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Involvement of opioid receptors in the systemic and peripheral antinociceptive actions of montelukast in the animal models of pain

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ABSTRACT

This study aimed to investigate the involvement of opioid receptors in the systemic and peripheral antinociceptive activities of montelukast in different animal models of pain.

Rats and mice were injected with montelukast to produce analgesia. The formalin and acetic acid-induced writhing tests were used to assess the nociceptive activity. The results showed that i.p. administration of montelukast (0.3–10 mg/kg) dose-dependently reduced flinching behavior in both the first and second phases of formalin test with mean ED_{50} of 0.55 and 5.31 mg/kg, respectively. Also, intraplantar administration of montelukast (3–30 μg/paw) produced antinociception against the two phases of formalin assay in a dose-dependent way with mean ED_{30} of 2.92 and 8.11 μg/paw, respectively. Furthermore, pre-treatment with naloxone (a non-selective opioid receptor antagonist) significantly inhibited both the systemic and also peripheral antinociceptive actions of montelukast in formalin test. In writhing test, the results showed that intraperitoneal administration of montelukast (3–10 mg/kg) significantly reduced the writhe number induced by acetic acid in mice. Moreover, co-administration of non-effective doses of montelukast (0.3 and 1 mg/kg; i.p.) and morphine (0.25 mg/kg; i.p.) significantly decreased the writhes number induced by acetic acid. Also, this effect was naloxone-reversible. These findings suggest that the systemic and peripheral antinociception produced by montelukast were mediated through the opioid receptors in central and peripheral nervous systems. Moreover, combination of montelukast and morphine could be noted as a new strategy for pain relief.

1. Introduction

Pain is one of the most frequent signs of different disorders and traces important medical and economic costs for the community (Jagerovic et al., 2002). Many pain suffers are not persuaded with their pain care and this makes investigating for new analgesics that can manage it more effectively which is an important challenge to drug research (Katz, 2002).

Growing body of evidences has demonstrated that the opioid receptors have an important role in the peripheral and central modulation of pain (Kanjhan 1995). Moreover, different inflammatory mediators are involved in the initiation and maintenance of pain/inflammatory pathway. In this regard, autacoids such as leukotrienes have pivotal role in mediating pain transmission, inflammation, ischemia, vascular injury, obstructive pulmonary disease, asthma, peptic ulcer, and also neuro-inflammation (Freiberg et al., 2009; Sood and Muthuraman, 2009; Zandman-Goddard et al., 2007; Jain et al., 2001). Also, it has been shown that the leukotrienes reduce the C-nociceptors threshold of activation and sensitizes intrapulpal A-delta fibers in hairy skin of rat hind limbs (Martin et al., 1987).

Montelukast is clinically applied for treatment of asthma by inhibiting the leukotriene receptors in bronchial smooth muscle. This drug acts through reduction of neutrophil infiltration, balancing oxidant-antioxidant status, and regulating the inflammatory mediators generation (Tugtepe et al., 2007). Recent preclinical studies have shown that lipooxygenase (LOX) enzyme inhibition by zileuton or leukotriene receptor antagonism by zafirlukast and montelukast caused antinociception in different inflammatory pain models (Singh et al., 2004, 2005). Moreover, Singh et al. (2004) reported that co-administration of zileuton and indomethacin resulted in enhanced anti-hyperalgesia when

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compared to each either alone. More importantly, clinical studies have indicated the antinociceptive effect of montelukast in children and adolescents with dyspepsia and duodenal eosinophilia (Friesen et al., 2004).

Further, some data have shown that sub-analgesic doses of morphine in combination with a leucotrienes antagonist could produce antinociception in hot-plate test (Gök et al., 1999). Capasso (1999) demonstrated in isolated guinea pig ileum, LOX inhibitors antagonize the contractions induced by naloxone challenge following acute opiate exposure. In the rat midbrain periaqueductal gray, the presynaptic inhibition of GABA release by opioid peptides is mediated by arachidonic acid metabolites generated by the activity of LOX enzyme (Vaughan et al., 1997; Christie et al., 2000). Trang et al. (2003) reported that activity of LOX metabolites, at the spinal level, contributes to the induction and expression of opioid physical dependence. Leukotrienes antagonists could also inhibit the opioid-induced withdrawal syndrome and induced neuroprotection (Rehni et al., 2008; Rehni and Singh, 2011).

Based on the above, the present study had the following objectives; (1) to determine the effects of systemic and peripheral administered montelukast on pain behaviors in rat formalin test, (2) to determine the effects of montelukast on visceral pain behaviors in mouse acetic acid induced writhing test, (3) to determine a potential involvement of opioid pathway in the systemic and local action of montelukast using naloxone as non-selective opioid antagonist, (4) to determine whether a combination of montelukast with morphine could enhances the antinociceptive effect.

