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Pharmacological modulation of leukotriene D_4 attenuates the development of opioid dependence in a mouse model of naloxone-induced opioid withdrawal syndrome

Ashish K. Rehni [a,*], Inderbir Singh [a], Nirmal Singh [b], Nitin Bansal [c], Seema Bansal [c], Manoj Kumar [a]

[a] Chitkara College of Pharmacy, Chandigarh-Patiala National Highway, Rajpura-140401, Patiala, Punjab, India
[b] Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala-147002, India
[c] Rajendra Institute of Technology and Sciences, 4th Milestone, Hisar Road, Sirsa-125055, India

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ABSTRACT

The present study was designed to investigate the effect of montelukast sodium, a leukotriene D_4 receptor antagonist, and 1,2,3,4,tetrahydroisoquinoline, a leukotriene D_4 synthetic pathway inhibitor, on the development of morphine dependence in a mouse model of naloxone-induced opioid withdrawal syndrome. Morphine (5 mg/kg, i.p.) was administered twice daily for a period of 5 days following which a single injection of naloxone (8 mg/kg, i.p.) precipitated the opioid withdrawal syndrome in mice. Behavioral observations were made for a period of 30 min immediately after naloxone treatment. The withdrawal syndrome was quantitatively assessed in terms of withdrawal severity score and the frequency of jumping, rearing, fore paw licking and circling. Montelukast sodium as well as 1,2,3,4,tetrahydroisoquinoline, markedly and dose dependently ($p<0.01$) attenuated the morphine-naloxone-induced opioid withdrawal syndrome in mice. However, administration of montelukast sodium or 1,2,3,4,tetrahydroisoquinoline did not alter the activity of the central nervous system, assessed in terms of locomotor activity count thus ruling out any *per se* sedative action of montelukast sodium. Further, pretreatment with montelukast sodium or 1,2,3,4, tetrahydroisoquinoline did not alter the acute analgesic effect of morphine. Thus, leukotriene D_4 may be involved in the development of opioid dependence and the precipitation of its withdrawal syndrome and thus may serve as a viable pharmacological target to tackle the problem of opioid addiction.

1. Introduction

Opioid drugs are used primarily for the treatment of pain (Akil and Lewis, 1987). Some of the CNS mechanisms that reduce the perception of pain also produce a state of well-being or euphoria (Hardman et al., 2001). Thus, opioid drugs are also abused for the purpose of obtaining these effects on mood. Continued use of opioids elicits certain physiological aberrations characteristic of substance dependence (Van Ree et al., 1999). Moreover, the abrupt withdrawal of opioids causes the precipitation of abstinence syndrome, a major problem when treating substance dependence (Williams et al., 2001). Various approaches are used to contain the acute aspects of the withdrawal syndrome. The first is based on cross tolerance of other opioids, followed by gradual dose reduction (Hardman et al., 2001; Krantz and Mehler, 2004). The second is based on the use of α_2-adrenergic agonists, such as clonidine and lofexidine, by virtue of their direct inhibitory effect on the locus ceruleus system (Lee et al., 1987), and the third is based on the activation of the endogenous opioid system by non-pharmacological methods such as acupuncture or transcutaneous electrical stimulation (Hardman et al., 2001). Moreover, opioid replacement has also been shown to be useful in limiting the protracted aspects of opioid withdrawal syndrome (Krantz and Mehler, 2004). However, none of the available options promises to conclusively treat the condition of opioid dependence and its related abstinence syndrome. Recently, our group has shown the role of nuclear factor kappa-B (NF-κB) in the pathogenesis of morphine dependence (Rehni et al., in press). It is further noted that leukotriene receptor stimulation causes transcriptional and functional activation of NF-κB (Thompson et al., 2006). Moreover, pharmacological inhibition of 5-lipooxygenase, an enzyme mediating the general biosynthesis of leukotrienes, has been shown to block opioid dependence both *in vitro* using a guinea pig ileum preparation experiment and *in vivo* in a rodent model of opioid dependence, thus indicating the possible involvement of leukotrienes in the development of opioid withdrawal (Capasso, 1997; Trang et al., 2003). 5-Lipoxygenase mediates the conversion of arachidonic acid into leukotriene A4 which then either gets converted into leukotriene B_4 or causes the synthesis of other cysteinyl leukotrienes (leukotriene C_4, D_4 and E_4). Gamma glutamyl transpeptidase is an enzyme responsible for the conversion of leukotriene C_4 to leukotriene D_4 and 1,2,3,4,tetrahydroisoquinoline selectively suppresses the activity of this enzyme (Lorenc-Koci et al., 2001, 2005; Hardman et al., 2001). Therefore, the effect of 1,2,3,4,

* Corresponding author. 39, Gurmat Colony, Sular Road, Patiala-147001, India. Tel.: +919872535671.
E-mail address: ashishkrehni@gmail.com (A.K. Rehni).

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tetrahydroisoquinoline on experimental morphine withdrawal syndrome was tested in order to evaluate the role of leukotriene D_4 in the pathogenesis of opioid dependence. Montelukast sodium is a potent, oral, specific leukotriene D_4-receptor antagonist (cysteinyl leukotriene [CysLT1]-receptor antagonist) recently approved for the treatment of chronic asthma (Jones et al., 1995; LaBelle et al., 1995). Thus, the possible effect of montelukast sodium on experimental morphine withdrawal syndrome was assessed to confirm the involvement of a leukotriene D_4 receptor activation-linked mechanism in the pathogenesis of opioid dependence. Therefore, the present study has been designed to investigate the effect of montelukast sodium, a selective antagonist of leukotriene D_4 receptors, and 1,2,3,4,tetrahydroisoquinoline, an inhibitor of leukotriene D_4 synthetic pathway, on the development of morphine dependence in a mouse model of naloxone-induced opioid withdrawal syndrome.

