

Dendrosomal Curcumin Nanoformulation Attenuates Naloxone Precipitated Morphine Withdrawal Signs in Rats

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Abstract

Curcumin is known to possess a wide range of therapeutic and pharmacological properties as anti-inflammatory, antitumor, antinociceptive and neuroprotective agents. The recent achievements of nanotechnology illustrate a novel approach in treatment of drug abuse and addiction. Curcumin as a new candidate can be exploited for drug abuse treatment, but its low bioavailability and water solubility is the most important obstacles and limits its application. Here, curcumin was efficiently encapsulated in a nontoxic nanocarrier, labeled dendrosome, to overcome these problems. The present study is designed to investigate the effect of intraperitoneal (i.p.) administration of Dendrosomal curcumin (DNC) on the behavioral signs of morphine withdrawal syndrome.

Male Wistar rats weighing 250-300 g were rendered dependent on morphine by subcutaneous (s.c.) administration of morphine sulfate (6, 16, 26, 36, 46, 56 and 66 mg/kg, 2 ml/kg) at set intervals of 24 h for 7 days. The animals received i.p. administration of DNC (12.5 mg/kg) immediately after each morphine injection. On day 8, naloxone (2 mg/kg, i.p.) was injected and behavioral signs of morphine withdrawal were assessed for 25 min. Our results show that i.p. injection of DNC significantly decreases naloxone precipitated morphine withdrawal signs. The present study indicates that intraperitoneal administration of Dendrosomal curcumin exerts a significant effect on morphine dependency in rats.

Keywords: Dendrosomal curcumin; Morphine withdrawal; Rat

Introduction

Chronic use of opioid drugs leads to the development of dependence and tolerance which in turn limits their therapeutic application and brings about serious social and health issues [1]. In many models of drug dependence, positive and negative reinforcement are two major components. Positive reinforcement of euphoric effects results in a compulsive and relentless desire for drug taking while negative reinforcement of withdrawal signs occurs following cessation of opioid receiving [2]. Opiate withdrawal syndrome is a multifaceted phenomenon involving various regions of the brain and is characterized by physiological and behavioral symptoms [3-7]. Curcumin is a hydrophobic compound derived from the rhizome of *Curcuma longa*, which is commonly used as a spice in most Asian countries [8]. Curcumin has been reported to have anti-inflammatory, antioxidant, antitumor, antinociceptive and neuroprotective activities [9,10]. The findings have been shown that daily administration of curcumin could prevent morphine analgesic tolerance [11]. However, low water solubility, poor uptake and tissue distribution remain major impediments, limiting the application of curcumin as treatment agent [12]. Recently, scientists have exposed several strategies, such as loading synthetic analogs from turmeric, designing metabolic inhibitors and liposomal formulations, and nanoparticles of curcumin, to overcome these problems [13-15]. Dendrosome was presented as a novel neutral, amphipathic, and biodegradable nanocarrier for a gene delivery system [16,17]. The high potential of dendrosome as a gene porter has led to the hypothesis that it can be applied as a vehicle for curcumin delivery. In this study, we aimed to investigate the effect of intraperitoneal (i.p.) administration of Dendrosomal curcumin (DNC) on the behavioral signs of morphine withdrawal syndrome.

Materials and Methods

Animals

Male Wistar rats weighing 250-300 g (Pasteur Institute, Tehran, Iran) were used in this study. They were housed three per cage in a

temperature-controlled colony room with a regular 12-h light/dark cycle (lights on at 07:00 am) and free access to food and water. This study was performed in accordance with ethical guidelines of the Faculty of Medical Sciences Ethics Committee, Tarbiat Modares University, which are based on NIH Guide for the Care and Use of Laboratory Animals.

Drugs

The main drugs used in this study are as follows: Morphine sulfate (Temad, Tehran, Iran), naloxone hydrochloride (Sigma-Aldrich, St. Louis, USA), Curcumin (Sigma-Aldrich, St. Louis, MO).

