Acta Anaesthesiologica Taiwanica 54 (2016) 65–69

Contents lists available at ScienceDirect

Acta Anaesthesiologica Taiwanica

journal homepage: www.e-aat.com

Research paper

A new pharmacological role for thalidomide: Attenuation of morphine-induced tolerance in rats

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ARTICLE INFO

Article history:
Received 15 February 2016
Received in revised form
14 May 2016
Accepted 16 June 2016

Keywords:
analgesia;
morphine tolerance;
thalidomide

ABSTRACT

Objective: Tolerance to the analgesic effect is the main side effect of chronic administration of opioids. Several drugs have been studied to try to find agents to prevent the development of this phenomenon. In the present study we aimed to evaluate the effect of thalidomide on morphine-induced tolerance to the analgesic effect.

Methods: Groups of male rats were randomly rendered and received daily morphine in combination with thalidomide vehicle or thalidomide (2.5 mg/kg, 5 mg/kg, or 10 mg/kg, intraperitoneally). Nociception was measured using the plantar test apparatus. Latency time was recorded when the animal reacted to the light stimulus; licking or raising its hind paw. Treatments and evaluations continued until completion of tolerance to the analgesic effect of morphine.

Results: Our findings indicated that tolerance was achieved following 11 days of morphine administration, while thalidomide postponed the day of tolerance completion for 4 days (2.5 mg/kg and 5 mg/kg thalidomide) or 10 days (10 mg/kg thalidomide). Moreover, thalidomide prevented the morphine-induced shift to the right of the ED_{50} in the dose–response curve.

Conclusion: It was concluded that thalidomide attenuated the morphine-induced tolerance to the analgesic effect.

1. Introduction

Tolerance to the analgesic effect of opioids is one of the major side effects associated with their long-term administration. This phenomenon limits the beneficial therapeutic use of opioids. Many attempts have been made to find agents that can prevent this adverse effect.

In recent years, inflammatory mediators have been reported to play a key role in pathways of opioid-tolerance induction.[1–3] Morphine augments the secretion of inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), and interleukin 6 through glial cell activation.[4] These inflammatory products enhance the neuronal excitability and sensitize the pain transmission neurons.[5,6] TNF-α may have a pivotal role in the genesis of mechanical inflammatory hyperalgesia in rats.[7] It augments glutamate neurotoxicity, upregulates the expression of the NMDA receptors and enhances the glutamatergic transmission.[5,8]

Several lines of evidence indicate the benefit of agents that interfere with the inflammatory signaling pathways as a strategic approach to attenuate development of morphine tolerance.[9,10] It has been shown recently that neuroprotective and anti-inflammatory agents, such as ketamine, minocycline, riluzole, donepezil, and pioglitazone, prevented morphine-induced tolerance and apoptosis in the rat central nervous system.[11–15] Researchers indicated that etanercept, a TNF-α inhibitor, restored the antinociceptive effect of morphine by inhibiting the spinal proinflammatory cytokine (e.g., TNF-α, IL-1β, and IL-6) expression in the morphine-tolerant rats.[9]

Conflict of interest: There is no conflict of interest.

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http://dx.doi.org/10.1016/j.aat.2016.06.002

There is evidence indicating that thalidomide inhibits the production of human monocyte TNF-α and alveolar macrophages.[16,17] Thalidomide, a glutamic acid derivative, was approved in 1998 by the US Food and Drug Administration for erythema nodosum leprosum and in 2006 for multiple myeloma.[18] This drug was a common over-the-counter sedative and antiemetic until 1961, when it was withdrawn because of teratogenicity.[19] In 1965 an accidental discovery of its immunomodulatory effects was made in erythema nodosum leprosum patients.[20,21] Today thalidomide and its analogs have shown efficacy against a wide variety of diseases, including inflammation and cancer.[22] Thalidomide exerts its inhibitory action on TNF-α by enhancing mRNA degradation.[23] In addition, it was reported to inhibit inflammatory hyperalgesia in rats and the writhing nociceptive response in mice, possibly due to inhibition of TNF-α production.[7]

The evidence from these studies prompted us to investigate the effect of thalidomide on morphine-induced tolerance to the analgesic effect.

2. Materials and methods

2.1. Drugs

Morphine sulfate was purchased from Temad Company (Tehran, Iran). It was dissolved in normal saline and injected using 1-mL insulin syringes. Thalidomide (Sigma-Aldrich Inc., Chemie GmbH, Germany) was dissolved in vehicle (dimethyl sulfoxide + saline, 4:1) and injected intraperitoneally (i.p.). All the solutions were freshly prepared on the day of the experiment.