2. Materials and methods

Swiss albino mice of either sex weighing 25±2 g obtained from Central Research Institute, Kasauli, India, maintained on standard laboratory diet (Kisan Feeds Ltd., Mumbai, India) and having free access to tap water, were used in the present study. They were housed in the departmental animal house and were exposed to a 12-h cycle of light and dark. The experiments were conducted in a semi-soundproof laboratory. The observer was blind to the treatment group assignment. The experimental protocol was approved by the institutional animal ethics committee and the animals were cared as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Reg. No. 107/1999/CPCSEA).

2.1. Drugs and chemicals

Montelukast sodium (Cipla Ltd., Solan, India), 1,2,3,4-tetrahydroisoquinoline (Sigma-Aldrich Chemicals Pvt. Ltd., St. Louis, USA), naloxone (Samarth Life Science Pvt. Ltd., Mumbai, India) and morphine sulphate (Jackson Laboratories, Amritsar, India) were dissolved/diluted in normal saline. The chemicals used were of analytical grade and all drug solutions were freshly prepared before use.

2.2. Induction of morphine withdrawal syndrome in mice

Morphine was administered (5 mg/kg, i.p.) twice daily for a period of 5 days. On the fifth day a single injection of naloxone (8 mg/kg, i.p.) precipitated the withdrawal syndrome in mice. Behavioral observations were made for a period of 30 min immediately after naloxone treatment (Way et al., 1969; Rehni et al., in press). The observations were made in a transparent perspex observation chamber with dimensions of 30 cm×30 cm×30 cm. Two observers blind to the treatment schedule simultaneously observed each animal for all the withdrawal measures and the mean value of both observations was recorded.

2.3. Assessment of morphine withdrawal syndrome in terms of jumping frequency

Stereotyped jumps precipitated by the opioid antagonist, naloxone are considered a predominant sign for quantification of morphine withdrawal syndrome in mice (Way et al., 1969; Marshall and Graham-Smith, 1971; Rehni et al., in press). Jumping frequency observed in a period of 30 min was used as a quantitative symptom of morphine withdrawal.

2.4. Assessment of withdrawal severity score (WSS)

A withdrawal severity score (WSS) was used to quantitate the severity of the withdrawal syndrome in mice in terms of the earlier reported characteristic behavioral patterns seen in mice suffering from experimental opioid withdrawal syndrome viz., fore paw tremor, wet-dog shake, straightening, ptosis and sneezing, all in a composite manner (Inoue et al., 2003; Shaw-Lutchman et al., 2002; Georgescu et al., 2003; Liu et al., 2007; Rehni et al., in press)(Table 1). The severity of the opioid withdrawal phenomenon was graded on a scale of 0–15 (normal score, 0; maximal withdrawal severity score, 15). For each individual behavioural component of the WSS 0 score point was awarded for no change in the normal behavior of mice with respect to each observation criteria, 1 score point was awarded for a mild increase in the respective observation criteria in mice, 2 score point was awarded for a moderate increase in the respective observation criteria in mice and 3 score point was awarded for a severe increase in the respective observation criteria in mice. Thus, the higher the score, the more severe the withdrawal syndrome was. The test was performed immediately after naloxone administration and the results were based on observations spanning first 30 min.

2.5. Assessment of the effect of morphine withdrawal syndrome on rearing, fore paw licking and circling frequency

The frequency of rearing, fore paw licking and circling was assessed over a period of 30 min to quantitate the severity of the experimental withdrawal phenomenon. These parameters have been noted to be indicative of the intensity of withdrawal syndrome (Glick and Morihisa, 1976; Patkina and Zvartau, 1978; Falls and Kelsey, 1989; Rehni et al., in press).

2.6. Assessment of the effect of various drug treatment(s) on locomotor activity in mice

Locomotor activity was monitored using an actophotometer (INCO, Ambala, India). Before the animals were subjected to various drug treatment(s), they were individually placed in the activity meter and total activity count was registered for each animal for a period of 10 min. Locomotor activity is expressed in terms of total photo beam interruption counts/10 min per animal (Reddy and Kulkarni, 1998; Rehni et al., in press).

Table 1
Withdrawal severity score

	Points
Fore paw tremor (normal=0; maximum=3)	3
0 No paw tremor	
1 Mild increase in paw tremor	
2 Moderate increase in paw tremor	
3 Severe increase in paw tremor	
Wet-dog shake (normal=0; maximum=3)	3
0 Normal behavior	
1 Mild increase in shaking behavior	
2 Moderate increase in shaking behavior	
3 Severe increase in shaking behavior	
Straightening (normal=0; maximum=3)	3
0 No body straightening behavior	
1 Mild increase in straightening behavior	
2 Moderate increase in straightening behavior	
3 Severe increase in straightening behavior	
Ptosis (normal=0; maximum=3)	3
0 No ptosis	
1 Mild increase in ptosis	
2 Moderate increase in ptosis	
3 Severe increase in ptosis	
Sneezing (normal=0; maximum=3)	3
0 No sneezing behavior	
1 Mild increase in sneezing behavior	
2 Moderate increase in sneezing behavior	
3 Severe increase in sneezing behavior	
Maximum points	15