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Short communication

Effect of licofelone against mechanical hyperalgesia and cold allodynia in the rat model of incisional pain

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Abstract :
Hyperalgesia from an incisional pain is evoked by noxious stimuli (mechanical and cold). The present study was aimed to examine the effect of licofelone, a dual inhibitor of cyclooxygenases (COX-1/COX-2) and 5-lipoxygenase (5-LOX) against mechanical hyperalgesia and cold allodynia in the rat model of incisional pain. Mechanical hyperalgesia and cold allodynia was assessed employing Randall and Sellitto analgesymeter and cold water maintained at 10°C, respectively. Zileuton (25–100 mg/kg, *po*), a 5-LOX inhibitor, indomethacin (1–30 mg/kg, *po*), a non-selective COX inhibitor, and licofelone (10–100 mg/kg, *po*) a dual inhibitor, significantly reversed the mechanical hyperalgesia and also caused an increase in cold allodynia threshold with different pharmacologic profile. The rank order of potency based on ED_{50} values in both the paradigms was found to be licofelone > indomethacin > zileuton.
The results of the present study are indicative of the role of leukotrienes along with prostaglandins in the rat model of incisional pain (a paradigm of postoperative pain). The results suggested that dual inhibition approach of simultaneous inhibition of COX and LOX pathways might prove beneficial in combating hyperalgesia of postoperative pain.

Key words:
incisional pain, mechanical hyperalgesia, cold allodynia, lipoxygenase, cyclooxygenase, dual inhibitor, licofelone

Introduction

Postoperative pain is a hyperalgesic condition that affects one of every two patients undergoing surgery. Hyperalgesia after tissue injury is modulated by locally released mediators. The essential role of arachidonic acid (AA) metabolites, prostaglandins (PGs) derived from cyclooxygenase (COX) action and leukotrienes (LTs) derived by lipoxygenase (LOX) pathway during hyperalgesia is evidenced [1, 8]. Peripherally acting PGs sensitize the nociceptors leading to lower threshold of polymodal C- and A-δ fibers. At central level, activation of spinal PG receptor sensitizes primary afferent fibers and increases the excitability of the dorsal horn neurons. Thus, the use of non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit PG synthesis have been shown to be effective in postoperative pain [15]. However, other studies demonstrate that PGs cannot account for all of the hyperalgesia associated with tissue injury. Studies in animals and humans have demonstrated that LTs, the products of NSAIDs resistant 5-LOX-catalyzed metabolism of AA, also produce hyperalgesia. Peripher-

ally, LTs have been shown to possess similar effect as PGs in regulating hyperalgesia [7]. Recently, Trang et al. reported the central effect of LTs in modulating hyperalgesia in formalin test in mice [12].

The present study was aimed to evaluate the efficacy of licofelone, a dual COX/LOX inhibitor, against mechanical hypersensitivity and allodynia (the hallmarks of postoperative pain) in the rat model of incisional pain (an animal paradigm of postoperative pain).

Materials and Methods

Animals

Female Wistar rats (Central Animal House, Panacea Biotec, Lalru, India) weighing 180–200 g, housed under ideal conditions (25 ± 0.5°C) and 12 h light : 12 h dark cycle were used. Animals had free access to food and water *ad libitum*. Experiments were carried out between 09:00 to 12:00 h. The experimental protocols were approved by the Institutional Animal Ethics Committee.

Drugs

Licofelone, indomethacin (Panacea Biotec Ltd., New Delhi, India) and zileuton (Archechem, Mumbai, India) were suspended in Tween 80 and administered in a constant dose volume of 10 ml/kg. Control animals received Tween 80.

Surgery (induction of incisional pain in rats)

Incisional pain was induced in rats as previously described by Brennan et al. [2] with minor modifications. Briefly, rats were anesthetized by thiopental sodium (25 mg/kg, *ip*) and the plantar surface of the left hind paw was prepared in the sterile manner. A 1 cm longitudinal incision was made with a no.11 blade, starting 0.5 cm from the proximal edge to the heel extending towards the toes. The plantaris muscle was elevated and incised longitudinally. Following hemostasis with gentle pressure, the skin was apposed with two single nylon sutures. The wound site was covered with povidine-iodine solution and animals were allowed to recover in their home cage.