2.2. Animals

Male Wistar rats ($n = 88$) weighing 250–300 g were purchased from the Razi Institute (Tehran, Iran). The animals were kept in temperature-controlled conditions (25 ± 2C) and standard cages (four rats per cage), on a 12-h light/dark cycle with free access to food and water *ad libitum*. They were randomly divided into several experimental groups, eight animals per group. In order to minimize the nonspecific stress response, animals were habituated to the testing environment, including transfer to the experimental laboratory, weighing, and handling. All the experiments were in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85–23, revised 1985), and were approved by the research and ethics committee of Kurdistan University of Medical Sciences.

2.3. Experimental details

The experimental groups are described in Table 1.

2.3.1. Model of tolerance induction

In order to induce a tolerance to the analgesic effect, morphine (10 mg/kg, i.p.) was injected daily according to our previous study.[24] It was injected daily 30 minutes after the thalidomide administration.

2.3.2. Assessment of nociception

The nociception was assessed using a plantar test apparatus (IITC Inc. Life Science, Los Angeles, USA) (Hargreaves method).[25] Rats were placed on a glass plate on the plantar test apparatus and a noxious heat source was held directly under the hind paw. As soon as it was started, the device supplied a continuous beam stimulus to the paw and the withdrawal reflex was produced. The latency time between exposure to the radiant and the paw withdrawal was measured as the analgesia. For each animal the average for three measurements of the baseline paw-withdrawal latency was determined as the baseline latency.

The intensity of the light was adjusted so that the baseline latencies were 8–10 seconds, with a cut-off time of 20 seconds in order to avoid tissue damage. Two measurements of the latency were averaged for each hind paw in each test session. Maximal possible effect (%MPE) using the following equation was measured for the latency-withdrawal response for each rat:

$$\%MPE = [\text{postdrug latency (s)} - \text{baseline latency (s)}] / [\text{cut-off value (s)} - \text{baseline latency (s)}] \times 100$$

It is worth noting that the baseline latency was determined once per day for each rat before the daily injection of morphine (10 mg/kg). Later, the drugs or their vehicles were injected. Thirty minutes after drug/vehicle administration, morphine was injected. Finally, 30 minutes after morphine administration, the postdrug latency was measured. Moreover, the baseline and the latency time were registered daily and the %MPE was calculated. The experiments were continued until there was no significant difference in the % MPE between the vehicle- or the drug-treated groups (the tolerant animals) and the vehicle-received group.[26]

2.3.3. Total analgesic effect assessment

In order to assess the total analgesic effect in different groups, the area under the curve (AUC) for %MPE against the time was calculated. This analysis allows a comparison of the effects from different analgesic tests. The AUC was calculated from the observed values using the trapezoidal rule.

2.3.4. Analgesic dose–response curves

The dose–response curve was plotted for each group for assessment of tolerance induction. Rats received the thalidomide vehicle or the morphine + thalidomide vehicle or morphine + thalidomide once a day for 11 days. On Day 12 (1 day after the morphine-tolerance

Table 1
The experimental groups.

Study sections	Treatment groups ($n = 8$ per group)	
Tolerance evaluation groups	Saline (1 mL/kg, i.p.) + thalidomide vehicle[a] (1 mL/kg, i.p.)	
	Morphine (10 mg/kg, i.p.) + thalidomide vehicle (1 mL/kg, i.p.)	
	Morphine (10 mg/kg, i.p.) + thalidomide (2.5 mg/kg, i.p.)	
	Morphine (10 mg/kg, i.p.) + thalidomide (5 mg/kg, i.p.)	
	Morphine (10 mg/kg, i.p.) + thalidomide (10 mg/kg, i.p.)	
	Groups for dose–response curves:	
	Animals received opposite treatments for 11 days, on Day 12, in separate groups, logarithmic doses of morphine (1/10/100 mg/kg, i.p.) were administered to generate analgesic dose–response curves	Saline (1 mL/kg, i.p.) + thalidomide vehicle (1 mL/kg, i.p.) Morphine (10 mg/kg, i.p.) + thalidomide vehicle (1 mL/kg, i.p.) Morphine (10 mg/kg, i.p.) + thalidomide (10 mg/kg, i.p.)

i.p. = intraperitoneally.
[a] Thalidomide vehicle = dimethyl sulfoxide + saline, 4:1.