Acta Anaesthesiologica Taiwanica 52 (2014) 163–168

Contents lists available at ScienceDirect

Acta Anaesthesiologica Taiwanica

journal homepage: www.e-aat.com

Original Article

Attenuation of morphine-induced dependence and tolerance by ceftriaxone and amitriptyline in mice

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ARTICLE INFO

Article history:
Received 9 April 2014
Received in revised form
7 November 2014
Accepted 12 November 2014

Key words:
amitriptyline;
β-lactam antibiotic;
ceftriaxone;
dependency;
morphine;
tolerance

ABSTRACT

Introduction: Tolerance to and dependence on the analgesic effect of opioids is a pharmacological phenomenon that occurs after their prolonged administration.
Objective: The aim of this study was to evaluate the protective effects of ceftriaxone and amitriptyline on the development of morphine-induced tolerance and dependence.
Methods: In this study, 18 groups (9 groups each for tolerance and dependency tests) of mice ($n = 8$) received saline [10 mL/kg, intraperitoneally (i.p.)], morphine (50 mg/kg, i.p.), ceftriaxone (50 mg/kg, i.p., 100 mg/kg, i.p., and 200 mg/kg, i.p.), amitriptyline (5 mg/kg, i.p., 10 mg/kg, i.p., and 15 mg/kg, i.p.), or a combination of ceftriaxone (50 mg/kg, i.p.) and amitriptyline (5 mg/kg, i.p.) once per day for 4 days for investigation and comparison of the effects of ceftriaxone and amitriptyline on the prevention of dependency and tolerance to morphine. Tolerance was assessed with administration of morphine (9 mg/kg, i.p.) and using the hot plate test on the 5th day. In dependency tests, withdrawal symptoms were assessed on the 4th day for each animal 30 minutes after the administration of naloxone (4 mg/kg, i.p.; 2 hours after the last dose of morphine).
Results: It was found that treatment with ceftriaxone or amitriptyline attenuated the development of tolerance to the antinociceptive effect of morphine and also reduced naloxone-precipitated withdrawal jumping and standing on feet. Furthermore, coadministration of ceftriaxone and amitriptyline at low doses (50 mg/kg, i.p. and 5 mg/kg, i.p., respectively) prior to morphine injection also decreased both morphine-induced tolerance and dependence.
Conclusion: Results indicate that the treatment with ceftriaxone and amitriptyline, alone or in combination, could attenuate the development of morphine-induced tolerance and dependence.

1. Introduction

Millions of people worldwide suffer from chronic pain brought on by diseases such as arthritis and cancer. The management of chronic pain is one of the greatest challenges in modern medicine. Opiates such as morphine have been widely used to treat various kinds of pain for decades. Long-term use of morphine is limited because of unwanted side effects including tolerance and dependence. Development of antinociceptive tolerance leads to increasing doses to control pain, and in some cases narcotics become ineffective and a higher drug dose is required to maintain the same level of effectiveness.[1,2] Dependence is a continuous need for a drug to maintain a state of physical equilibrium following repeated consumption of opiates, and is indicated by withdrawal symptoms when opiate administration is terminated.[3,4] In the past decades, many studies were conducted to clarify the mechanisms involved in morphine-induced tolerance or dependence and have focused on the attenuation of these effects for management of chronic pain treatment. Morphine-induced tolerance and dependence is a complex physiological response that involves a within-system and a between-system adaptation. The within-system adaptations include opioid receptors uncoupling from G proteins and receptor downregulation. Between-system adaptations, such as the

Conflicts of interest: The authors declare that they have no conflicts of interest.

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http://dx.doi.org/10.1016/j.aat.2014.11.001

pain facilitatory systems (opiate-activated opponent systems), also play an important role in the development of opioid-induced tolerance and dependence.[5–8] The activation of the ionotropic *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors has been implicated in the development of morphine analgesic tolerance and dependence.[7,9–11] Chronic opioid treatment resulted in the activation of protein kinase C and translocation that phosphorylates the NMDA receptor-gated Ca channel, leading to potentiation of NMDA receptor activity. Opening of these channel leads to an influx and increases intracellular Ca^{2+} concentration, which produces several effects.[10,12–15] Furthermore, excitatory amino acids, their activated receptors, such as NMDA receptors, and the subsequent downstream signals [such as nitric oxide (NO)] are probably involved in opioid-induced tolerance and dependence. Other studies showed that proinflammatory cytokines, released from activated glial cells after repeated opioid administration, participate in the between-system mechanism.[16–19]

Glutamate transporters are critical for glutamate removal from the extracellular space and are essential for maintaining homeostatic levels of extracellular glutamate. Decreasing extracellular glutamate by overexpressing the predominant astrocytic glutamate transporter was found to be effective in animal models of both visceral and neuropathic pain. Recently, it was found that β-lactam antibiotics upregulate glutamate transporters and increasing glutamate uptake through glutamate transporter subtype 1 (GLT-1) activation. Among all β-lactam antibiotics studies, ceftriaxone showed the highest potency in the upregulation of glutamate transporter. Ceftriaxone, a β-lactam antibiotic, is one of the members of third-generation cephalosporins. It is readily transported across the blood–brain barrier and is effective against gram-negative and gram-positive bacteria through the inhibition of cell wall synthesis. Ceftriaxone enhances both protein expression and functional activity of GLT-1 via a mechanism involving the nuclear factor-κ B signaling pathway. The glutamate transporter plays a major role in the maintenance of glutamate homeostasis. Among the five glutamate transporters, GLT-1 is responsible for 90% of glutamate uptake in the central nervous system (CNS). These findings suggest the high efficiency of ceftriaxone in the upregulation of GLT-1 and the reduction of glutamate excitotoxicity.[20–22]