Hyperalgesia testing

Two tests were used to assess the pain behavior: mechanical hyperalgesia (Randall and Sellitto analgesymeter) and cold allodynia. Animals were tested for

Tab. 1.. Summary of the action of licofelone, zileuton and indomethacin against mechanical hyperalgesia (a) and cold allodynia (b) in the rat model of incisional pain

(a) Mechanical hyperalgesia							
Compound	Dose (mg/kg)	Route	MED (mg/kg)	Onset	Duration of action	Maximum percent reversal	ED_{50} (mg/kg)
Licofelone	10, 30, 100	*po*	10	3 h	Long	91.6 (3 h)	2.92
Zileuton	25, 50, 75, 100	*po*	25	3 h	Short	77.2 (5 h)	49.88
Indomethacin	1, 3, 10, 30	*po*	10	I h	Medium	80 (1 h)	2.4
(b) Cold allodynia							
Licofelone	10, 30, 100	*po*	30	1 h	Long	80.4 (3 h)	36.77
Zileuton	25, 50, 75, 100	*po*	25	3 h	Medium	78.36 (3 h)	49.85
Indomethacin	1, 3, 10, 30	*po*	10	3 h	Short	46 (5 h)	41

MED is defined as minimum dose that elicits a statistically significant reversal as compared to vehicle-treated controls. Onset of action is defined as the earliest detected statistically significant reversal of MED. Duration of action is defined as *short* if the MED elicited a statistically significant reversal at only one of the post-administration time points, *medium* if the MED elicited a statistically significant reversal at two of the post-administration time points, *long* if the MED elicited a statistically significant reversal at three of the post-administration time points. Maximum percent reversal is reported for the highest dose studied. ED_{50} is defined as the dose that produces 50% of the maximum effect. ED_{50} was calculated by linear regression [13]. *po – peroral*

pain behavior before surgery, immediately after surgery, and at 1, 3, 5 and 12 h after surgery. The mechanical hyperalgesia was assessed by measuring the paw withdrawal threshold (PPWT) (both ipsilateral and contralateral) when exposed to mechanical stimulation (Randall and Sellitto) [3, 9]. The cold allodynia in both ipsilateral and contralateral paw was determined by measuring the paw withdrawal latency (PWL) when dipped in cold water maintained at 10°C [4]. The data (PPWT or PWL) were transformed to percent reversal by using formula:

$$\% \text{ reversal} = \frac{\text{postdose threshold} - \text{predose threshold}}{\text{baseline threshold} - \text{predose threshold}} \times 100$$

and ED_{50} was calculated using linear regression analysis.

Statistical analysis

The data were analyzed by one-way ANOVA followed by *post hoc* Dunnett's test and values with $p < 0.05$ were considered statistically significant as compared to vehicle-treated (naive) controls.

