of two drugs are lower, equal or greater than the sum of effect of each agent given alone, respectively.\(^\text{(20)}\) Synergism requires that drugs have different mechanisms of actions.\(^\text{(21)}\) The synergistic interaction usually occurs between two drugs with different mechanisms of actions which additively takes place when two agents potentiate each other’s activity profile at their target sites. Consequently, ketorolac and opioid seem to be consistent with therapeutic consideration. However the combinations of ketorolac fentanyl and ketorolac tramadol were more effective in tail flick method and writhing method, respectively. These findings may offer advantages while using combination of ketorolac with opioid drugs in selecting different opioid in different types of pain. The reason behind such type of interaction might be related to the potency of the drugs used such as fentanyl which is more potent among the drugs used or to their additional mechanisms of actions such as inhibition of norepinephrine and serotonin uptake in case of tramadol in addition to its opioid mechanism of action. The reason behind this kind of difference in interaction remains unclear. It has been proposed that this synergistic interaction between NSAIDs and opioids occur at intracellular sites of convergence between prostanoid and opioid receptor transduction mechanisms.\(^\text{(22)}\) Vaughan et al 1997\(^\text{(23)}\) proposed a novel cellular mechanism underlying the synergism between opioids and NSAIDs in the midbrain periaqueductal gray, a brain region critical for analgesic action of both opioids and NSAIDs.

**CONCLUSION**

These findings suggest that ketorolac exhibits synergistic action with opioid when used in combination even at subeffective doses. In clinical practice this would allow use of combination for effective analgesia according to the type of pain. Such combination would have reduced incidence of adverse effects.

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**REFERENCES**

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Figure 1(A)

% analgesia

Figure 1(B)

% inhibition of writhes
**Figure 2(A)**

![Bar Graph for % Analgesia](image1)

**Figure 2(B)**

![Bar Graph for % Inhibition of Writhes](image2)