Preparation of the DNC

DNC preparation was performed using optimized protocol in the authors' lab [18,19]. Briefly, different weight/weight ratios of dendrosome/curcumin ranging from 50:1 to 10:1 were examined before settling on a suitable ratio of 25:1. Curcumin was dissolved in various amounts of dendrosome and checked for absorbance spectra by ultraviolet spectrophotometry (Infinite[®] 200 PRO, Tecan, Mannedorf, Switzerland). Then, the appropriate mixture of dendrosome and curcumin was evaluated for excitation/emission value in comparison with curcumin dissolved in PBS and 1% methanol as the control samples [18]. The loading of dendrosome nanocarriers with curcumin molecules was performed using Gou et al's protocol [20]. Briefly,

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Received February 03, 2015; **Accepted** March 09, 2015; **Published** March 14, 2015

Citation: Ghaemi-Jandabi M, Abdollahi H, Azizi H, Sadeghizadeh M, Semnianian S (2015) Dendrosomal Curcumin Nanoformulation Attenuates Naloxone Precipitated Morphine Withdrawal Signs in Rats. J Addict Res Ther 6: 211. doi: [10.4172/2155-6105.1000211](https://doi.org/10.4172/2155-6105.1000211)

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curcumin and dendrosome were co-dissolved in 5 mL acetone; this solution was added into 5 mL PBS while stirring constantly. Then, the acetone was evaporated in a rotary evaporator. The curcumin/dendrosome micelle solution was sterilized using a 0.22 μ m syringe filter (EMD Millipore) [20]. Finally, the prepared DNC were stored at 4°C in a light-protected condition until used [18].

Induction of morphine dependency

Animals were rendered dependent by subcutaneous injection of incremental doses of morphine for 7 days (6, 16, 26, 36, 46, 56 and 66 mg/kg, 2 ml/kg) [21]. Non-dependent animals received the same volume of saline. Injections were done at a fixed time during the whole period of study. Opioid withdrawal syndrome was induced by naloxone hydrochloride (2 mg/kg, i.p.) in the day after last morphine injection.

Apparatus

Behavioral signs of morphine withdrawal were assessed in a transparent cylindrical Plexiglas test chamber (30 cm diameter, 50 cm height and bedded with wood chip) for 25 min[22]. All animals' behaviors were evaluated by the same observer and statistical analyses were performed by an experienced researcher who was blind to the treatment.

Experimental groups

Animals were randomly divided into 4 experimental groups; Group 1, saline treated rats received naloxone to find out if there is any withdrawal sign (n=6); Group 2, morphine treated rats received

