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Research Article

Attenuation of Morphine-Induced Tolerance and Dependency by Pretreatment with Magnesium Sulfate and Amitriptyline in Male Mice

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ABSTRACT

Background: Clinical use of Morphine in pain management is a controversial issue and long-term exposure to opiates induces physical dependency and tolerance. The aim of this study was to evaluate the attenuation effects of Magnesium sulfate and Amitriptyline on the development of Morphine-induced dependency and tolerance. ***Methods:*** In this study different groups of mice were received saline (10 mL/kg,ip), Morphine (50 mg/kg,ip), Magnesium sulfate (20, 40 and 60 mg/kg,ip), Amitriptyline (5, 10 and 15 or 20 mg/kg,ip), or combination of Magnesium sulfate (20 mg/kg,ip) and Amitriptyline (5 mg/kg,ip) once a day for 4 and 10 continues days, respectively for investigation of the effects of Magnesium sulfate and Amitriptyline on the prevention of dependency and tolerance. Tolerance was assessed by administration of Morphine (9 mg/kg,ip) and using hot plate test on eleventh day. Withdrawal symptoms were assessed in fifth day by administration of naloxone (4 mg/kg,ip), 2 hrs after last dose of Morphine during 30 minutes for each animal. ***Results:*** It was found that pretreatment with Magnesium sulfate or Amitriptyline decreased the development of tolerance to the antinociceptive action of Morphine and also reduced naloxone-precipitated withdrawal jumps and standing on feet. Additionally, pretreatment by co-administration of Magnesium sulfate and Amitriptyline, before morphine administration, decreased the dependency and tolerance more significantly. ***Conclusion:*** From these results it could be concluded that pretreatment of Magnesium sulfate via blocking the N-methyl-D-Aspartate receptor-operated calcium channel and Amitriptyline by Glutamate transporter activation property alone or in combination could prevent the development of Morphine-induced dependency and tolerance.

Introduction

Narcotics such as Morphine are used in the chronic pain treatment for decades. By long-term usage of narcotics such as Morphine, leading to the development of antinociceptive tolerance, it is necessary to increase administered doses to control pain, where in some cases, narcotics become low effective and even ineffective. Besides, a higher dose of narcotics should be administered over time to maintain the same level of analgesia. In the pharmacological terms, it is a shift to the right in the dose-response curve.[1-4] On the other hand, repeated use of opiates lead to the development of dependency. Dependency is defined as continued necessity for the opiates administration to maintain a state of physical equilibrium following repeated use of the opiates which is revealed by withdrawal signs when drug administration is ended.[5-8] Identification of innovative strategies to attenuate opioid tolerance and development of dependency could offer important benefits in the controlling of chronic pain treatment. For decades, many studies have focused to clarify the mechanisms involved in the Morphine tolerance and dependency. The ionotropic N-methyl-d-aspartate (NMDA) receptor blockers such as Magnesium sulfate, which is widely used in patients with preeclampsia, have sedative and antinociceptive effect where to be useful in anesthesia and also as an adjunctive therapy in the clinical management of severe manic agitation, where could attenuate opioid tolerance and dependency. Furthermore, they were evaluated for the treatment of acute stroke. Magnesium also antagonizes Ca at the NMDA subtype of glutamate receptor site and

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decline calcium influx into the cells. Tricyclic antidepressants such as Amitriptyline are widely used to treat of chronic pain such as neuropathic and inflammatory pain conditions. Tricyclic antidepressants produce analgesia by various mechanisms involving NMDA receptors, biogenic amines, opioids, inflammatory mediators, and also substance P. Amitriptyline is a glutamate transporter activator and used in the treatment of depression, nocturnal enuresis in children and hiccup. Numerous studies suggested that, Amitriptyline can be effective in prevention of Morphine-induced dependency and tolerance. The proposed involved mechanisms are include: inhibition of pro-inflammatory cytokine, prevention of glutamate transporter down-regulation and enhancing glutamate transporters activity.[9-13] The present study was conducted to evaluate the attenuation effects of pretreatment with different doses of Magnesium sulfate and Amitriptyline as well as combined administration of these drugs on the development of Morphine-induced tolerance and dependency.

Materials and Methods

Drugs

Morphine sulfate and Naloxone were obtained from Darupakhsh and Tolid Daru pharmaceutical Companies, respectively (Iran). Magnesium sulfate was obtained from Pasteur institute (Iran). Amitriptyline was purchased from Sobhan pharmaceutical Company (Iran).

