



Effects of Magnesium Oxide Nanoparticles on Depression- Like Behavior Induced by Naloxone and Morphine Withdrawal

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Abstract

Background: Our previous studies indicated that nanoparticles of magnesium oxide (nano-MgO) can affect some of morphine withdrawal signs such as anxiety, but their interaction with opioidergic system activity in depression- like behavior induced by morphine withdrawal is not clear.

Objectives: The present study aimed at investigating the effects of nano-MgO on depression induced by naloxone and morphine withdrawal in animals.

Methods: Adult male NMRI mice were divided in 2 main groups. For the control group, intraperitoneal (i.p.) injection of nano-MgO (1, 2.5 and 5 mg/kg) or naloxone (3 and 5 mg/kg) and coinjections of naloxone before and after nano-MgO 2.5 mg/kg were done. The morphine withdrawal group received saline or nano-MgO 2.5, 5 and 10 mg/kg as acute (a single injection at the test day) and chronic injection (coinjected with morphine for 4 days). To develop morphine dependency, increasing doses of 20, 40, and 80 mg/kg morphine were injected subcutaneously for 4 days. Then, mice received a final morphine injection (40mg/kg) 3 hours prior to naloxone (5 mg/kg (i.p.)) on the test day. Depression- like behavior was assessed by the tail suspension test.

Results: Naloxone 3 and 5mg/kg induced depression ($P < 0.05$ and $P < 0.01$, respectively), while acute injection of nano-MgO 2.5mg/kg after naloxone 5 mg/kg improved depression ($P < 0.01$). Morphine withdrawal has induced depression in addicted mice ($P < 0.05$), while acute injection of nano-MgO 2.5 mg/kg ($P < 0.05$) and nano-MgO 5 and 10 mg/kg ($P < 0.01$) reduced it.

Conclusions: Nano-MgO can reduce depression- like behavior induced by naloxone and morphine withdrawal in animals probably by interaction with opioid receptors.

Keywords: Depression, Naloxone, Nanoparticles, MgO, Morphine Withdrawal

1. Background

As a divalent cation, magnesium is an essential element for the normal function of the body and nervous system activity, and its deficiency can induce depression- like behavior in rodents and humans (1, 2). Oral magnesium administration to animals indicated antidepressant- like effects similar to antidepressant drugs (3). On the other hand, there is an association between depression and opioid receptor activity as well as depression and addiction (4, 5). Human and animal studies have mainly indicated that Mu (μ) opioid receptors (MORs), one type of G-protein coupled opioid receptor, are involved in the etiology of depressive disorders and that the general opioid receptor antagonist, naloxone, has depression- like effects (5). In clinical studies, it has been shown that the risk of development of depression increased in patients as the duration of opi-

oid analgesic exposure increased (6, 7). Also, it has been demonstrated that pretreatment by a magnesium supplement in animals can decrease both morphine-induced place preference and naloxone-induced place aversion (8). Our previous study has revealed that nano-MgO improves some of morphine withdrawal signs as well as anxiety- like behavior in addicted male mice (9).

2. Objectives

To determine the efficacy of nano-MgO in mood disorders such as depression and its interaction with the opioid system in the brain, in this study, we aimed at assessing the effect of acute usage of nano-MgO in the presence and absence of opioid receptor antagonist on depression- like behavior in healthy animals and acute or chronic use of nano-

MgO on depression induced by morphine withdrawal in dependant animal models.

3. Methods

3.1. Animals and Drugs

Adult male mice weighting 27 ± 3 g were purchased from animal house of faculty of veterinary medicine at Shahid Chamran University of Ahvaz, Iran. Animals were kept in a room with 12/12 h dark/light cycle and temperature $23 \pm 1^\circ\text{C}$ and were given free access to food and tap water except during the test time. Nano-MgO (Lolitech Co, Germany, particle size < 50 nm (Figure 1)) dispersed in saline 0.9% by ultrasonic bath (Parsnahand Co, Iran) for 20 minutes and shaken for 1 minute before each injection. Morphine sulphate (Temad Co, Iran) and naloxone hydrochloride (Sigma Co, Germany) dissolved in saline 0.9%.

