Iranian Journal of Pharmaceutical Sciences
Autumn 2008: 4(4): 253-260
www.ijps.ir

Original Article

# Evaluation of Effects of Dextrometorphan and Midazolam on Morphin-Induced Tolerance and Dependence in Mice

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**Abstract**

Long-term exposure to opiates induces physical dependence and tolerance. The main goal of this study was to evaluate the effects of dextromethorphan and midazolam and their combination on morphine tolerance and dependence in mice. Different groups of mice were rendered randomly and received morphine (50 mg/kg, sc), morphine (50 mg/kg, sc) + dextromethorphan (25,50 or 75 mg/kg, ip), morphine (50 mg/kg, sc) + midazolam (0.5, 1, 2 mg/kg, ip), morphine (50 mg/kg, sc) + dextromethorphan (25 mg/kg, ip) + midazolam (0.5 mg/kg, ip) once a day for four days. Tolerance was assessed by administration of morphine (9 mg/kg, ip) on the fifth day. Withdrawal symptoms (markers for dependence) was assessed by administration of naloxone (4 mg/kg, ip) two hours after co-administration of morphine with either dextromethorphan or midazolam or their combination groups. Results showed that pretreatment with dextromethorphan or midazolam decreased the degree of tolerance and withdrawal symptoms significantly. Additionally, co-administration of dextromethorphan and midazolam before morphine administration decreased the tolerance and dependence but it was not significant. From these results, it may be concluded that dextromethorphan and midazolam alone or in combination could prevent the development of tolerance and dependence to the analgesic effects of morphine. These effects can be related to the N-methyl-D-aspartate (NMDA) receptor antagonist behavior of dextromethorphan and GABA-receptor agonist behavior of midazolam.

*Keywords:* Dependence; Dextromethorphan; Midazolam; Morphine; Tolerance.
*Received*: December 16, 2007; *Accepted:* August 7, 2008.

## 1. Introduction

Long-term exposure to opiates induces physical dependence and tolerance. The neurobiological mechanisms of these phenomena are not completely clear. On the other hand, these drugs are widely used in clinical management of pain. Thus in patients who use opiates for example morphine in order to have analgesic effects, tolerance and dependence limit the therapeutic efficacy of opioids [1, 2]. It is generally believed that chronic opiate treatment induces neuroadap-

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tations in intracellular signaling elements at multiple levels. Therefore, it is possible to modulate the development of opiate tolerance and dependence by regulating the intracellular neurotransmitters, ion channels and intracellular messenger pathways [3-8]. Chronic opioid treatment leads to protein kinas C (PKC) activation and translocation [5-7] which phosphorylates the NMDA receptor-gated Ca channel, resulting in potentiation of NMDA receptor activity [2-4, 8-10]. Opening of these channel leads to influx and increases intracellular Ca concentration, which produces several effects. NMDA receptor antagonists such as dextromethorphan and ketamine and MK801 have been shown in animal models and clinical trials to attenuate opioid tolerance and dependence [11-19]. On the other hand, it is known that γ- amino butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS). Several evidences have shown that GABAergic system plays an important role in the development of tolerance and dependence in morphine therapy [19-27].

Midazolam as a benzodiazepine receptor agonist has been widely used for induction and maintenances of anesthesia with opioids or inhaled anesthetic in clinics [21, 26]. This medication suppresses withdrawal responses by inhibition of the hypersensitization of the spinal cord nervous [17].

Midazolam may occupy the benzodiazepine receptor on the benzodiazepine GABA-Cl channel complex and, therefore, facilitates the inhibitory action of GABA on neuronal transmission. Midazolam could prolong the antinociceptive effect of morphine by delaying the chronic morphine-induced development of tolerance to antinociception in rats [19, 20, 22]. In the present investigation, the possible interaction between opiate and NMDA and GABA receptors regarding tolerance and withdrawal were studied.

## 2. Materials and methods

### *2.1. Animals and drugs*

Male albino mice (20-30g) were studied. Pain sensitivity was evaluated by hot-plate test. Morphine sulfate from Darupakhsh, Iran, dextromethorphan hydrochloride from Rotexmedica, Germany, midazolam hydrochloride from Dormicum, Canada and naloxone hydrochloride from Tolid Daru, Iran were used in this study.

