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Ellagic acid enhances morphine analgesia and attenuates the development of morphine tolerance and dependence in mice

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ABSTRACT

According to our previous study, ellagic acid has both dose-related central and peripheral antinociceptive effect through the opioidergic and L-arginine-NO-cGMP-ATP sensitive K^+ channel pathways. In the present study, the systemic antinociceptive effects of ellagic acid in animal models of pain, and functional interactions between ellagic acid and morphine in terms of analgesia, tolerance and dependence were investigated. Ellagic acid (1–30 mg/kg; i.p.) showed significant and dose-dependent antinociceptive effects in the acetic acid-induced writhing test. Intraperitoneal ellagic acid acutely interacted with morphine analgesia in a synergistic manner in this assay. Ellagic acid (1–10 mg/kg; i.p.) also exerted analgesic activity in the hot-plate test. Pre-treatment with naloxone (1 mg/kg; i.p.) significantly reversed ellagic acid, morphine as well as ellagic acid-morphine combination-induced antinociceptin in these two tests. More importantly, when co-administered with morphine, ellagic acid (1–10 mg/kg) effectively blocked the development of tolerance to morphine analgesia in the hot-plate test. Likewise, ellagic acid dose-dependently prevented naloxone-precipitated withdrawal signs including jumping and weight loss. Ellagic acid treatment (1–30 mg/kg; i.p.) had no significant effect on the locomotion activity of animals using open-field task. Therefore, these results showed that ellagic acid has notable systemic antinociceptive activity for both tonic and phasic pain models. Altogether, ellagic acid might be used in pain relief alone or in combination with opioid drugs because of enhancing morphine analgesia and preventing morphine-induced tolerance to analgesia and dependence.

1. Introduction

Opioids are used in the management of recurring acute pain, chronic non-malignant pain with organic origin, severe neuropathic pain and moderate to severe pain associated with cancer (Cherny, 1996; Martin and Eisenach, 2001). However, clinical usefulness of these drugs in the treatment of chronic pain is limited by their side effects in particular analgesic tolerance and physical dependence (Bhargava, 1994). As other analgesics are often used in combination with morphine to minimize the dose, several criteria should be met for an ideal fixed dose combination of morphine with them. For example, synergistic or at least additive interaction between morphine and other analgesics is expected and ideally the combined analgesics should be able to block morphine tolerance (Meert, 1996).

Growing pieces of evidence have been demonstrated that nitric oxide (NO) and prostaglandins play a role in the development of tolerance to analgesic effect of morphine. Enhancement of NO production results in increasing the rate and extent of tolerance development to morphine analgesia (Bhargava and Bian, 1998). Likewise, inhibition of NO production attenuates the tolerance with time (Majeed et al., 1994). In addition, Adams et al. (1993) reported that nitric oxide synthase (NOS) inhibitors prevent some aspects of the naloxone-precipitated withdrawal syndrome. Moreover, it has been shown that the free radical scavenging agents as well as cyclooxygenase enzyme inhibitors could be potential tools in the prevention of morphine tolerance and withdrawal syndrome (Powell et al., 1999).

Flavonoids are bioactive polyphenols which possess multiple protective actions in some central nervous pathophysiological conditions, especially the modulation of pain transmission (Ullah and Khan, 2008). Ellagic acid (2,3,7,8 tetrahydroxy [1] benzopyranol [5,4,3-cde] [1] benzopyran-5,10-dione) is a polyphenolic compound that occurs largely as ellagitannins in plants such as raspberries, the stem and bark of eucalyptus species and

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nuts (Clifford and Scalbert, 2000). Also, ellagic acid, a major component of pomegranate juice, is an increasingly popular dietary supplement used by the American adult population (Corbett et al., 2010). This bioflavonoid has been reported to have antioxidant, antifibrotic, anti-inflammatory, cardioprotective, anticancer and antinociceptive properties (Giris et al., 2012; Gainok et al., 2011). We have recently established the analgesic effect of ellagic acid in different animal models of pain. According to our results, the dose-related antinociceptive effect of ellagic acid has both peripheral and central components which are mediated by opioidergic and L-arginine-NO-cGMP-K^{+}_{ATP} mechanisms (Mansouri et al., 2013). Moreover, some reports have indicated that flavonoids such as quercetin, flavone, catechin, and chrysin could reverse or prevent the naloxone-precipitated withdrawal contractures of the acute morphine-dependent guinea pig ileum (Cappaso et al., 1998).

Based on the above findings, this study was planned to elucidate possible interaction between morphine and ellagic acid in terms of pain, tolerance to morphine analgesia, and dependence in mice. The specific protocols were used to determine: (1) the acute antinociceptive interaction between systemic administration of ellagic acid and morphine and the nature of this interaction by isobolographic method; (2) the sub-acute (5-day) interaction between morphine and ellagic acid in tolerance to analgesia; (3) whether systemic treatment with ellagic acid could prevent naloxone-precipitated withdrawal signs.