Animals and treatment

Adult male Albino mice weighing 20 - 30 g, aged 8 weeks, were allocated into different groups (n = 8). Animals had free access to food and water under standard lighting conditions (12h: 12h, light: darkness) and at room temperature (24 ± 0.5 ºC).

All experiments were performed 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). The study was conducted in Faculty of Pharmacy of Tabriz University of Medical Science.

For evaluation of the effects of different doses of Magnesium sulfate and Amitriptyline on the Morphine-induced tolerance, mice were pretreated for 5 continuous days with saline (10 mL/kg, ip), Magnesium sulfate (20, 40 and 60 mg/kg, ip), Amitriptyline (5, 10 and 15 mg/kg, ip) and Magnesium sulfate (20 mg/kg, ip) + Amitriptyline (5 mg/kg, ip). Subsequently, for next 5 continuous days mice were received Morphine (50 mg/kg, ip), 30 minutes before daily administration of mentioned medicines. Tolerance was assessed using hot-plate after administration of the test dose of Morphine (9 mg/kg, ip) on eleventh day.

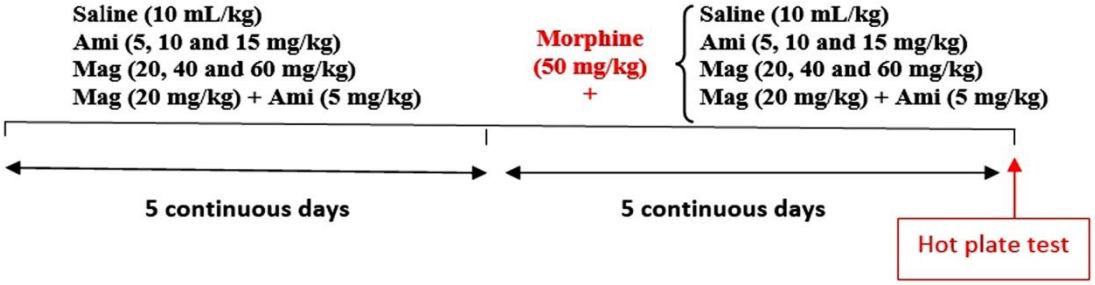

Figure 1. The procedure of monitoring morphine induced nociception by hot-plate test. Ami: Amitriptyline and Mag: Magnesium sulphate.

To assess the effects of different doses of Magnesium sulfate and Amitriptyline on the Morphine-induced dependency, different groups of mice were received saline (10 mL/kg, ip)+ saline (10 mL/kg, ip), Morphine (50 mg/kg, ip) + saline (10 mL/kg, ip), Morphine (50 mg/kg, ip) + Magnesium sulfate (20, 40 and 60 mg/kg, ip), Morphine (50 mg/kg, ip) + Amitriptyline (5, 10 and 20 mg/kg, ip) and Morphine (50 mg/kg, ip) + Magnesium sulfate (20 mg/kg, ip) + Amitriptyline (5 mg/kg, ip) once a day for 4 continuous days.

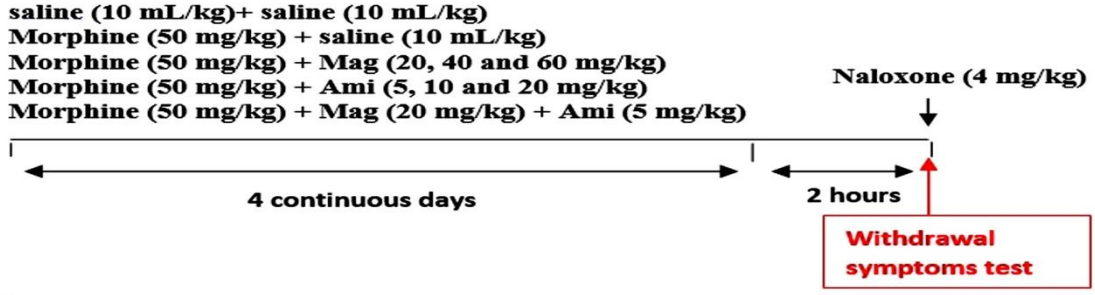

Figure 2. The procedure of monitoring morphine induced dependency by withdrawal test. Ami: Amitriptyline and Mag: Magnesium sulphate.