### *2.2. Methods*

#### *2.2.1. Hot - plate test*

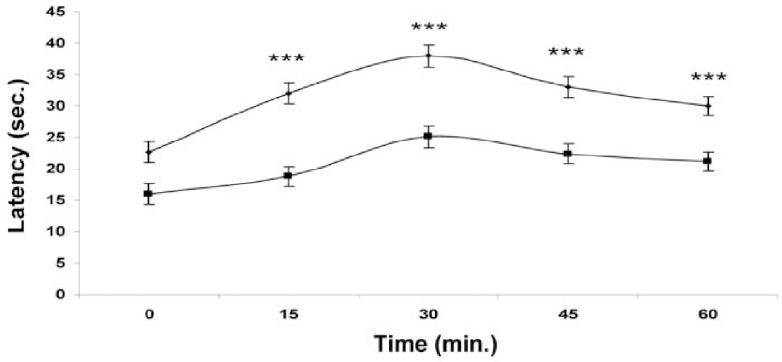


**Figure 1.** Effects of morphine on tolerant and non tolerant mice. Animals received either (◆) saline (10 ml/kg, sc) or (■) [morphine (50 mg/kg, sc) +saline (10 ml/kg, sc)] for 4 days. Antinociception of a test dose of morphine (9 mg/kg, sc) was tested 24 h after the last dose of morphine (50 mg/kg, sc) in tolerant and non tolerant mice. Each group had at least 9 mice. Results are expressed as Mean±SE. ***$p<0.001$, significantly different from the respective non tolerant control group.

Each animal was placed on a surface (23×23 cm) maintained at 55±2 ºC surrounded by a Plexiglas wall 20 Cm high. Licking of hands was used at the end point for determination of response latencies. Failure to respond by 45 sec. was a marker for termination of the test (cut off).

*2.2.2. Induction of tolerance*

In order to induce tolerance, groups of 9 mice were chosen randomly. Mice were treated subcutaneously (sc) by morphine (50 mg/kg) in combination with either dextromethorphan or midazolam or both dextromethorphan and midazolam once a day for four days. To evaluate the degree of tolerance, the antinociceptive effect of a test dose of morphine (9 mg/kg) was measured 24 h after the last dose of morphine in combination with dextromethorphan or midazolam or both dextromethorphan and midazolam.

2.2.3. Induction of dependence

Groups of 9 mice were chosen randomly. Mice were treated subcutaneously (sc) with morphine (50 mg/kg) in a combination with dextromethorphan (ip) or midazolam (ip) or both dextromethorphan and midazolam once a day for four days. To evaluate the effect of different doses of dextromethorphan and midazolam in dependence (jumping and standing on feet) a dose of naloxone (4 mg/kg, ip) was injected 2 h after the last dose of morphine on the fourth day.

*2.2.4. Evaluation of the withdrawal syndrome*

After naloxone injection, withdrawal symptoms (number of jumping and number of standing on feet) in 30 min. were evaluated.

*2.2.5. Statistical analysis*

The results are expressed as the Mean±SEM. Differences between the individual mean values in different groups were analyzed by one-way analysis of variance (ANOVA) and Tukey test as a post hoc analysis. Differences with a $p<0.05$ were considered significant.

## 3. Results

*3.1. Development of tolerance to the morphine antinociception*

Animals received morphine (50 mg/kg, sc) for 4 days. In each group antinociceptive response of a test dose of morphine (9 mg/kg, ip) was assayed 24 h after the last dose of morphine (50 mg/kg, sc). Animals that

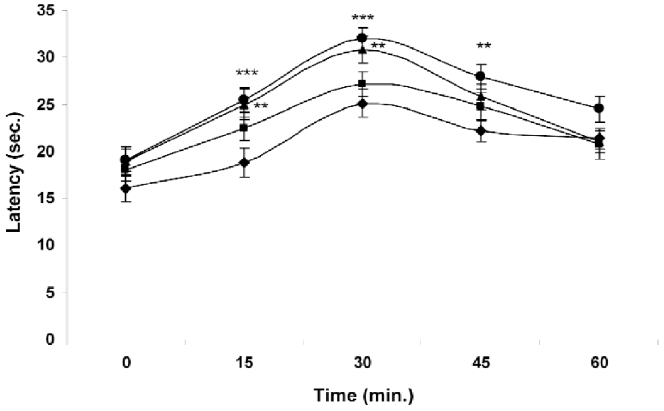


**Figure 2.** Effects of different doses of dextromethorphan (■) 25, (▲) 50, (●) 75 mg/kg, ip) on tolerance determined by hot-plate test in morphine-tolerant mice. Each group had at least 9 mice. Results are expressed as Mean±SE.**$p<0.01$, ***$p<0.001$, significantly different from the (◆) control group.

became tolerant to effects of morphine exhibited only a small antinociceptive effect (Figure 1).

