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Effect of Magnesium Oxide Nanoparticles on Atropine-Induced Memory Impairment in Adult Male Mice

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Abstract

Background: Previous studies have shown that magnesium oxide nanoparticles (MgO-n) improve passive avoidance memory in adult male mice. Alternatively, muscarinic receptors of the cholinergic system have a primary role in memory formation but their relationship with the improvement effects of magnesium on memory is not clear.
Objectives: The aim of this study was to investigate the effect of nano magnesium oxide on memory deficits induced by atropine as a muscarinic receptor antagonist in passive avoidance memory tests.
Materials and Methods: In this experimental study, NMRI male mice were placed in groups receiving atropine (0.1 and 1 mg/kg), recipient of MgO-n (1, 2.5, and 5 mg/kg) and groups receiving atropine in effective dose and different doses of MgO-n were used. Saline was used as a vehicle for drugs in the control groups. Memory was evaluated with a step-down apparatus to determine the coming down latency from a safe platform on days 1, 3, and 7 after training. Locomotor activity was also evaluated through an open field test in all groups after memory measurements.
Results: The results showed that atropine (1 mg/kg) decreased the latency time of coming down from the podium and induced memory deficits ($P < 0.01$). MgO-n in doses of 2.5 and 5 mg/kg caused a significant increase in latency time of coming down from the podium over one week ($P < 0.001$). MgO-n was able to reverse memory impairments resulting from atropine (1 mg/kg) ($P < 0.001$). Locomotor activity did not change in any of the groups.
Conclusions: It seems that the potentiating effect of MgO-n on memory is due to interference with the cholinergic pathway.

Keywords: Atropine, Magnesium, Memory, Nanoparticles

1. Background

Among the systems involved in the modulation of learning, memory and cognitive functions have shown clear roles for the cholinergic system (1). This system has a main role in associated learning and memory formation through the nicotinic and muscarinic receptors (2).

Some studies have shown that an inhibition of acetylcholine muscarinic receptors leads to impaired learning and memory performance in humans and animals (3). Atropine is an anticholinergic drug that is a cholinergic muscarinic receptor antagonist. Clinical and experimental evidence suggests that the central or systemic administration of anticholinergic drugs such as atropine leads to an impaired memory (4). In one experiment, mice treated with atropine resulted in both decreased levels of hippocampal acetylcholine and spatial memory impairment (5). Drugs that increase cholinergic activity can improve memory (6). It has also been shown that after the end of the atropine action, the ability to learn is improved and returns to normal states in animals (7). On the other hand, there is evidence for a positive effect of magnesium on memory (8). Recent evidence suggests that magnesium plays an important role in the release of neurotransmitters, neuronal excitability, and synaptic plasticity (8). One of the important activities of magnesium in the brain is its effect on NMDA (N- methyl- D-aspartate) receptors, which are ionotropic receptors of glutamate (9, 10). In various concentrations, magnesium has different effects on the central nervous system and intellectual and neuronal functions by biochemical and neuronal modulation (11). Previous studies have suggested that the magnesium ion is a positive regulator of synaptic plasticity because it increases the number of pre-synaptic release sites, resulting in increased synaptic transmissions and improvements in learning and memory (11, 12). However, magnesium does not easily pass through the blood-brain barrier and intravenous injections lead to small increases in the amount of these ions in the cerebrospinal fluid. Thus, peripheral administration of magnesium due to the restrictions in crossing of the blood-brain barrier to treat problems caused by magnesium defi-

ciency in central nervous system is underlying (12). Therefore, to study magnesium's effect on learning and memory in the brain we need the appropriate compound that increases the flow of ions into the brain. Nanotechnology is currently an important approach in the world's scientific and industrial. With this technology, the combination of drugs with sizes less than 100 nm has been used in different instances (8). The use of nanoparticles, for various reasons, has attracted many researchers (13, 14). The important properties of nanoparticles, such as nano drugs, include a high surface-to-volume ratio compared to the conventional drug forms, thus, making it easier to cross the blood-brain barrier, and producing increased solubility and absorption rates with an increased efficiency (8, 14). MgO-n is one of the metal oxide nanoparticles used in medicine and industry, and its effects on physiological functions such as memory are not clear and need to be further investigated (14-16).

According to the new features of nanoparticles on human health, check their effects are necessary. Despite the fact that a lot of research has been performed on magnesium's effects on memory, few studies have examined the effects of MgO-n on memory. Abdolahzadeh et al. have shown that the acute administration of MgO-n before and after training improves memory in male mice and prevents morphine-induced amnesia (17).

