European Journal of Pharmacology 702 (2013) 62–70

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and analgesia

# Inhibition of brain oxidative stress and inducible nitric oxide synthase expression by thymoquinone attenuates the development of morphine tolerance and dependence in mice

Ahmed O. Abdel-Zaher [a,*], Mostafa G. Mostafa [b], Hanan M. Farghly [a], Mostafa M. Hamdy [a], Ghada A. Omran [c], Najlaà K.M. Al-Shaibani [a]

[a] Department of Pharmacology, Faculty of Medicine, Assiut University, Assiut, Egypt
[b] Department of Anesthesia, South Egypt Cancer Institute, Assiut University, Assiut, Egypt
[c] Department of Forensic Medicine and Toxicology, Faculty of Medicine, Assiut University, Assiut, Egypt

ARTICLE INFO

*Article history:*
Received 6 August 2012
Received in revised form
10 January 2013
Accepted 16 January 2013
Available online 30 January 2013

*Keywords:*
Thymoquinone
Morphine
Tolerance
Dependence

ABSTRACT

In this study, the effect of thymoquinone on morphine-induced tolerance and dependence in mice was investigated. Repeated administration of thymoquinone along with morphine attenuated the development of morphine tolerance, as measured by the hot plate test, and dependence, as assessed by naloxone-precipitated withdrawal manifestations. Concurrently, morphine-induced progressive increase in brain malondialdehyde (MDA) level and nitric oxide (NO) production as well as progressive decrease in brain intracellular reduced glutathione (GSH) level and glutathione peroxidase (GSH-Px) activity were inhibited by co-administration of thymoquinone. Morphine-induced progressive increase in brain glutamate level was not inhibited by concomitant administration of thymoquinone. Similarly, co-administration of thymoquinone inhibited naloxone-induced increase in brain MDA level, NO overproduction and decrease in brain intracellular GSH level and GSH-Px activities but it did not inhibit naloxone-induced elevation of brain glutamate level in morphine-dependent mice. The inhibitory effect of thymoquinone on morphine-induced tolerance and dependence on naloxone-induced biochemical alterations in morphine-dependent mice was enhanced by concurrent i.p. administration of the NMDA receptor antagonist, dizocilpine, the antioxidant, N-acetylcysteine or the NO synthase inhibitor, L-N (G)-nitroarginine methyl ester. On the other hand, this inhibitory effect of thymoquinone was antagonized by concurrent i.p. administration of NO precursor, L-arginine. In addition, concomitant administration of thymoquinone inhibited morphine tolerance and dependence-induced increase in inducible but not in neuronal NO synthase mRNA expression in mice brain. These results demonstrate that inhibition of morphine-induced oxidative stress, increase in the expression of brain inducible NO synthase and NO overproduction by thymoquinone can attenuate the development of morphine tolerance and dependence.

© 2013 Elsevier B.V. All rights reserved.

## 1. Introduction

Thymoquinone, the major active component of the volatile oil of *Nigella sativa* seed, has been reported to prevent $CCl_4$-induced oxidative injury in isolated rat hepatocytes (Mansour et al., 2001) and in rat liver (Kanter et al., 2005). Similarly, thymoquinone was found to ameliorate liver dysfunction induced by bile duct ligation (Oguz et al., 2012), cyclophosphamide (Alenzi et al., 2010) and acute endotoxemia (Helal, 2010) in rats by up-regulation of antioxidant mechanisms. Furthermore, Hosseinzadeha et al. (2007) reported that thymoquinone has antioxidant effects during cerebral ischemia-reperfusion injury in rat hippocampus. In addition, thymoquinone was demonstrated to be effective in protecting mice against acetaminophen-induced hepatotoxicity possibly via increased resistance to oxidative and nitrosative stresses (Nagi et al., 2010). In rat kidney, thymoquinone was also found to inhibit gentamicin (Sayed-Ahmed and Nagi, 2007) and cyclosporine (Uz et al., 2008)-induced oxidative stress and nitric oxide (NO) overproduction. Morever, It has been reported that thymoquinone suppresses inducible NO synthase expression (El-Mahmoudy et al., 2002) in lipopolysaccharide-stimulated rat peritoneal macrophages and produce antianxiety-like effects in mice through modulation of NO levels (Gilhotra and Dhingra, 2011).

The augmented glutamate release has been found to play an important role in morphine tolerance, dependence and withdrawal symptoms (Sepulveda et al., 1998; Wen et al., 2004).

* Corresponding author. Fax: +20 88 2332278.
*E-mail address:* ahmedosmanaz@hotmail.com (A.O. Abdel-Zaher).

0014-2999/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2013.01.036

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 (Bajo et al., 2006; Murray et al., 2007; Wang et al., 2004). Overactivation of glutamatergic system is accompanied by increased formation of reactive oxygen species (Alekseenko et al., 2009). Furthermore, morphine was found to induce oxidative stress in brain (Guzmán et al., 2006; Ibi et al., 2011; Ozmen et al., 2007). Also, it has been established that many glutamate actions mediated through NMDA receptors result from the subsequent activation of NO synthase and the formation of NO (Kone et al., 2003). In addition, a variety of non-competitive and competitive NMDA receptor antagonists, free radical scavengers and NO synthase inhibitors were found to suppress the development of morphine tolerance and dependence (Homayoun et al., 2003; Huang et al., 2012; Liu et al., 2011; Mori et al., 2007; Wang et al., 2004). Thus, oxidative stress and NO play a role in morphine tolerance and dependence (Mori et al., 2007; Ozek et al., 2003; Ozdemir et al., 2011).