*3.2. Naloxone-induced withdrawal*

Animals were rendered dependent to morphine by administration of morphine (50 mg/kg, sc) once a day for four days. The dose of 4 mg/kg, ip of naloxone was chosen for induction of withdrawal symptoms. Naloxone induced withdrawal signs: Jumping and standing on feet (Figures 5 and 6).

*3.3. Effect of pretreatment with dextromethorphan on tolerance and dependence to chronic morphine therapy*

As shown in Figure 2, dextromethorphan injection (25, 50, 75 mg/kg, ip) 30 min. before daily morphine administration, decreased tolerance to the analgesic effects of morphine significantly. Figures 5 and 6 show that pretreatment with dextromethorphan (25, 50, 75 mg/kg, ip) dose dependently decreased the withdrawal symptoms significantly.

*3.4. Effect of pretreatment with midazolam on tolerance and dependence to chronic morphine therapy*

As shown in Figure 3, injection of midazolam (0.5, 1, 2 mg/kg, ip) 30 min. before daily morphine administration decreased tolerance to the analgesic effect of morphine significantly with dose of 2 mg/kg. Figures 5 and 6 show that pretreatment with midazolam (0.5 , 1, 2 mg/kg, ip) dose dependently decreased the withdrawal symptoms significantly.

*3.5. Effect of pretreatment with dextromethorphan and midazolam on tolerance and dependence to chronic morphine therapy*

As shown in Figure 4, co-administration of dextromethorphan (25 mg/kg, ip) and midazolam (0.5 mg/kg, ip) 30 min. before daily morphine administration decreased tolerance phenomenon but it was not significant and Figure 5 and 6 show that this combination decreased withdrawal symptoms significantly.

## 4. Discussion

The principle aim of this study was to evaluate the effects of dextromethorphan (as a non competitive NMDA receptor antagonist) and midazolam (as a benzodiazepine receptor agonist) on development of morphine tolerance and dependence. It has been proposed that repeated administration of

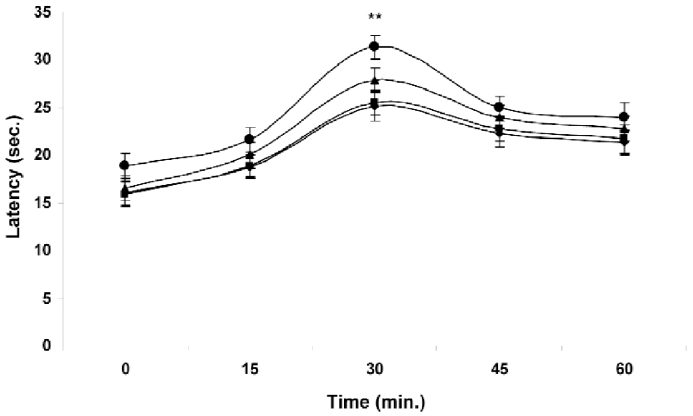


**Figure 3.** Effects of different doses of midazolam (■) 0.5, (▲) 1, (●) 2 mg/kg, ip on tolerance determined by hot-plate test in morphine-tolerant mice. Each group had at least 9 mice. Results are expressed as Mean±SE. ** $p<0.01$, significantly different from the (♦) control group.

opiate may activate the NMDA receptor through G protein associated with opioid receptor and/or intracellular mechanisms [1, 2, 4-8, 10].

This opiate related activation of NMDA receptors may initiate subsequent intracellular changes such as production of nitric oxide (NO) and/or the activation of protein kinas C (PKC) [2, 6, 8]. Both NO and PKC have been shown to be critical for development of morphine tolerance [6, 8]. The results of the present study show that the NMDA receptor antagonists such as dextromethorphan (25, 50, 75 mg/kg, ip) may attenuates development of morphine tolerance and dependence in mice.