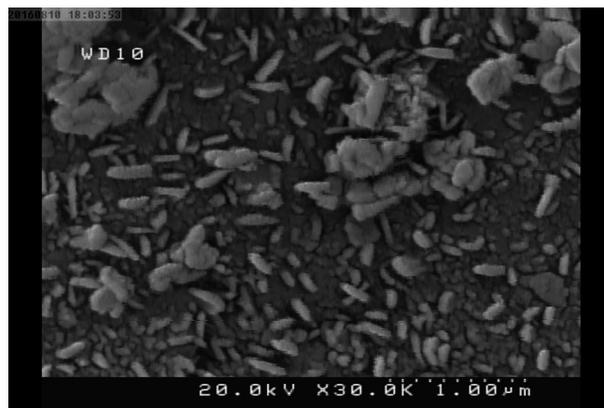


Figure 1. Scanning Electron Microscopic Image of Nano-MgO Dry Powder

3.2. Animals Grouping

Mice were randomly divided into 2 main groups: The first group included healthy mice (controls) receiving saline 0.9% and the groups receiving nano-MgO (1, 2.5 and 5 mg/kg) or naloxone 3, and 5 mg/kg alone, and groups receiving a coinjection of naloxone 5 mg/kg before and after the injection of nano-MgO 2.5 mg/kg; all drugs were injected intraperitoneally (i.p.) as an acute form. The interval time between the 2 injections was 30 minutes. The second group included dependent mice that received saline 0.9% or received i.p. injection of nano-MgO (2.5, 5, 10 mg/kg) (a single injection 30 minutes before naloxone for withdrawal induction at the test day (as an acute injection) and 4 injections during morphine administration from first to fourth days (as chronic injection)). Table 1 demonstrates animal grouping. The doses of drugs were selected according to our previous study (9).

3.3. Tail Suspension Test

Depression-like behavior was evaluated in all groups by tail suspension test 30 minutes after the second injection. The tail suspension test is used to measure depression level in mice. In this test, mice were suspended on the metal rod stand 50 to 75 cm above the table top by the adhesive tape placed approximately 1 cm from the top of the tail. After 1 minute of acclimatization, immobility duration was recorded for 5 minutes from side view using small cameras. Mice were considered immobile only when they hung passively and were completely motionless (10). All procedures were performed in accordance with institutional guidelines for animal care and use by Shahid Chamran University of Ahvaz. Number of animals in each group was 8 (N = 8).

3.4. Addiction Protocol

To induce dependence in mice, morphine was administered subcutaneously 3 times per day at 9 a.m. (20 mg/kg), 1 p.m. (40 mg/kg), and 5 p.m. (80 mg/kg) for 3 consecutive days. On the fourth day, 3 hours after the injection of the morphine 40 mg/kg, naloxone (5 mg/kg, i.p.), the selective antagonist of opioid receptors, was injected into the addicted animals for morphine withdrawal induction (9).

3.5. Data Analysis

Data were expressed as mean \pm SEM. Student's t test was used to compare the means of unpaired data. ANOVA was used for multiple comparisons between groups and Tukey post hoc test was performed using instate 3 software. Significance level was set at $P < 0.05$.

4. Results

4.1. The Effects of Acute Injections of Nano- MgO (1, 2.5 and 5 mg/kg) on Immobility Time

Data in Figure 2 demonstrate that acute injection of all doses of nano-MgO (1, 2.5 and 5 mg/kg) could not change immobility time in the tail suspension test. Below, we selected nano-MgO 2.5 mg/kg as an ineffective dose on depression for further experimentation.

4.2. The effects of Acute Injections of Naloxone Before and After Injection of Nano-MgO on Immobility Time

Results in Figure 3 revealed that the acute injection naloxone 3 and 5 mg/kg significantly increased immobility time in the tail suspension test ($P < 0.05$, $P < 0.01$, respectively); and we selected naloxone 5 mg/kg for coinjection with nano-MgO 2.5 mg/kg. Data revealed that acute injection of nano-MgO 2.5 mg/kg after naloxone 5 mg/kg

Table 1. Animals Grouping

Animals Grouping								
1. Healthy Animals								
Groups (acute injection)	1	2	3	4	5	6	7 ^a	8 ^b
Control (saline)	+	-	-	-	-	-	-	-
Nano-MgO 2.5 mg/kg	-	+	-	-	-	-	+	+
Nano-MgO 5 mg/kg	-	-	+	-	-	-	-	-
Nano-MgO 10 mg/kg	-	-	-	+	-	-	-	-
Naloxone 3 mg/kg	-	-	-	-	+	-	-	-
Naloxone 5 mg/kg	-	-	-	-	-	+	+	+
2. Addicted Animals								
Days of injections	Acute Injection				Chronic Injections			
	1. Morphine (20, 40, 80 mg/kg)	2. Morphine (20, 40, 80 mg/kg)	3. Morphine (20, 40, 80 mg/kg)	4. Morphine 40 mg/kg + naloxone 5 mg/kg	1. Morphine (20, 40, 80 mg/kg)	2. Morphine (20, 40, 80 mg/kg)	3. Morphine (20, 40, 80 mg/kg)	4. Morphine 40 mg/kg + naloxone 5 mg/kg
Control (saline)	-	-	-	+	+	+	+	+
Nano-MgO 2.5 mg/kg	-	-	-	+	+	+	+	+
Nano-MgO 5 mg/kg	-	-	-	+	+	+	+	+
Nano-MgO 10 mg/kg	-	-	-	+	+	+	+	+

^aReceived naloxone 5 mg/kg before injection of nano-MgO 2.5 mg/kg.