In light of these consideraions, the aim of this work was to evaluate the potential role of thymoquinone in attenuation of morphine tolerance and dependence. In addition, an attempt was undertaken to clarify the possible role of glutamate, oxidative stress and NO synthase isoforms in these effects.

## 2. Materials and methods

### 2.1. Animals and treatments

Male adult Swiss-Webster mice weighing 22–30 g from the animal house of Assiut University were used in all experiments. The animals were housed in stainless steel cages under a 12 h light/dark cycle at 25 °C and allowed water and food (laboratory chow) ad libitum. They were divided into groups, 8 animals each. The research was conducted in accordance with the internationally accepted principles for Guide for the Care and Use of Laboratory Animals. The experiments reported here were approved by our institutional ethics committee.

Animals of Group-I were treated subcutaneously (s.c.) with 5 mg/kg morphine (0.1% solution in saline) twice daily at 12 h intervals for seven days. Groups-II mice received 10 mg/kg thymoquinone [1% solution in normal saline in presence of 0.1% (v/v) tween 80] intraperitoneally (i.p.), 30 min before each morphine injection for seven days.

Animals of groups III to VI were pretreated, 30 min before each morphine injection for seven days, with 10 mg/kg thymoquinone in combination with dizocilpine (MK-801), N-acetylcysteine (NAC), L-arginine (L-ARG) or L-N (G)-nitroarginine methyl ester (L-NAME). One group of animals was used for each treatment. MK-801(0.005% solution in saline), NAC (1% solution in saline), L-ARG (1% solution in saline) and L-NAME (0.25% solution in saline) were injected i.p. at dose levels of 0.25, 50, 300, and 10 mg/kg, respectively.

Control groups of animals were treated likewise with the pure vehicles [normal saline or normal saline in presence of 0.1% (v/v) tween 80].

### 2.2. Hot plate test

In this method, the time taken by the mouse to lick its hind paws or to jump with all four feet within a plexiglass cylinder placed on a hot plate surface (55 °C) was determined. This reaction time was taken as the end point and the increase in hot plate latency was taken as a measure of the analgesic activity. Prior to administration of drugs, mice were tested on the hot plate for four days in order to obtain a stable control response level. The animals were removed from the hot plate if they did not respond within 30 s in order to avoid tissue damage. Any animal failed to respond within 30 s was excluded immediately and retested again after 30 min. The antinociceptive effect of morphine was determined 60 min after the first treatment on the first, third, fifth, and seventh day.

### 2.3. Induction of withdrawal syndrome

On the eighth day, two hours after the first treatment, each mice was injected with 5 mg/kg naloxone (0.1% solution in saline) i.p. Immediately after naloxone injection each animal was placed in a transparent acryl cylinder (20 cm in diameter, 35 cm in height) to observe withdrawal manifestations (jumping, rearing, paw tremor and teeth chattering) for 30 min. The withdrawal manifestations were manually evaluated by co-workers blind to the treatment protocol. Control animals were tested before and after naloxone administration.

### 2.4. Biochemical measurements

After recording of the withdrawal manifestations, animals were sacrificed by decapitation. Blood and brain tissues were obtained from each animal for biochemical measurements. Blood and brain tissues were also obtained on the first, third,\fifth and seventh day of treatment of other groups of mice twice daily with 5 mg/kg morphine or 5 mg/kg morphine 30 min after administration of 10 mg/kg thymoquinone i.p. These animals were not subjected to hot plate test or naloxone challenge.

The brain was rinsed in ice-cold saline, blotted carefully and weighed. Then the brain was placed in a glass homogenizer containing 2 ml phosphate buffer (pH 7.4) or saline. After homogenization, by using motor-driven Teflon pestle, the homogenate was divided into two equal portions. The first portion was centrifuged for 10 min and the supernatant was used for estimation of malondialdehyde (MDA) level, and glutathione peroxidase (GSH-Px) activity. To the second part of the homogenate an equal volume of perchloric acid (1 mol/l) was added and mixed by vortexing. The mixture was allowed to stand for 5 min at room temperature. After centrifugation for 10 min, the supernatant was collected carefully and used for estimation of glutamate and intracellular reduced glutathione (GSH) levels directly or stored at −20 °C until assay.

For determination of glutamate, the supernatant fluid was adjusted to pH 9 with phosphate solution [1.93 mol/l tripotassium phosphate ($K_3PO_4.3\,H_2O$) solution]. The glutamate content in the supernatant was measured spectrophotometrically via its enzymatic dehydrogenation with conversion of β-nicotinamide adenine dinucleotide ($NAD^+$, oxidized form), to β-nicotinamide adenine dinucleotide (NADH, reduced form) according to the method of Lund (1986). A standard reference curve was prepared for each assay.

The intracellular GSH content of the neutralized supernatant was assayed using Ellman's reagent [5, 5-dithio-bis-2-nitrobenzoic acid (DTNB solution)] according to the method of Grififith (1980). A standard reference curve was prepared for each assay.

Lipid peroxidation, a major indicator of oxidative stress, was determined by measuring of the MDA level in tissue homogenates. MDA is an end product of lipid peroxidation and its level was determined spectrophotometrically by using the thiobarbituric acid reactive substances method previously described by Ohkawa et al. (1979). A standard curve was run simultaneously with each set of samples by using 1, 1, 3, 3-tetramethoxypropane as an external standard.

The GSH-Px activity was measured by the method of Paglia and Valentine (1967). The enzyme reaction in the tube which contains β-nicotinamide adenine dinucleotide phosphate (β-NADPH, reduced form), GSH, glutathione reducates and a sample or a standard was initiated by addition of hydrogen peroxide. The change

Download English Version:

https://daneshyari.com/en/article/5828677

Download Persian Version:

https://daneshyari.com/article/5828677

Daneshyari.com