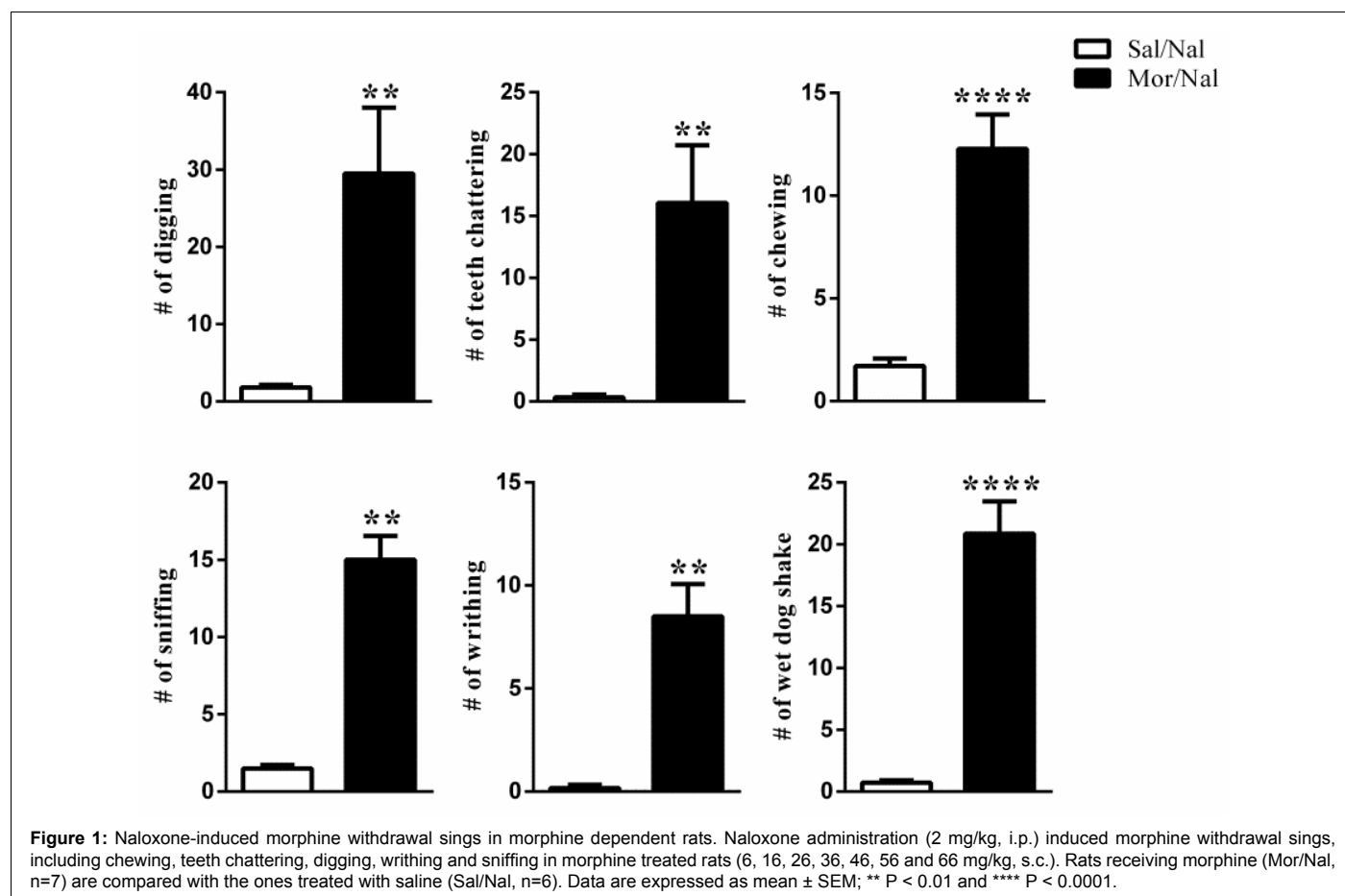
naloxone to evaluate morphine withdrawal signs (n=7); Group 3, animals received concurrent injection of Dendrosomal curcumin (DNC, 12.5 mg/kg, i.p.) with morphine injection for 7 days (n=6). They received naloxone 24h after last morphine injection. Animals in group 4 received concurrent injection of dendrosome (vehicle) with morphine injection and received naloxone 24h after last morphine injection (n=7).

Statistical analysis

Morphine withdrawal symptoms were compared in different experimental groups using unpaired two-tailed Student's t-test or the non-parametric Mann-Whitney U test when necessary (when counts were not normally distributed). Quantal (all or none) behavioral data were analyzed by the chi-square test. Differences amongst means were considered statistically significant if the p-value was less than 0.05. The results are shown as mean \pm SEM.

Results

Morphine withdrawal was precipitated by intraperitoneal injection of naloxone hydrochloride (2 mg/kg, i.p.) in morphine dependent rats. These rats showed pronounced withdrawal signs including chewing, digging, teeth chattering, wet-dog shake and writhing following naloxone injection. Non-dependent or saline treated rats (Group 1) exhibited no withdrawal signs following naloxone administration (Figures 1 and 2). Moreover, morphine pre-treated vehicle animals also exhibited pronounced withdrawal signs following naloxone administration. There were no significant differences between morphine pre-treated vehicle animals and morphine treated ones



(the data is not represented). Animals received concurrent injection of Dendrosomal curcumin (DNC) with morphine injection (Group 3) showed significant reduction in several signs of naloxone induced

morphine withdrawal symptoms. Some withdrawal signs, including chewing, teeth chattering, digging, writhing and sniffing were reduced significantly (Figure 3). Wet dog shake sign was also reduced but its

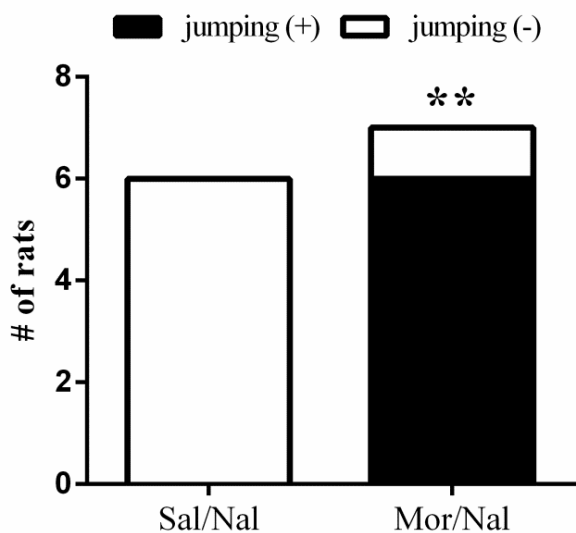


Figure 2: Expression of naloxone (2 mg/kg, i.p.)-induced jumping in morphine dependent rats. Rats receiving morphine (Mor/Nal, n=7) are compared with the ones treated with saline (Sal/Nal, n=6). Ordinate represents the number of rats. ** P < 0.01, chi-square test.