Previous studies [1, 11, 14-16] showed that administration of dextromethorphan attenuated intracellular of Ca influx both in NMDA receptor gated channel as well as in voltage- gated Ca channel. Some studies showed a significant increase in dopamine metabolites following morphine administration was demonstrated in the nucleus accumbens (NAc) [12, 29]. This increase by morphine could be attenuated by co-administered dextromethorphan. Further more neurochemical analysis revealed that the effect of dextromethorphan could be through its action on the dopaminergic mesolimbic pathway, which could be activated by morphine and attributed to the cause of rewarding [12, 29, 30]. Dextromethorphan also potentiates the antinociceptive effects of the mu-opioid receptor agonist morphine under some conditions [9, 14]

Other studies have shown that there is an interaction between GABA and opioid system and GABAergic system has a role in opioid tolerance and dependence [17, 18, 20-26, 31, 32]. Both GABAA- and GABAB-mediated synaptic potentials in dopamine cells of the ventral tegmental area (VTA) were inhibited presynaptically by opioids [6, 29]. The GABAA-mediated synaptic potential is thought to arise from inter neurons that are hyperpolarized by opioids [12, 29, 30, 32]. The inhibition of spontaneous activity recorded from interneurons correlated with the inhibition of tetrodotoxin-sensitive GABA-mediated IPSPs recorded in dopamine cells [29]. It was concluded that cells that were hyperpolarized by μ-opioid receptors in the VTA were GABA interneurons [29]. Studies show that selective GABAA receptor antagonist such as bicuculline enhanced cGMP production, revealing that the cortical NOS/sGC system is tonically inhibited by

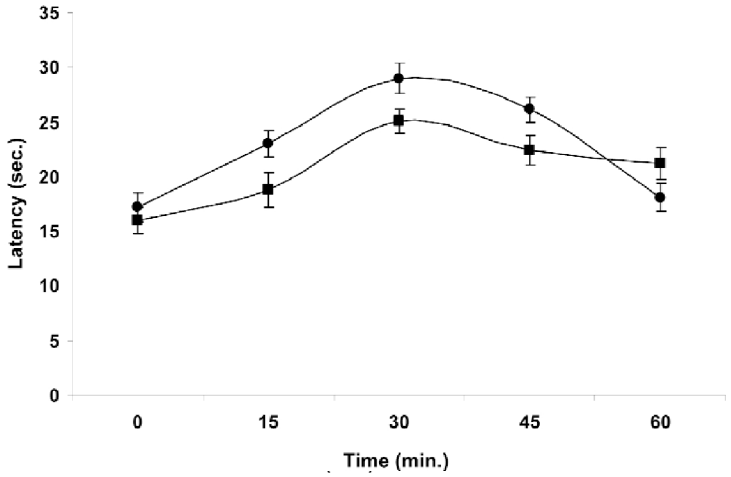


**Figure 4.** Effects of the use of (●) [dextromethorphan (25 mg/kg, ip) + midazolam (0.5 mg/kg, ip)] on tolerance determined by hot-plate test in morphine-tolerant mice. Each group had at least 9 mice. Results are expressed as Mean± SE. (■) control group.