2. Objectives

Due to the positive role in promoting the memory formation of MgO-n and cholinergic system, the relationship between them has been less research attention. The purpose of this study was to investigate the effect of MgO-n on atropine-induced memory impairment in adult male mice.

3. Materials and Methods

In this experimental study, we used NMRI male mice (weighing 25 - 35 g) from the center of the proliferation of laboratory animals, Jondishapour University of Medical Sciences. Animals were housed for about a week to become accustomed to their new environment before the start of the experiment. The animals were then transported to the animal house. All of the animals were in good condition and maintained in a 12-hour light/dark cycle at 23 ± 2°C) in a special cage. Except during testing, all animals had free access to adequate water and food. Learning and memory tests were conducted in the light and in the range of 8 - 14 hours and each animal was used once for testing. The drugs used were prepared before the test.

The animals were divided into groups receiving atropine (manufacture Co., Rasht, Iran) as a cholinergic muscarinic receptor antagonist (0.1 and 1 mg/kg), MgO-n (Lolitech Co., Germany, particle size < 50 nm) receiver (1, 2.5, and 5 mg/kg), receiving effective doses of atropine groups with different doses of MgO-n. Saline was used in the control groups. Each group had seven animals (n = 7).

All ethical principles for the care and use of laboratory animals were followed for this study. All injections were performed intraperitoneally (IP). Long-term memory was evaluated in mice within a week on days 1, 3, and 7 after the intervention (a shock) by a step-down device and passive avoidance learning was assessed.

The step-down device consisted of a box made of Plexiglas with dimensions of $40 \times 30 \times 30$ cm with a floor of steel bars. Each of the steel bars was 0.3 inches in diameter with a spacing of 1 cm). A wooden platform with dimensions of $4 \times 4 \times 4$ cm in the bottom center of the floor was provided. Electric shocks with a frequency 1 Hz at 15 volts for 15 seconds using a stimulator connected to the floor bars transmitted a shock to the animals' hands and feet. When the animal was placed on the podium, the natural tendency of the animal was to get down on the floor bars. However, if the animal received a shock in his place is unlike innate desire to go down from on the platform avoids. In other words, an inhibitory avoidance learning has taken place. The latency time of coming down from the safe podium (step-down latency) was considered as memory retrieval (18). This involved two stages of training and testing. In the training phase, animals were slowly placed on the wooden platform in the middle of the device and the delay of coming down from the platform was recorded by a chronometer. After coming down from the plate, the animal immediately received an electric shock for 15 seconds. Before ending of the shock, the animal was removed from the device and the expected injection was performed (18, 19).

The test phase was conducted for 24 hours, 3 and 7 days after the training phase and was similar to the training phase; however, one difference was that no shock was given to the animal during this phase. Thus, each animal was slowly placed on the wooden platform again and the delay in coming down from the platform was considered as a memory retrieval. In these experiments, the time limit for stopping the mouse on the podium was a maximum of 300 seconds (18).

The open field device was used to ensure there was no effect of the drug on locomotor activity. The device consisted of a rectangular box on a wooden plate, which was divided into nine sections by four intersecting lines. Each time the animal's head and two of the anterior extremities crossed one of the lines, a number was specified for the an-

imal. The number of disconnected lines during the 5 minutes of the experiment depicted the animal's motor activity.

All experiments in this study included: three groups of animals that received IP injections of MgO-n (1, 2.5, and 5 mg/ kg), two groups of animals that received different doses of atropine (0.1 and 1 mg/ kg, IP), and two groups of animals that received an effective dose of atropine (1 mg/kg) and the effective and ineffective doses of MgO-n (5 and 1 mg/kg, respectively). The control groups received only saline. All drugs were injected after the training phase, and long term memory was assessed at 1, 3, and 7 days after training. Locomotor activity was also measured on those days for all groups.