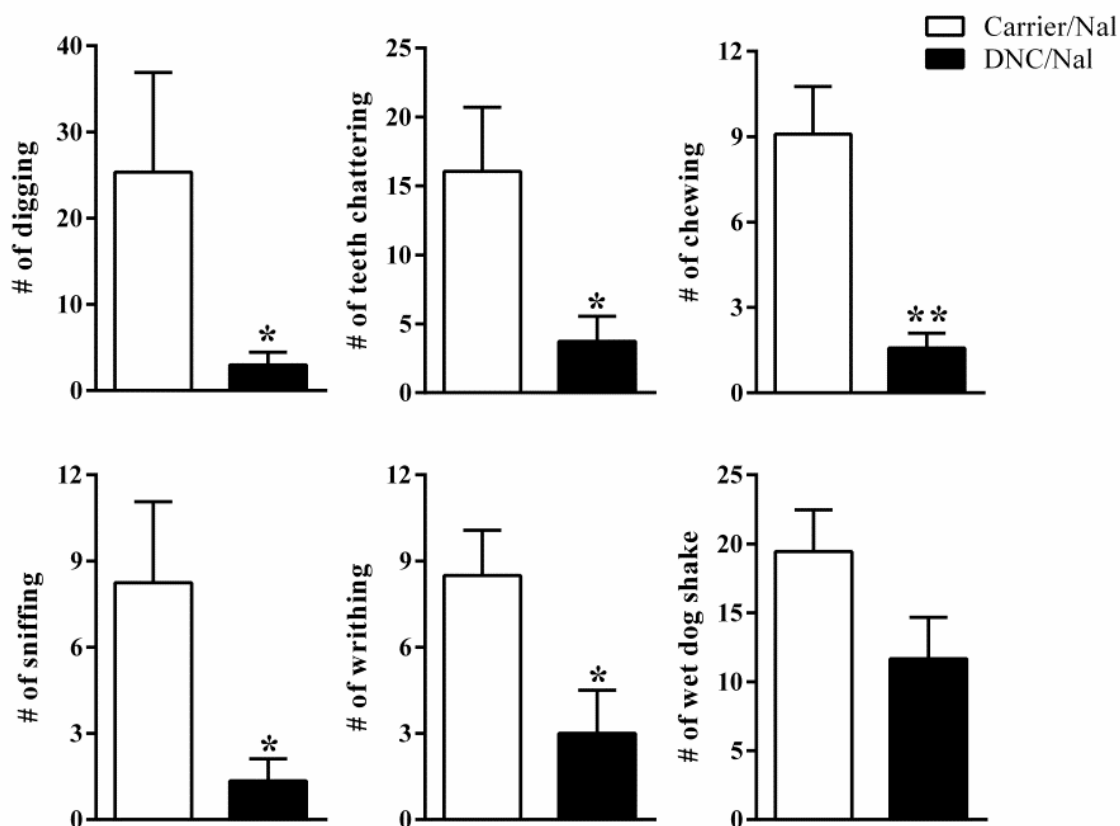


Figure 3: Effect of concurrent injection of dendrosomal curcumin (DNC) with morphine on naloxone-precipitated withdrawal symptoms. Intraperitoneal (i.p.) administration of dendrosomal curcumin (DNC) with each daily morphine injection (6, 16, 26, 36, 46, 56 and 66 mg/kg, s.c.) significantly decreased the withdrawal symptoms, including chewing, teeth chattering, digging, writhing and sniffing (DNC/Nal, n=6). Rats receiving DNC with morphine were compared with the ones pretreated with vehicle (Carrier/Nal, n=7). Data are expressed as mean±SEM. * P < 0.05 and ** P < 0.01.