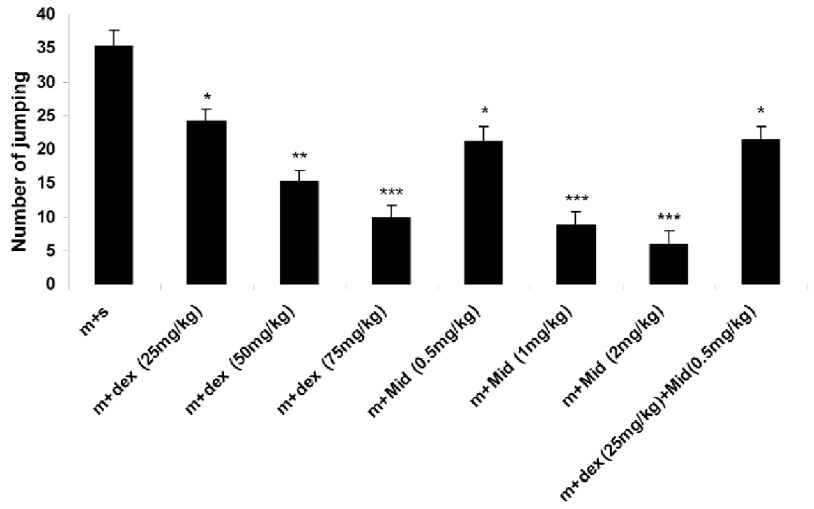


**Figure 5.** Effects of different doses of dextromethorphan (25,50,75 mg/kg, ip) and midazolam(0.5,1,2 mg/kg, ip) and dextromethorphan (25 mg/kg, ip)+ midazolam (0.5mg/kg, ip) on jumping induced by naloxone (4 mg/kg, ip) in morphine-dependent mice. Each group had at least 9 mice. Results are expressed as Mean ± SE.*$p$<0.05, **$p$<0.01,***$p$<0.001, significantly different from the morphine control group.

endogenous GABA [27]. Thus production of NO is decreased in attendance of midazolam as an GABAA agonist. Furthermore, previously studies showed that administration of midazolam can increase or decrease morphine-induced antinociception, depending upon relative concentration of these drugs by modulating spinal opioid receptors, and it also can inhibit morphine-induced tolerance and dependence in the rat [29].

The GABAB IPSP is thought to arise from fibers originating in the nucleus accumbens or ventral pallidum. On the basis of selective effects of 5-HT and dopamine on GABAA and GABAB IPSPs, separate terminals are thought to mediate these synaptic responses [33, 34]. The GABAB IPSP was increased by D1-dopamine agonists and decreased by 5-

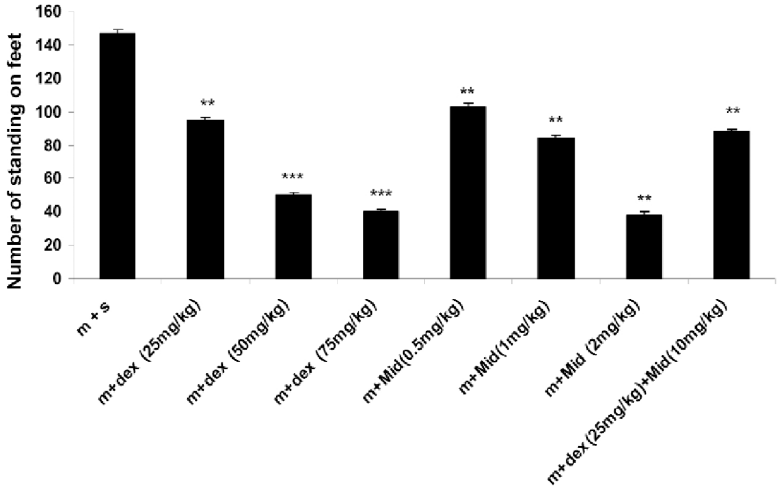


**Figure 6.** Effects of different doses of dextromethorphan (25, 50, 75 mg/kg, ip) and midazolam (0.5, 1, 2 mg/kg, ip) and dextromethorphan (25 mg/kg, ip)+midazolam (0.5 mg/kg, ip) on standing on feet induced by naloxone (4 mg/kg, ip) in morphine-dependent mice. Each group had at least 9 mice. Results are expressed as Mean±SE. **$p$<0.01, ***$p$<0.001, significantly different from the morphine group.

HT1B agonists, whereas the GABAA IPSP was insensitive to both agonists. Unlike inhibition of the GABAA IPSP, both mu and kappa opioid agonists decreased the GABAB IPSP by a presynaptic mechanism [28, 31]. Thus the activation of opioid receptors on at least two types of GABA-releasing terminals decrease GABA-mediated inhibition, allowing an increase in activity through disinhibition [29, 30]. In the present study, pretreatment with midazolam ( 0.5, 1, 2 mg/kg, ip) 30 min. before daily morphine administration prevented the development of morphine tolerance and dependence. Previous studies showed that concomitant administration of diazepam to morphine results in the inhibition of the development of morphine tolerance and dependence. Some mechanisms are proposed [23-25].

Furthermore co-administration of dextromethorphan and midazolam has showen that the use of this combination decrease the development of morphine tolerance and dependence but results was not significant. Previous studies showed the reduction the subunits of GABAA receptors in attendance of NMDA antagonist [31], and other studies have showen that NMDA-receptor antagonists such as dextromethorphan or phencyclidine have some psychotic adverse effects which are treated by benzodiazepine and therefore this combination may be useful.

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