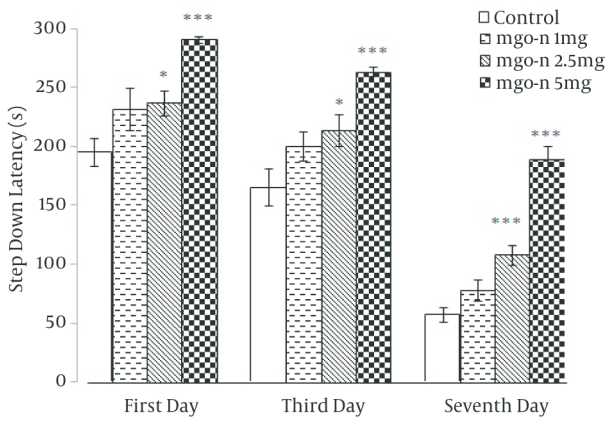
An analysis of variance with repeated measures, one-way analysis of variance, and Dunnett C additional tests were performed. Significance was considered when p values were less than 0.05. SPSS version 17 was used to perform statistical calculations and graph drawing.

4. Results

4.1. The Effect of Different Doses of MgO-n (1, 2.5, and 5 mg/kg) on Long-Term Memory

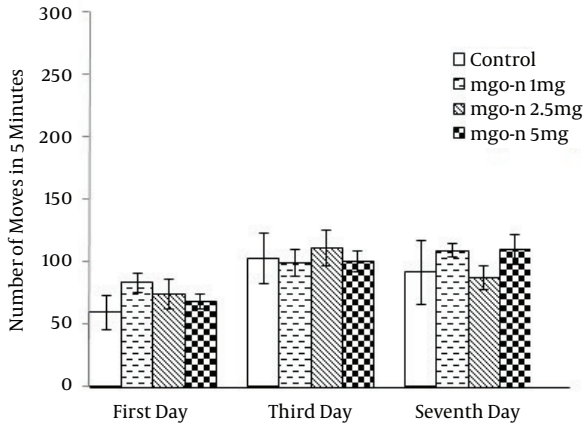
MgO-n at doses of 2.5 and 5 mg/kg, in comparison with the control group, improved memory on all test days by increasing the latency time in the step-down test ($P < 0.001$ and $P < 0.05$, respectively), while MgO-n (1 mg/kg) did not show a significant effect (Figure 1 and 2).

Figure 1. The Effect of Different Doses of MgO-n After Training (1, 2.5, and 5 mg/kg) on Step Down Latency



Each column shows the mean ± SEM. There were significant differences between the groups receiving 2.5 and 5 mg/kg of MgO-n and controls on each of the three test days (*$P < 0.05$) and (***$P < 0.001$); (N = 7).

Figure 2. The Effect of Different Doses of MgO-n (1, 2.5 and 5 mg / kg) on Locomotor Activity in the Open Field Test

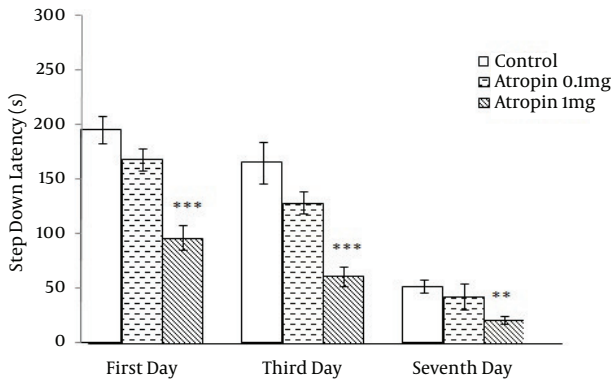


This illustration shows the effect of various doses of MgO-n after training (1, 2.5, and 5 mg/kg) on locomotor activity in the open field test 1, 3, and 7 days after training. Each column shows the mean ± SEM. There were no significant differences between the groups receiving different doses of MgO-n and the control group in the number of movements; (N = 7).

4.2. The Effect of Different Doses of Atropine (0.1 and 1 mg/kg) on Long Term Memory

Statistical analysis showed that the atropine at 1 mg/kg significantly decreased the latency time in the step-down test ($P < 0.001$) and produced an impairment of memory compared with the control group for all days after training (Figure 3).

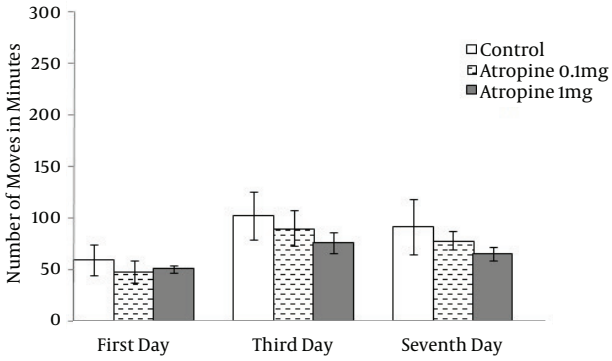
Figure 3. The Effect of Different Doses of Atropine (0.1 and 1 mg/kg) After Training on the Step Down Latency on Days 1, 3, and 7 After Training



Each column shows the mean ± SEM. There were significant differences between the atropine group (1 mg/kg) and the control group (**$P < 0.01$, ***$P < 0.001$); (N = 7).