2. Materials and methods

2.1. Drugs and Chemicals

Ellagic acid was obtained from Sigma-Aldrich (St. Louis, MO, USA). Morphine sulfate was gifted from Temad Pharmaceutical Co. (Tehran, Iran), while naloxone hydrochloride was purchased from Tolidaru pharmaceutical Co. (Tehran, Iran). Acetic acid was obtained from Merck Co. (Darmstadt, Germany). All test drugs were freshly dissolved in sterile physiological normal saline solution with the pH adjusted to 7.3–7.5 by 0.1 N NaOH as needed, while ellagic acid was dissolved in normal saline containing 10% dimethylsulfoxide (DMSO). Doses and drug administration schedules were based on our experience in laboratory (Mansouri et al., 2013) and other previous reports (Beltz et al., 2008; Rogerio et al., 2006).

2.2. Animals

Experiments were conducted using adult male Swiss mice (25–30 g) obtained from the central animal house of Jundishapur University of Medical Sciences (Ahvaz-Iran). They were housed at 22 ± 2 °C and 12 h light/dark cycles (light from 7:00 to 19:00 h) with free access to food and water *ad libitum*. All animals were randomly divided into groups of eight in each, acclimatized and habituated to the laboratory environment for at least one week prior to the experiments, and used only once throughout the experiments. All behavioral observations were carried out by a blinded investigator.

Animal care and experimental procedures were in accordance with the NIH Guide for Care and Use of Laboratory Animals (Publication no. 85-23, revised 1985). Also, we followed the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983), as well as our institutional guidelines for experiments with animals, designed to avoid suffering and limit the number of animals.

2.3. The mouse acetic acid writhing test

The writhing test was selected as a model of acute visceral pain, because it can be a model of clinical relevant intestinal nociception in humans (Reichter et al., 2001). In brief, ellagic acid (1–30 mg/kg), morphine (0.25–3 mg/kg) or the corresponding vehicles were intraperitoneally (i.p.) administrated 30 min before acetic acid injection. Immediately after i.p. injection of 0.1 ml/10 g acetic acid (0.6% v/v in normal saline), the animals were isolated for observation. The numbers of abdominal writhing episodes were recorded for 30 min, starting 5 min after injection of acetic acid in each animal. A writhe was defined as a contraction of the abdominal muscles accompanied by an elongation of the body and extension of the hind limbs. Antinociceptive activity (reduction in writhes) was expressed as percent of maximal possible effect (% MPE) that was calculated by the following equation: %MPE = [100 × (mean writhes in control group − mean writhes in drug (s) treated group)]/mean of writhes in control group (Jain et al., 2001).

2.4. The mouse hot-plate test

Pain reflexes in response to thermal stimulus in the hot-plate test were evaluated according to Eddy and Leimbach's method (1953). Mice were individually placed on a 55 ± 1 °C hot-plate which was surrounded by a clear acrylic cage, and latency time (s) to either hind paw licking or jumping (whichever came first) was recorded. The cut-off time was set as 45 s to avoid tissue injury. Before drug administration, the hot-plate latency was measured 3 times, and the average of the second and third trials was used as the pre-drug reaction latency at 0 min. The latency was also measured at 30, 60, and 90 min following i.p. drug injection. After testing, the time-course of response curves was constructed. The antinociceptive effects of a single dose of morphine (10 mg/kg, s.c.) or ellagic acid (1–10 mg/kg, i.p.) were determined before the following studies.

2.5. Involvement of opioid receptors in ellagic acid-induced antinociception in the writhing and hot-plate tests

To evaluate the involvement of opioid receptors in the antinociceptive activity of ellagic acid, animals were pre-treated with naloxone (1 mg/kg, i.p.), a non selective opioid antagonist, 15 min before administration of the ellagic acid (3 mg/kg, i.p.) alone or combined with effective dose of morphine (1 mg/kg, i.p.) and tested by using acetic acid induced writhing test (Santos et al., 1999). The number of writhing was measured as mentioned above.

In a separate set of experiments, to assess the possible contribution of the opioid receptor in the hot-plate test, mice were pre-treated with naloxone (1 mg/kg, i.p.) and after 15 min they received ellagic acid (10 mg/kg, i.p.), morphine (10 mg/kg, i.p.) or vehicle (Santos et al., 1999). Other groups of animals received only ellagic acid, morphine, or vehicle injection.

2.6. Induction and assessment of the morphine tolerance and dependence

The loss of analgesic activity of morphine in the hot-plate test was used to assess the degree of tolerance. In this set of experiments, 40 mice were given saline or morphine. Mice that treated with morphine, were subdivided into four groups of eight mice each and received i.p. saline containing 10% DMSO as vehicle or ellagic acid (1, 3 and 10 mg/kg, i.p.) and then, 30 min later, injected with morphine (10 mg/kg, s.c.) twice daily (09:00 and 17.00) for 5 consecutive days (Ren et al., 2004). The control group was treated with saline containing 10% DMSO as vehicle and then,