^bReceived naloxone 5 mg/kg after injection of nano-MgO 2.5 mg/kg.

significantly reversed immobility time to the level of control (saline) group ($P < 0.01$), while acute injection of nano-MgO 2.5 mg/kg before naloxone could not affect depression induced by the naloxone 5 mg/kg alone (Figure 3).

4.3. The Effects of Acute Injections of Nano-MgO on Immobility Time in Morphine Withdrawal Induced by Naloxone

As shown in Figure 4, morphine withdrawal increased immobility time and induced depression in addicted mice ($P < 0.05$). Moreover, acute injections of nano-MgO 2.5 mg/kg ($P < 0.05$) and 5 and 10 mg/kg ($P < 0.01$) significantly reduced immobility time in a dose-dependent manner.

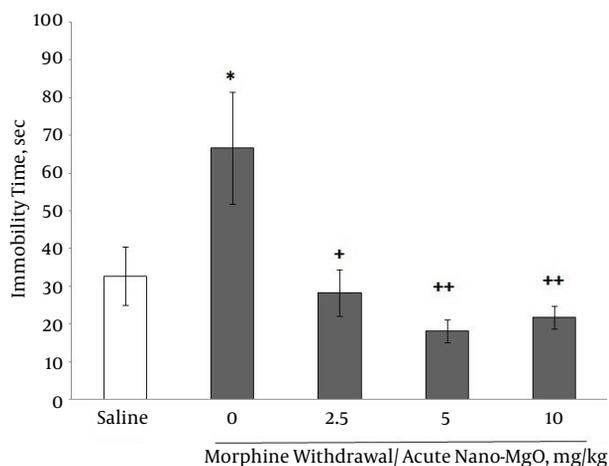
4.4. The Comparison Between Acute and Chronic Injections of Nano-MgO on Depression-like Behavior in Morphine Withdrawal Mice

Table 2 demonstrates no difference between acute and chronic injection of nano-MgO in the reduction of immobility time; it was just at the dose of 5 mg/kg that chronic injection increased immobility time in comparison with acute injection ($*P < 0.05$).

5. Discussion

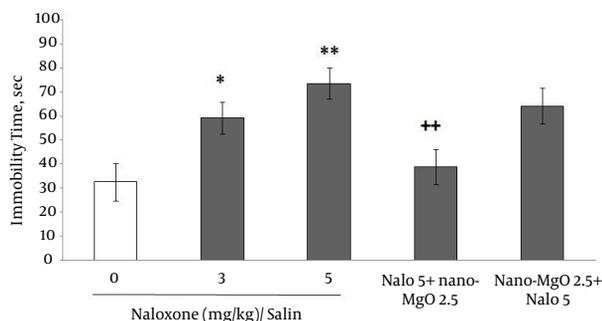
In this study, it was found that acute injection of nano-MgO at doses of 1, 2.5, and 5 mg/kg had no effect on the level of depression in the tail suspension test (Figure 2), while naloxone could induce depression in doses of 3 and 5 mg/kg and acute injection of nano-MgO 2.5 mg/kg after

Figure 2. The Effect of Acute Injection of Nano-MgO (1, 2.5 and 5 mg/kg) on Immobility Time



No differences were found between the groups. All data were expressed as mean \pm SEM, (N = 8).

Figure 3. The effect of Acute Injection of Naloxone Before and After Injection of nano-MgO on Immobility Time



*P < 0.05 and **P < 0.01 revealed a significant difference in comparison with the control (saline) group, ++P < 0.01, showing significant data in comparison with naloxone 5 mg/kg. All data were expressed as mean \pm SEM, (N = 8).