2. Material and methods

2.1. Animals

Experiments were carried out using adult male Wistar rats (130–180 g) and Swiss mice (25–30 g) provided from the central animal house of Ahvaz Jundishapur University of Medical Sciences (Ahvaz-Iran). They were housed at 22 ± 2 °C under 12 h light/dark cycles (light from 7:00 to 19:00 h) with free access to food and water. The experimental protocol was approved by an Institutional Review Committee for the use of Human or Animal Subjects and the procedures were in accordance with at least the Declaration of Helsinki for human subjects or the National Institutes of Health's Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). The animals were used only once and then euthanized.

2.2. Chemicals

Montelukast and indomethacin was kindly gifted by Sobhan Pharmaceutical Co. (Tehran, Iran). Naloxone hydrochloride and morphine sulfate ($5H_2O$) were provided from Temad Pharmaceutical Co. (Tehran, Iran). montelukast, Naloxone hydrochloride and morphine sulfate were dissolved in physiological saline (0.9%). Indomethacin was dissolved in saline alkalinized with sodium bicarbonate. Drug concentrations were freshly prepared in such a way that the necessary dose could be injected.

2.3. Nociceptive tests

2.3.1. Formalin test

The antinociceptive effect was evaluated using the rat formalin model, which consisted of administering 50 μl of formalin 2.5% in the subplantar region of the right hind paw. In brief, immediately after formalin injection the animals were placed into the observation chamber and nociceptive behaviors were determined using the method described by Dubuisson and Dennis (Dubuisson and Dennis, 1977). The number of flinching (an elevation and shrinking back of the injected paw) of the hind paw was recorded every five minutes for a total of 60 min. The first 5 min post formalin injection is known as the first phase (phase 1) and the period between 15–60 min as the second phase (phase 2).

2.3.2. Writhing test

All animals were acclimatized to laboratory environment for at least 2 h before testing. The abdominal writhing induced by intraperitoneal administration of acetic acid in mice as described by Siegmund et al. (1957). The numbers of abdominal writhing episodes (contraction of the abdominal area with elongation of hind legs) were recorded for 30 min starting from 5 min after intraperitoneal injection of acetic acid in each animal.

2.4. Experimental protocols

2.4.1. Antinociceptive action of systemic montelukast in formalin test

To determine the systemic antinociceptive activity of montelukast during both phases of the test, doses of montelukast at 0.3, 1, 3 and 10 mg/kg or its vehicle (5 ml/kg) was intraperitoneally administered 30 min prior to formalin injection. Morphine (5 mg/kg) and indomethacin (10 mg/kg) were also used as standard drugs. The doses of drugs used have been selected based on literatures and our previous reports (Mansouri et al., 2014a; Hemmati et al., 2013; Daizo et al., 1999).

2.4.2. Antinociceptive action of peripheral montelukast in formalin test

The rats received a s.c. injection (50 μl) into the plantar surface of their right hind paw of either vehicles or montelukast (3, 6, 10 and 30 μg/paw) 20 min before formalin injection into the ipsilateral paw. To determine whether montelukast acted locally, it was administered to the left (contralateral) paw 20 min before injection of formalin into the right hind paw and the corresponding response on nociceptive behaviors was assessed. The reference drugs, morphine (25 μg/paw) and indomethacin (800 μg/paw), were also used for comparison. The doses of indomethacin and morphine used here have been selected according to previous reports and our experience in lab (Mansouri et al., 2014a; Ortiz, 2012; Rodrigues and Duarte, 2000).

2.4.3. Antinociceptive action of montelukast in writhing test

Briefly, montelukast (0.3, 1, 3, 6 and 10 mg/kg) or its vehicle were intraperitoneally administrated 30 min prior to acetic acid injection. Immediately after intraperitoneal injection of 0.1 ml/10 g acetic acid (0.6% v/v) in normal saline (0.9% NaCl), animals were isolated for observation.

2.5. Role of opioid receptors

2.5.1. Involvement of opioid receptors in the systemic antinociceptive action of montelukast

To determine the role of opioid receptors in the systemic antinociceptive action of montelukast (3 mg/kg), naloxone (non-selective opioid receptor antagonist) at dose 1 mg/kg was injected i.p. 15 min before montelukast administration.

2.5.2. Involvement of opioid receptors in the peripheral antinociceptive action of montelukast

To identify the participation of opioid receptors in the peripheral antinociceptive effect of montelukast (10 μg/paw), the rats were pre-treated with naloxone at dose 20 μg/paw (Mansouri et al., 2014a). Naloxone was injected into the formalin-injured paw

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