Assessment of nociception by hot-plate test

The hot plate test is commonly used for evaluating thermal pain sensitivity Mice were placed on a stainless steel surface (23×23 cm) which was maintained at 55 ± 2 ºC surrounded by a Plexiglas wall (20 cm height) and latency time was recorded.. Thirty minutes prior to the treatment of the nociceptive threshold was measured and the latency time has been used as the pre-drug latency for each test animal. The hot-plate latency was recorded when the animal licked its hind paw. A 40 sec cut-off time was considered to avoid any possible tissue injury.. Hot-plate response latency are expressed as the percentage of Maximal Possible Effect (MPE%) according to the following equation.[8]

MPE% = [(TL - BL) / (T cut-off - BL)] × 100

Where, TL = Test Latency time and BL = Base Latency time.

Withdrawal symptoms test

Mice were tested for the occurrence of withdrawal symptoms (jumping and standing on feet) after the injection of Morphine (50 mg/kg, ip) on fourth day. Two hours after the last dose of Morphine, withdrawal syndrome as an index of Morphine dependency, was precipitated by injection of Naloxone (4 mg/kg, ip). Animals were placed individually on the filter paper in an open Plexiglas chamber (25×25×40 cm) and the number of jumps and standing on feet were recorded by an observer over a 30 min period for each animal.[13]

Statistical Analysis

Statistical analysis of each data set was performed by SPSS software (version 17). All the results were presented as mean ± SEM for eight rats in each group. Statistical comparisons among the experimental groups were made by the one way analysis of variance (ANOVA) followed by Tukey post hoc test where differences with p values less that 0.05 were considered significant.

Results

Development of Morphine-induced tolerance to analgesic effect

Figure 3 shows the effects of administration of four doses of morphine (50 mg/kg, ip) in four consecutive days. As it is shown, administration of Morphine resulted in significant reduction in licking time and as result MPE% values, in all recorded times (P<0.001).

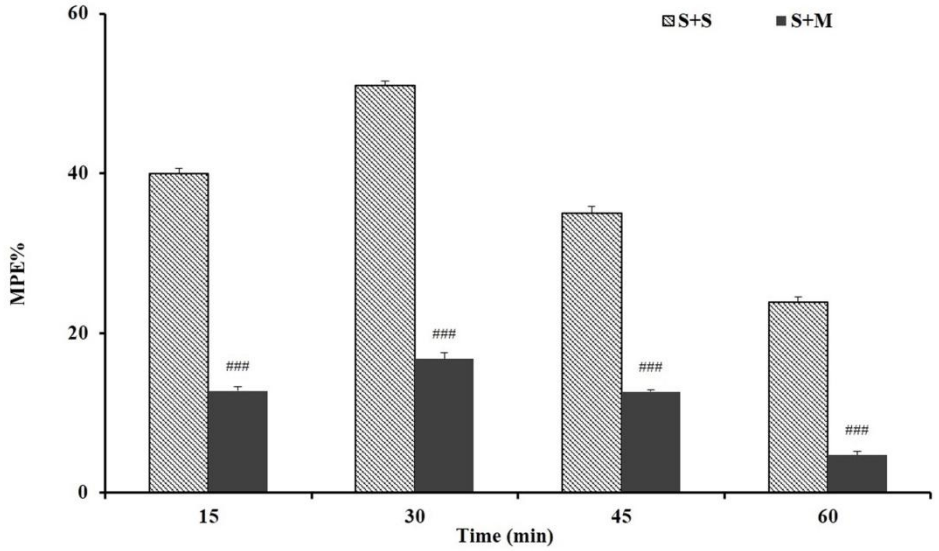

Figure 3. Effects of Morphine (M) on tolerant and non-tolerant mice. Saline (S) group was received saline (10 mL/kg, ip) for 10 days and tolerant group received saline (10 mL/kg, ip) for five days and Morphine (50 mg/kg, ip) + saline (10 mL/kg, ip) for the next five days. (###P<0.001 compared with S+S group).

Effects of administration of Magnesium sulfate on Morphine-induced tolerance

The effect of pretreatment with different doses of Magnesium sulfate on Morphine-induced tolerance is shown in Figure 4. Results indicated that, pretreatment with Magnesium sulfate reduced the tolerance to Morphine in all test times, especially in times 45 and 60 min. On the other hand, tolerance to Morphine was reduced by administration of Magnesium sulfate in a dose dependent manner.