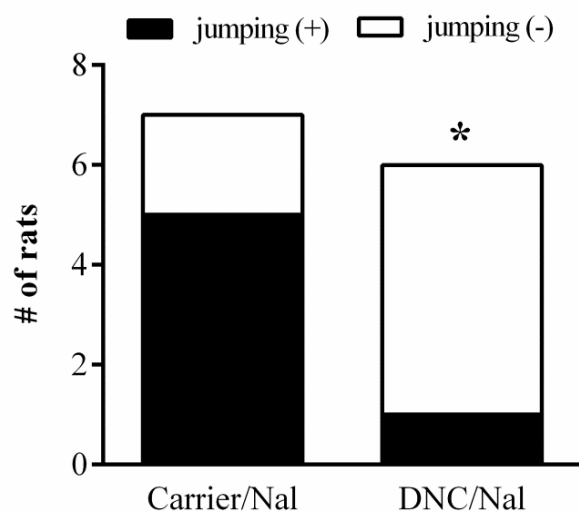


Figure 4: Effect of Intraperitoneal (i.p.) injection of dendrosomal curcumin (DNC) in rats chronically treated with morphine on the expression of naloxone (2 mg/kg, i.p.)-induced jumping. Rats receiving DNC with morphine (DNC/Nal, n=6) were compared with the ones pretreated with vehicle (Carrier/Nal, n=7). Ordinate represents the number of rats. * $P < 0.05$, chi-square test.

reduction was not statistically significant. The number of rats that exhibited jumping reduced significantly (Figure 4).

Discussion

There are various methods for induction of morphine dependency; it seems that injection methods allow more precise control over morphine administration [21]. The present study demonstrates that the concurrent injection of Dendrosomal curcumin (DNC) with morphine attenuates the development of morphine dependency in rats. These results indicate that i.p. administration of Dendrosomal curcumin (DNC) significantly decreases the somatic signs of naloxone-induced morphine withdrawal syndrome, including chewing, teeth chattering, digging, writhing, sniffing and jumping.

The remodeling of chromatin structure is implicated as epigenetic mechanisms play a regulatory role in gene expression and behavioral abnormalities that characterize drug addiction [23]. Many drugs of abuse induce modifications in histone acetylation and methylation in the brain, and evidences have been reported that these changes underlie some of the functional abnormalities found in addiction models [24,25]. It has been identified some alterations in histone acetylation and methylation in the nucleus accumbens (NAc) after acute or chronic exposure to cocaine [26–28]. Curcumin as an inhibitor of histone acetyl transferases (HATs) and DNA methyl transferases (DNMTs) activity may disrupt gene transcription and affect drug dependency.

Chronic exposure to drugs of misuse has been reported to cause adaptations in some intracellular messenger pathways. One of the best well-known adaptations is the upregulation of the cAMP pathway after chronic opiate application [29]. This upregulation and the subsequent activation of the transcription factor CREB seem to mediate features of tolerance and dependence [30,31]. The BDNF mutant mice showed marked attenuation of morphine-induced physical dependence, manifested as naloxone-precipitated withdrawal symptoms after repeated morphine treatments [32]. Matsushita and Ueda [11] have reported that daily administration of the CREB-binding protein inhibitor curcumin could prevent upregulation of BDNF transcription and morphine analgesic tolerance. It has also been shown that curcumin could decrease the activation of p-CREB in dorsal root ganglion [33].

With respect to the mentioned findings we can conclude that curcumin might affect morphine withdrawal and tolerance through reduction in CREB Activity and BDNF transcription.

The present study indicates that i.p. administration of Dendrosomal curcumin exerts a significant effect on morphine dependency in rats. Further studies are required to determine molecular and anatomical mediators which are thought to be involved in this phenomenon.

The integrated preparation process of Dendrosomal curcumin (DNC) has been characterized in our previous studies to generate polymeric nanoparticles with high drug loading, narrow size distribution and long-term stability [18,19]. The well-defined and controlled nanoparticles provide the platform for drug delivery and drug discovery.

Conclusion

Our results show that intraperitoneal administration of Dendrosomal curcumin attenuates morphine physical dependency. The recent achievements of nanotechnology illustrate a novel approach in treatment of drug abuse and addiction.

Acknowledgment

This work was supported by Faculty of Medical Sciences, Tarbiat Modares University.

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