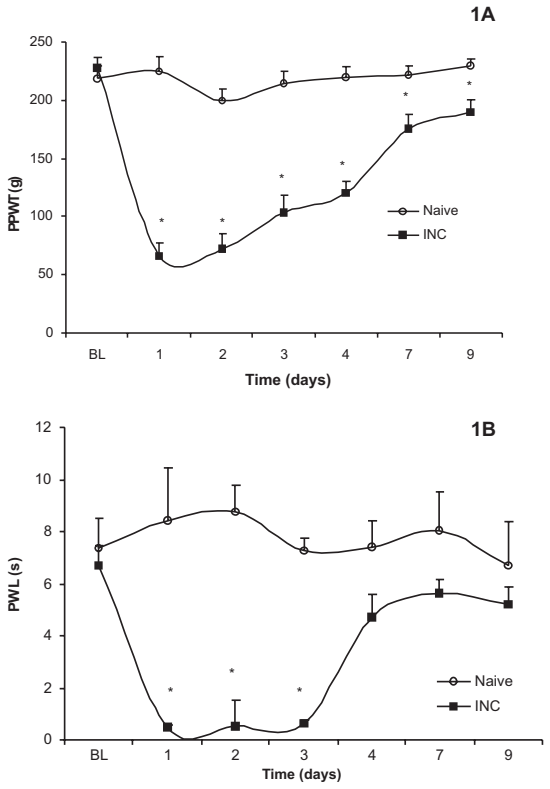

Fig. 1. Time course of (**A**) mechanical hyperalgesia, measured by Randall and Sellitto analgesymeter, (**B**) cold allodynia, measured by dipping hind paw in cold water (10°C) following incision of plantar surface of the rat hind paw. PPWT – paw withdrawal threshold; PWL – paw withdrawal latency. Values represent the mean ± SEM, n = 8–10 per group. * $p < 0.05$ as compared to naive rats. A significant mechanical hyperalgesia and cold allodynia was present at most of the postsurgical test time points. INC – incisional group, BL – basal

Results and Discussion

Mechanical hyperalgesia

Incision of the plantar surface of the rat hind paw produced a significant reduction in PPWT and PWL at most of the times studied up to 9 days (Fig. 1A and B). The reduction in PPWT was maximal on postincisional day 1 (66.75 ± 12.75 g) as compared to the baseline value (227.5 ± 10.9 g) and naive control animals (219 ± 9.34 g). The reduction in the response to cold allodynia (PWL) was maximal on post-incisional days 1–3 (1.71 ± 0.35 s) as compared to the baseline (6.72 ± 0.73 s) and naive controls (6.96 ± 0.85 s). No change in PPWT and PWL of contralateral paw was observed at any of the test times (data not shown).

Licofelone (10–100 mg/kg, *po*) produced a maximum reversal of 91.6% (100 mg/kg, Tab. 1, Fig. 2A) 3 h following drug administration (PPWT 277.5 ± 21.8 g *vs.* 84.75 ± 8.3 g for control). However, the onset of action of licofelone at 3 h was found to be long-acting (effective at all test points 3, 5, 24 h after administration). Indomethacin (3–30 mg/kg, *po*) and zileuton (25–100 mg/kg, *po*) produced a maximal reversal of 80% (30 mg/kg) and 77.2% (100 mg/kg, *po*), respectively at 1 and 5 h after their administration (PPWT indomethacin 130.5 ± 12.2 g *vs*. 79.5 ± 10.8 g for control and zileuton 176.25 ± 7.18 g *vs.* 86.25 ± 12.68 g for control) (Tab. 1, Fig. 2A). Based on ED_{50} values (Tab. 1), the rank of potency against mechanical hyperalgesia is icofelone > indomethacin > zileuton.

Cold allodynia

Licofelone (10–100 mg/kg) produced a maximum reversal of 80.4% (100 mg/kg, Tab. 1, Fig. 2B) 3 h following its administration (PWL 3.77 ± 0.44 s *vs.* 0.74 ± 0.16 s for control). The reversal of allodynia by licofelone was significant at 1, 3, 5 and 24 h after the treatment. Indomethacin (3–30 mg/kg) produced