Effect of administration of Amitriptyline on Morphine-induced tolerance

Figure 5 illustrates the effects of different pretreatment doses of Amitriptyline on Morphine-induced tolerance. Similar to the Magnesium sulfate, administration of Amitriptyline prior to the Morphine injection, attenuated the development of tolerance to the Morphine. Furthermore, its effect in times 15 min and 60 min was more significant. However, the effect of administration of Amitriptyline 10 mg/kg, ip was higher than Amitriptyline 15 mg/kg, ip, at times 15 min and 30 min.

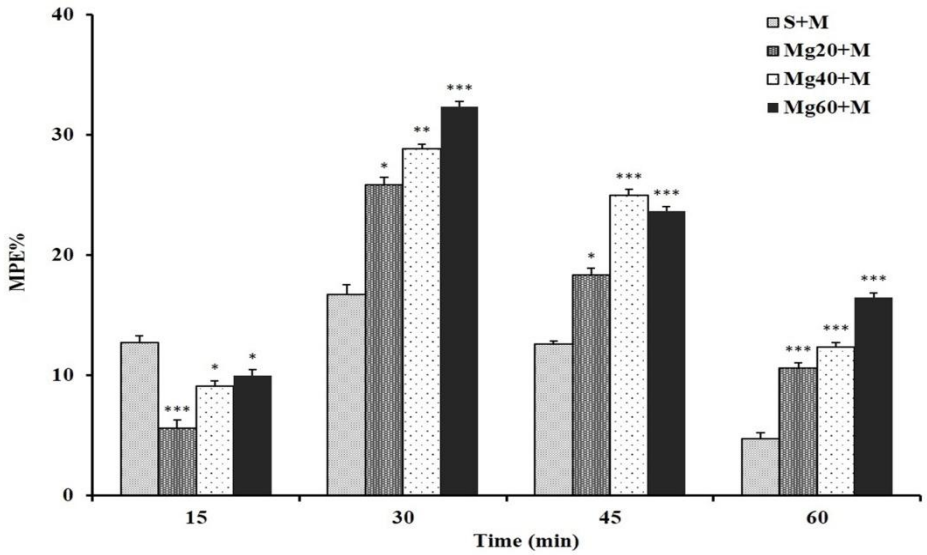

Figure 4. Effects of different doses of Magnesium sulfate (Mg) on Morphine-induced tolerance. Mice were received Magnesium sulfate (20, 40 and 60 mg/kg, ip) for five days and Magnesium sulfate (20, 40 and 60 mg/kg, ip) + Morphine (M) (50 mg/kg, ip) for the next five days. (*P<0.05, **P<0.01 and ***P<0.001 compared with M+S group).

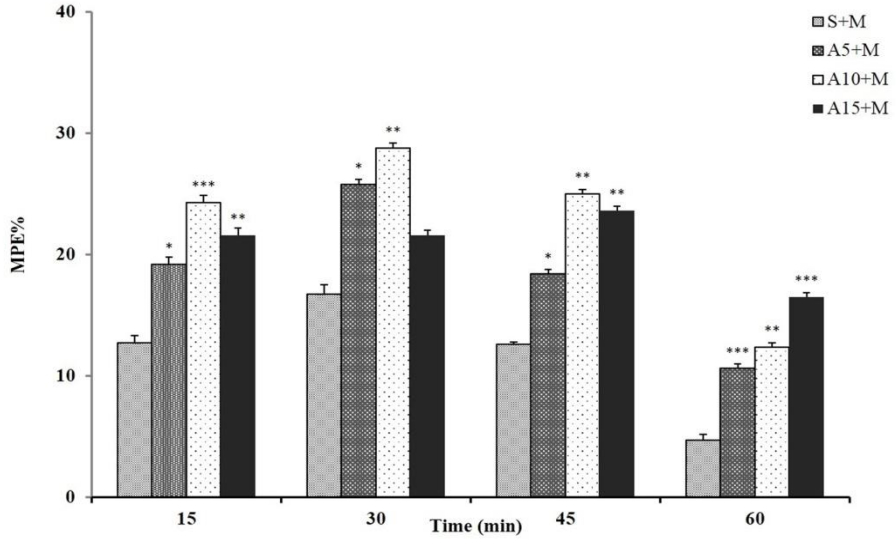

Figure 5. Effects of different doses of Amitriptyline (A) (5, 10 and 15 mg/kg, ip) on Morphine-induced tolerance. Mice were received Amitriptyline (5, 10 and 15 mg/kg, ip) for five days and Amitriptyline (5, 10 and 15 mg/kg, ip) + Morphine (M) (50 mg/kg, ip) for the next five days. (*P<0.05, **P<0.01 and ***P<0.001 compared with M+S group).