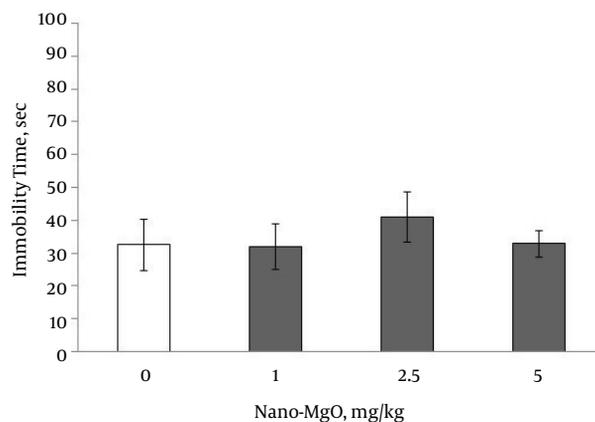
Table 2. The Comparison Between Acute and Chronic Injections of Nano-MgO on Depression-like Behavior in Morphine Withdrawal Mice

Groups	Nano-MgO 2.5 mg/kg	Nano-MgO 5 mg/kg	Nano-MgO 10 mg/kg
Acute	28.2 \pm 6.15	18 \pm 3.05	21.66 \pm 5.11
Chronic	38.33 \pm 8.62	36.25 \pm 7.11 ^a	26.66 \pm 7.6

^aP < 0.05 showed a significant difference in comparison with acute nano-MgO 5 mg/kg.

naloxone injection could reverse the depressive effect of naloxone 5 mg/kg (Figure 3). In support of the above results, it has been shown that there is an interaction be-

Figure 4. The Effect of Acute Injection of Nano-MgO on Immobility Time in Morphine Withdrawal Mice



*P < 0.05 revealed a significant difference in comparison with the control (saline) group, +P < 0.05 and ++P < 0.01, showing significant data compared with morphine withdrawal/ acute saline. All data were expressed as mean \pm SEM, (N = 8).

tween opioidergic system activity and depression-like behavior; however, opioidergic receptors agonists can show antidepressant effects, and naloxone, as a general opioid antagonist, can reverse their antidepressant effects (5). Also, there is evidence that magnesium induces opposite effects on the states of MOR agonist affinity, depending on which guanine nucleotide binds with G-proteins, while the affinity of opioid antagonist naloxone is not sensitive to regulation by magnesium (11). In our study, injection of nano-MgO before naloxone could not change the depressive effect of naloxone (Figure 3), meaning that perhaps injection of nano-MgO before naloxone could not change naloxone affinity for opioid receptors.

Opiates are used for their antinociception, sedative and antidepressant effects, while their chronic usage induced dependence and addiction (6, 12). Although opioids have antidepressant activity, their repeated usage can increase depression (6). Changes in cyclic adenosine monophosphate (cAMP) and downregulation of opiate receptors are possible adaptive mechanisms that could underlie opiate tolerance and dependence (13). Combination therapy is a way to reduce side effect of opioid dependence (14). We indicated that in addicted mice, pretreatment by nano-MgO could improve the depressive effect of morphine withdrawal and the efficacy of acute injection of nano-MgO was relatively higher than chronic injection (Figure 4 and Table 2).

It has been shown that central and peripheral N-methyl-D-aspartate (NMDA) glutamate receptors are involved in the withdrawal syndrome development in morphine-dependent mice (15). Activation of the NMDA

receptor has been implicated in the opioid tolerance mechanisms, particularly μ -opioid tolerance (16). Also, NMDA receptor activation increased influx of calcium ions and lead to neuronal dysfunction and depression (2).

Memantine (a noncompetitive antagonist of NMDA receptor) can decrease the morphine withdrawal signs in addicted mice (15). Moreover, memantine attenuated the expression of opioid physical dependence in humans (17). Magnesium is a physiological voltage-dependent blocker of NMDA receptor ion channel (3). It seems that magnesium by a block of NMDA receptor, similar to memantine, could induce an antidepressant effect in morphine dependent mice.

In the mouse periaqueductal gray (PAG), the MOR and the NMDAR can physically interact and this association appears to provide a significant conceptual advance as to how the NMDAR exerts its negative regulation of MOR function (18).

Indeed, in laboratory animals, chronic administration of opiates increases calcium uptake into various brain areas and calcium channel blocker can reduce morphine dependence and reduce the downregulation of MOR induced by opioid agonists (19).

On the other hand, morphine stimulates glutamate release in central nervous system (CNS) and glutamate-induced activation of NMDA receptors; this is while magnesium decreases glutamate release in CNS areas (8). Interaction between MOR and NMDA receptor and blockage of NMDA receptors by magnesium and reduction in calcium current are the main possible reasons for the antidepressant activity of nano-MgO in addicted mice.

In conclusion, it seems that acute use of nano-MgO can be a new supplement to reduce depression-like behavior induced by morphine dependence. Perhaps there is an interaction between opioid receptors and nano-MgO in the body, and finding the exact molecular mechanisms of nano-MgO function in depression-like behavior need to be further investigated.

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Footnotes

Authors' Contribution: Mahnaz Kesmati and Mozghan Torabi wrote the manuscript and performed the statistical calculations; Maryam Konani, performed behavioral experiments.

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Conflict of Interest: The authors declare no conflict of interest.

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