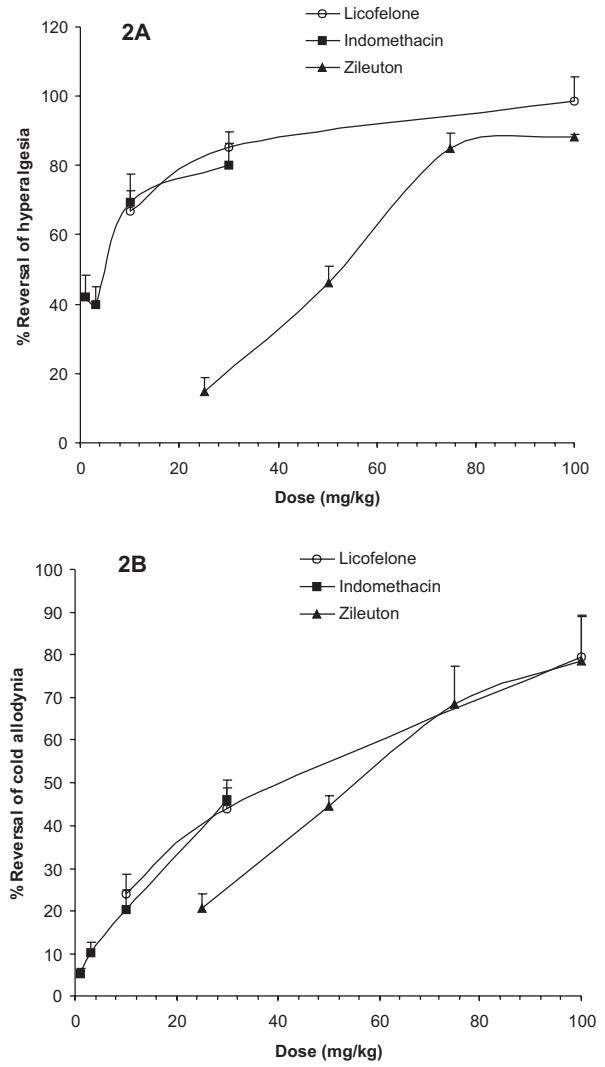

Fig. 2. Effect of licofelone (10–100 mg/kg, *po*), zileuton (25–100 mg/kg, *po*), and indomethacin (1–30 mg/kg, *po*) on (A) reversal of incision-induced mechanical hyperalgesia, (B) reversal of incision-induced cold allodynia in the incised rat paw. Data (mean ± SEM) at the time of maximal reversal for each compound. * $p < 0.05$ as compared to naive rats.

a maximal reversal of 46% (30 mg/kg) 5 h following administration (PWL indomethacin 2.98 ± 0.05 s *vs.* 0.51 ± 0.08 s for control) (Tab. 1, Fig. 2B). In contrast to mechanical hyperalgesia, the effect of indomethacin against cold allodynia was only significant at 3 h. Further, zileuton displayed maximal reversal of cold allodynia of 78.36% (100 mg/kg) 3 h after administration (PWL zileuton 2.45 ± 0.67 s *vs.* 0.51 ± 0.08 s for control) (Tab. 1, Fig. 2B). Based on ED_{50}, the rank order of potency against cold allodynia is licofelone > indomethacin > zileuton.

The present study established the potency of indomethacin, zileuton and licofelone against hyperalgesia of incisional pain. The *in vivo* rank order of compounds is suggestive of complementary and quantifiable role of metabolites of AA produced by COX and LOX in modulating hyperalgesia in this animal paradigm. Previous reports [10, 13, 14] demonstrated anti-hyperalgesic effect or anti-allodynic effect of selective or non-selective COX inhibitors in postoperative pain. Studies in animals and humans have demonstrated that LTs' products of NSAIDs resistant 5-LOX pathway of AA metabolism also produce hyperalgesia. Intraplantar injection of LT receptor agonist (LTB_4) or 8R-15(S)-dihydroxy-eicosa-5-cis-9,11,13-trans-tetraenoic acid (8R-15-diHETE), which are metabolites derived from 5- and 15- LOX pathways, respectively, evoked profound hyperalgesic response likely involving sensitization of C- and A-δ nociceptors [6]. Pretreatment with 5-LOX inhibitor or cysteinyl LT receptor antagonist reversed the carrageenan-induced hyperalgesia [5, 11]. We recently reported that LTs and PGs played a complementary role in radicular pain induced by herniated nucleus pulposus in rats [9].

However, we are unaware of any reports on the efficacy of LOX inhibitors in rat model of postoperative pain. Both, PGs and LTs, have been reported to modulate pain at peripheral and at central level. Thus, the anti-hyperalgesic and anti-allodynic effect of licofelone, a dual inhibitor of COX/LOX pathways, suggests that simultaneous dual inhibition of these two pathways helps to achieve an effective control of hyperalgesia of incisional pain.

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