Effect of co-administration of Magnesium sulfate and Amitriptyline on Morphine-induced tolerance

Figure 6 compares the combined effect of pretreatment with Magnesium sulfate (20 mg/kg, ip) and Amitriptyline (5 mg/kg, ip) with the individual effects of them. Results indicated that the co-administration of these drugs had higher protective effects on the development of tolerance than both drugs alone at all test times (P<0.001 compared with M+M group).

Development of Morphine-induced dependency to analgesic effect

Table 1 and 2 show the results of pretreatment with Magnesium sulfate and Amitriptyline on the development of dependency to the Morphine. Number of jumping and standing on feet, as withdrawal syndrome symptoms, in Morphine-dependent groups were increased significantly compared with the non-dependent group. Although, pretreatment with Magnesium sulfate and Amitriptyline in low doses had not protective effect on the development of the dependency, in higher doses, Magnesium sulfate and Amitriptyline were significantly decreased the number of jumping and standing on feet compared with morphine-dependent group (P<0.001). However, co-administration of Magnesium sulfate and Amitriptyline with low doses was also significantly reduced the both withdrawal syndrome symptoms (P<0.001).

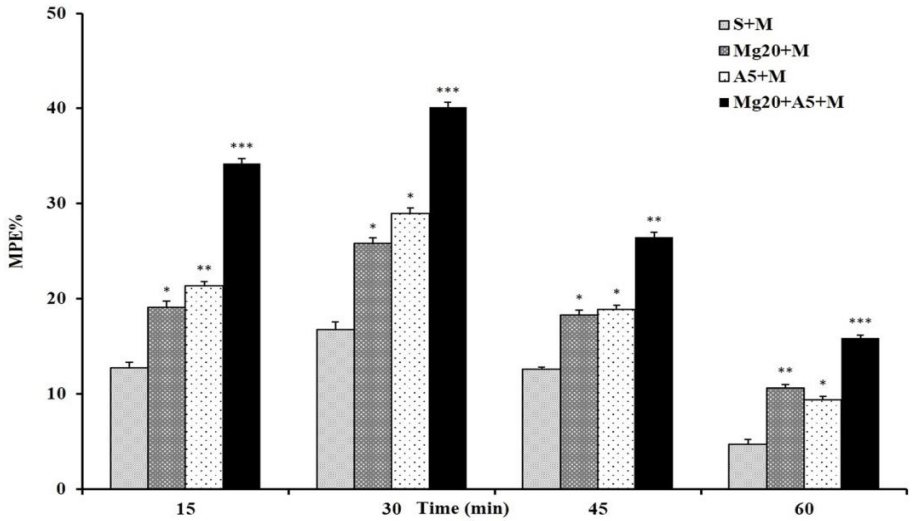

Figure 6. Effects of co-administration of Magnesium sulfate (Mg) (20 mg/kg, ip) and Amitriptyline (A) (5 mg/kg, ip) on morphine-induced tolerance. Mice were received Magnesium sulfate (20 mg/kg, ip) + Amitriptyline (5 mg/kg, ip) for five days and Magnesium sulfate (20 mg/kg, ip) + Amitriptyline (5 mg/kg, ip) + Morphine (M) (50 mg/kg, ip) for the next five days. (*P<0.05, **P<0.01 and ***P<0.001 compared with M+S group).

Table 1. Effects of different doses of Magnesium sulfate (Mg) (20, 40 and 60 mg/kg, ip) or Amitriptyline (A) (5, 10 and 20 mg/kg, ip) and Magnesium sulfate (20 mg/kg, ip) + Amitriptyline (5 mg/kg, ip) on standing on feet induced by Naloxone (4 mg/kg, ip) in Morphine-dependent mice.

Treatment (mg/kg, ip)	Number of mice	Number of standing on feet
S+S	8	43 ± 4.2
M+S	8	134 ± 9.0$^{###}$
Mg20+M	8	120 ± 7.4
Mg40+M	8	68 ± 6.3***
Mg60+M	8	59 ± 7.0***
A5+M	8	122 ± 5.3
A10+M	8	97 ± 7.0**
A20+M	8	70 ± 9.6***
A5+Mg20+M	8	48 ± 3.6***

Each group contained 8 mice. Results were expressed as Mean ± SD. (**P<0.01 and ***P<0.001 compared with M+S group and $^{###}$P<0.001 compared with S+S group).

