Evaluation of analgesic and anti-inflammatory effect of nanoparticles of magnesium oxide in mice with and without ketamine

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Abstract. – OBJECTIVES: According to importance and increasing application of nanoparticles and their toxicity, the identification effects of nanoparticles on physiological systems are essential. Some studies show magnesium has analgesic effect in some pain models but this evaluation was not carried on nano-magnesium oxide (MgO). Thus, present study was designed to evaluation effect of MgO nanoparticles alone and in combination with ketamine on two pain and inflammation model in mice.

MATERIALS AND METHODS: At this study, adult male mice was used which had 29±3 gram weight. Formalin and acetic acid tests were carried. Acetic acid (1%) was intraperitoneally injected 0.3ml and the abdominal writhing was counted from 10 to 30 minutes after it. Formalin (2.5%) was injected 0.04 ml/mouse subcutaneously in plantar site of mice. The time of licking was cumulatively measured 0-5 (acute phase) and 15-25 (chronic phase) minutes later. Control (negative control), ketamine (0.1 mg/kg), MgO nanoparticles (5 and 10 mg/kg), conventional MgO (5 and 10 mg/kg) and ketamine with conventional and nanoparticles MgO groups were studied in both tests.

RESULTS: Mean of writhing was significantly decreased by all drugs with comparison to control group (p = 0.0001). This decreasing was significant between conventional and nanoparticle MgO. The time of licking at both acute and chronic phases of formalin test was significantly decreased by all drugs with comparison to control group. However, this mean had significant difference with MgO nanoparticles.

CONCLUSIONS: It seems that the nano-MgO induces analgesic and anti-inflammatory effects through central and peripheral mechanisms at experimental formalin and acetic acid testes and potentiates effect of ketamine.

Key Words:
MgO nanoparticles, Formalin test, Acetic acid test, Ketamine, Mice.

Introduction

Magnesium as a important ion of body has a limited level in the serum (0.3% of total body magnesium), where it is in three states-ionized (62%), protein bound (33%), and those bound mainly to albumin and complexed to anions¹. Its half-life varying between 41 and 181 days and equilibrium between tissue pools is reached slowly².

Magnesium as a noncompetitive blocker of N-methyl D-aspartate (NMDA) receptor inhibits calcium entry into the cell³. Begon et al⁴ and Hasanein et al⁵ indicated that the magnesium and NMDA receptor involve in the modulation of pain. The NMDA receptor antagonism inhibits induction and maintenance of central sensitization after noxious stimuli. Magnesium is also a physiological calcium antagonist at different voltage-gated channels, which may be important in the mechanisms of antinociception¹. Bolcal et al⁶, showed that magnesium potentiates analgesic effect of opioids.

Ketamine is a NMDA receptor blocker and used as injectable anesthetic agent. At present study we used it as antagonist of NMDA receptor.

Conventional drugs suffer from the major limitation of adverse effects, the result of the non-specificity of their action, and from a lesser effectiveness due to improper or ineffective dosages, e.g., in cancer chemotherapy and anti-diabetic therapy⁷. Nanotechnology offers the possibility of designing new drugs with greater cell specificity and drug-release systems that act selectively on specific targets. This allows the administration of smaller but more effective doses, minimizing adverse effects. Nanotechnology can also be used to optimize drug formulations, in-
creasing drug solubility and altering the pharmacokinetics to sustain the release of the drug, thereby, prolonging its bioavailability\textsuperscript{6,8}.

Since the effect of nano-MgO did not evaluate for anti-inflammatory and analgesic; aim of present study was evaluation and comparison its analgesic and anti-inflammatory effects alone and in combination with ketamine and with conventional MgO in mice.

**Materials and Methods**

This study was conducted in Iran-Ahvaz-Shahid Chamran University in 2011.

Mice were purchased from Lab Animals’ Research Center, Jundishapour University of Ahvaz, Iran. Mice (NMRI strain) with 20 ± 3 g weight were divided 10 groups and 7 mice in each group. The mice were kept with feed and water ad libitum under 12 hours light and 12 hours dark condition. At this study, adult male mice was used which had 29±3 g weight.