Tricyclic antidepressants (TCAs) such as amitriptyline are primarily used for mood disorders. They are also widely used to treat chronic pain such as neuropathic pain conditions. TCAs increase both synaptic concentrations of serotonin or norepinephrine and therefore enhance neurotransmission. TCAs produce analgesia via various mechanisms involving NMDA receptors, biogenic amines, opioids, inflammatory mediators, and substance P. Amitriptyline is a glutamate transporter activator and used in the treatment of depression. Amitriptyline can be effective in prevention of morphine-induced dependence and tolerance. The proposed involved mechanisms are inhibition of proinflammatory cytokine, prevention of glutamate transporter downregulation, and enhancement of the activity of glutamate transporters.[23–27]

The aim of this study was to evaluate the attenuation effects of pretreatment with different doses of ceftriaxone, amitriptyline, and their combination on the development of morphine-induced tolerance and dependence.

2. Materials and methods

2.1. Drugs

Morphine sulfate and naloxone were purchased from Darou Pakhsh Company (Tehran, Iran) and Tolid Daru Company (Tehran, Iran), respectively. Ceftriaxone hydrochloride and amitriptyline were obtained from Jaber Ebne Hayyan Pharmaceutical Company (Tehran, Iran) and Sobhan Pharmaceutical Company (Tehran, Iran), respectively.

2.2. Animals and treatment

Adult male Albino mice (Provided from Pasteur Institute, Tehran, Iran) weighing 20–30 g (aged 8 weeks) were allocated randomly to different groups ($n = 8$). The animals were maintained under standard temperature (24 ± 0.5°C) and lighting conditions (12-hour light/12-hour darkness) with free access to food and water.

All experiments were executed in accordance with the Guide for Care and Use of Laboratory Animals of Tabriz University of Medical Sciences, Tabriz, Iran (National Institutes of Health Publication No 85-23, revised 1985). This study was conducted at the Faculty of Pharmacy of Tabriz University of Medical Science.

Nondependent and morphine-dependent control groups were administered intraperitoneally (i.p.) with normal saline (10 mL/kg + 10 mL/kg) as well as normal saline (10 mL/kg) and morphine (50 mg/kg, i.p.) for 4 days, respectively. For evaluation of the effects of different doses of ceftriaxone and amitriptyline on the prevention of the morphine-induced tolerance, mice were pretreated for 4 days with ceftriaxone (50 mg/kg, i.p., 100 mg/kg, i.p., and 200 mg/kg, i.p.), amitriptyline (5 mg/kg, i.p., 10 mg/kg, i.p., and 15 mg/kg, i.p.) and ceftriaxone (50 mg/kg, i.p.) + amitriptyline (5 mg/kg, i.p.) 30 minutes prior to morphine injection (50 mg/kg, i.p.). Subsequently, to evaluate the degree of tolerance, the antinociceptive effect of a test dose of morphine (9 mg/kg, i.p.) was measured 24 hours after the last dose of morphine using a hot plate.

Furthermore, to assess the effects of the different doses of ceftriaxone and amitriptyline on the attenuation of dependence to morphine, withdrawal symptoms (number of jumping and standing on feet) for each animal (in the 9 groups; such as tolerance test) during 30 minutes were assessed on the 4th day after the administration of naloxone (4 mg/kg, i.p., 2 hours after the last dose of morphine).

2.3. Hot plate test

The antinociceptive activities of ceftriaxone and amitriptyline were determined by exposing the animals to potentially painful stimuli such as heat or electric shock and measuring either the time it takes these animals to respond to the stimuli or the intensity with which they respond. In this method, the time taken by the mice to lick its hind paws placed on a hot plate's stainless steel surface (23 cm × 23 cm and 55 ± 2°C) was determined. This reaction time was taken as the end point, and the increase in hot plate latency (seconds) was taken as a measure of the analgesic activity. The animals were removed from the hot plate if they did not respond within 30 seconds (cutoff time) in order to avoid tissue damage. Thirty minutes prior to treatment the nociceptive threshold was measured, and the latency time was used as the predrug latency for each test animal. Hot plate response latency is expressed as the percentage of maximal possible effect (MPE%) according to the following equation:

$$\mathrm{MPE\%} = [(\mathrm{TL} - \mathrm{BL})/(T\ \mathrm{cutoff} - \mathrm{BL})] \times 100,$$

where TL denotes test latency time and BL is the base latency time.

2.4. Withdrawal symptoms test

Mice were tested for the degree of dependence after administration of morphine (50 mg/kg, i.p.) for 4 consecutive days. Injection of naloxone (4 mg/kg, i.p.), 2 hours after the last dose of morphine, precipitated severe withdrawal symptoms in morphine-