Table 2. Effects of different doses of Magnesium sulfate (20, 40 and 60 mg/kg, ip), Amitriptyline (5, 10 and 20 mg/kg, ip) and Magnesium sulfate (20 mg/kg, ip) + Amitriptyline (5 mg/kg, ip) on jumping induced by Naloxone (4 mg/kg, ip) in Morphine-dependent mice.

Treatment (mg/kg, ip)	Number of mice	Number of Jumping (Mean± SD)
S+S	8	13 ± 2.1
M+S	8	60 ± 5.5$^{###}$
Mg20+M	8	50 ± 5.9
Mg40+M	8	33 ± 3.4***
Mg60+M	8	25 ± 3.0***
A5+M	8	53 ± 5.0$^{###}$
A10+M	8	35 ± 2.8***
A20+M	8	28 ± 4.8***
A5+Mg20+M	8	24 ± 4.2***

Each group contained 8 mice. Results were expressed as Mean ± SD. (*P<0.05 and ***P<0.001 compared with M+S group and $^{###}$P<0.001 compared with S+S group).

Discussion

Chronic treatment with opioids leads to the activation of protein kinas C and translocation which phosphorylates the NMDA receptor-gated Ca channel, resulting in potentiation of NMDA receptor activity. Opening of these channel leads to an influx and increases intracellular Ca concentration, which displays several effects.[10,12-15] Furthermore, other studies demonstrated that pro-inflammatory cytokines released from activated glial cells after repeated Morphine injections participates as between-system mechanism.[16-19] Morphine tolerance and dependency is a complex physiological response that involves a within-system and a between-system adaptation. The within-system adaptations, which include opioid receptors uncoupling from G-proteins and receptor down-regulation, are well-known mechanisms of opioid tolerance.[14-17] Furthermore, recent studies have also proposed that between-system adaptations, such as the pain facilitatory systems may play an important role in the development of opioid dependency. In addition, it is well known that, activation of the NMDA subtype of glutamate receptors, through G protein associated with opioid receptor and intracellular mechanisms such as the subsequent downstream signals (like nitric oxide), and the activation of protein kinas C (PKC) where both NO and PKC are involved in the development of Morphine analgesic tolerance and dependency.[7,9-11] Evidences from previous studies suggested that NMDA receptors are involved in the plasticity that arises from the long-term administration of morphine.[16,18-22] Long term administration of opiate leads to removing the Magnesium blocked in the Calcium channel and opening the Calcium channel of NMDA receptors and increasing in intracellular Calcium. Previous studies have shown that

administration of magnesium can attenuate the tolerance and dependency to the analgesic effects of morphine and its Mechanism was related to the property of Magnesium to block the Ca channel of NMDA receptors. So it could be suggested that magnesium may have a potential role in prevention of morphine dependency.[20-24] In the present study pretreatment with Magnesium sulfate (20, 40 and 60 mg/kg, ip) 30 min before daily Morphine administration reduced tolerance and dependency of Morphine. Chronic Morphine administration induces down regulation of spinal GTs and as a result Morphine-induced GT down regulation would increase the availability of extracellular glutamate. Increased glutamate availability at the extracellular level could increase the probability of excitatory amino acid receptor activation including NMDARs.[20,23-26] Amitriptyline might attenuate the development of morphine dependency and tolerance. The proposed mechanisms are including: Inhibiting of the expression of pro-inflammatory cytokines such as TNFα, IL-1β, and IL-6 and increasing expression of IL-10 via the p38 MAPK-HO-1 signal transduction cascade; Activation of NF-κB, inhibiting glutamate transporter down-regulation, and expression of up-regulating of glutamate transporters GLAST and GLT-1 in glial cells; and finally preventing phospho-PKA and PKC expression, thus promoting GLAST and GLT-1 trafficking to the glial cell surface. Normal or over-expression of GLAST and GLT-1 in glial cell membranes preserves glutamate transporter uptake activity, decreases spinal excitatory amino acid release, and reduction of NMDA receptor activation.[12,26-29]

Conclusion

In conclusion, our study indicated that pretreatment with Amitriptyline alone or in combination with Magnesium sulfate could reduce the morphine-induced tolerance and dependency which suggesting a probable synergistic effect of these drugs. Further studies with different doses and different exposure time are needed to clarify the exact effect of co-administration of these medicines.

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Conflict of interests

The authors declare that they had no conflict of interests.

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