**Acetic Acid-Induced Abdominal Writhing Test**

In the writhing test, acetic acid (1\%) was intraperitoneally injected 0.3 ml and the abdominal writhing was counted from 10 to 30 minutes after it. In other groups, the nano-MgO (Iolitec, Hallbronn, Germany with 40 nm size) at dose 5 and 10 mg/kg, conventional MgO (Merk, Darmstat, Germany) (5 and 10 mg/kg) and ketamine (0.1 mg/kg) alone and with conventional and nanoparticles MgO was intraperitoneally administrated 20 minutes before acetic acid injection and the number of writhing was counted as later group. Control (without any drug) group was studied.

**Formalin Test**

In formalin test, the formalin (2.5\%) was subcutaneously injected 0.04 ml/mouse in plantar site of mice. The licking time of foot was calculated 5 (acute phase) and 15-25 minutes (chronic phase) after formalin injection. All drugs were administrated similar to writhing test (as above).

**Statistical Analysis**

The mean of data was compared between groups by SPSS software (version 16, Chicago, IL, USA) and \( p \) value was significantly concerned at 0.05 levels. Graphical data are expressed as means ± SEM. The data analysis was performed by one-way analysis of variance (ANOVA) followed by LSD test for assessing specific group comparisons.

**Results**

The results showed the mean number of writhing was decreased by all drugs with comparison to control group (\( p = 0.0001 \)). This decreasing was significant between conventional and nanoparticle MgO (Figure 1). Also ketamine decreased number of writhing (\( p < 0.0001 \)). Co-administration of conventional or nanoparticle MgO potentiated effect of ketamine (\( p < 0.0001 \)).

The time of licking at both acute and chronic phases of formalin test was significantly decreased by all drugs with comparison to control group (\( p = 0.0001 \)) (Figures 2 and 3). However, this mean had significant difference with MgO nanoparticles. The mice did not show signs of pain in chronic phase when nano-MgO was administrated at dose 10 mg/kg and ketamine was co-administrated with conventional and nanoparticle MgO (Figure 3).

**Discussion**

At present study, we used acetic acid and formalin model for analgesic and anti-inflammatory effect of nano-MgO. The acetic acid induces inflammation in peritoneum and causes writhing position. Acetic acid is an animal model for acute and tonic peripheral visceral pain in rats and mice. It induces a stereotypic response pattern in form of constrictions composed of abdominal contractions, twisting and turning of the trunk and extension of the hind limbs, known as the *writhing test*\textsuperscript{10}. However, formalin test is more reliable than acetic acid test. The formalin model is widely used for evaluating the effects of analgesic compounds in laboratory animals. Injection of formalin into the hind paw induces a biphasic pain response; the first phase is thought to result from direct activation of primary afferent sensory neurons, whereas the second phase has been proposed to reflect the combined effects of afferent input and central sensitization in the dorsal horn\textsuperscript{11}.

We demonstrated that MgO has analgesic effect in both writhing and formalin test in mice. This effect was reported by other researchers\textsuperscript{12-15}. Gupta et al\textsuperscript{16} demonstrated magnesium sulfate...
significantly increased the mean and maximum duration of analgesia in thoracic epidural block. The MgO nanoparticles had greater analgesic effect than conventional MgO in our study. This may be related to better absorption or more penetration of nanoparticles especially in central nervous system. This effect was dose dependent so that 10 mg/kg had greater analgesic effect than 5 mg/kg of MgO nanoparticles.

Administration of magnesium potentiated effect of ketamine. This subject is related to effect of magnesium on action of ketamine as NMDA receptor blocker\textsuperscript{17}. Rondon et al\textsuperscript{18} showed that the neuropathic pain symptoms

![Figure 1](image1.png)

**Figure 1.** Mean number of writhing in different groups of mice. The letters show significant difference between groups (n = 7 and \( p < 0.05 \)).

![Figure 2](image2.png)

**Figure 2.** Mean time of licking in acute phase of formalin test in different groups of mice. The letters show significant difference between groups (n = 7 and \( p < 0.05 \)).
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References


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Conclusions

Nano-MgO induces greater analgesic and anti-inflammatory effects through central and peripheral mechanisms at experimental formalin and acetic acid tests and potentiates effect of ketamine.

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Conflict of Interest

The Authors declare that they have no conflict of interests.