Figure 4 shows that different doses of atropine (0.1 and 1 mg/kg) had no effect on locomotor activity.

Figure 4. The Effect of Different Doses of Atropine (0.1 and 1 mg/kg) on Locomotor Activity of Mice in the Open Field Test on Days 1, 3, and 7 After Training

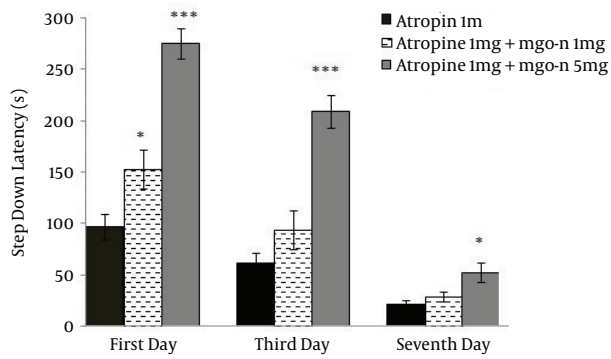


Each column shows the mean ± SEM. There were no significant differences between the groups; (N = 7).

4.3. Effect of MgO-n on Atropine-Induced Memory Impairment

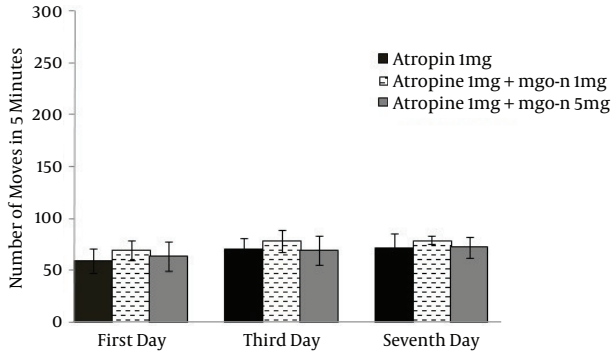
Figure 5 shows the results of effective and ineffective doses of MgO-n (5 and 1 mg/kg, respectively) and an effective dose of atropine (1 mg/kg). Statistical analysis revealed a significant difference between groups simultaneously injected with MgO-n (1 and/or 5 mg/kg) and atropine (1 mg/kg) in comparison to atropine (1 mg/kg) alone ($P < 0.05$ and $P < 0.001$, respectively). The results shown in Figure 6 did not show any changes in locomotor activity in these groups.

Figure 5. The Results of Effective and Ineffective Doses of MgO-n (5 and 1 mg/kg, respectively) on the Amnesia Induced by Atropine (1 mg/kg) on Days 1, 3, and 7 Following Training in Step-Down Latency



Each column shows mean ± SEM. Significant differences between the groups are represented as *P < 0.05 and ***P < 0.001; (N = 7).

Figure 6. The Results of Effective and Ineffective Doses of MgO-n (5 and 1 mg/kg, Respectively) on the Presence of Amnesia Induced by Atropine (1 mg/kg) on Locomotor Activity on Days 1, 3, and 7 Following Training



Each column shows the mean ± SEM. There were no significant differences between the groups receiving MgO-n and the control group in the number of movements; (N = 7).

5. Discussion

The results of this study showed that the MgO-n in doses of 2.5 and 5 mg/kg improved memory retrieval in a passive avoidance learning task (Figure 1). Our results were confirmed by previous studies based on the effect of magnesium compounds on memory and related structures, and that acute prescriptions of MgO-n before and after training cause memory improvements in male mice (16, 17).

Another result of this study suggests that atropine (1 mg/kg) causes damage in passive avoidance memory without any change in locomotor activity (Figures 3 and 4).

Previous studies have shown that after training, injections of atropine reduce the memory in a step-down test, but before the test, they were unable to change memory (18). In explaining the role of atropine in its effects on memory, it can be noted that atropine is an anticholinergic agent that blocks muscarinic-type receptors (20). It has been also been shown that blocking these receptors disrupts memory performance and learning in both humans and animals (3). All of the above-mentioned studies confirm our results concerning atropine's effect on memory.

Our results showed that MgO-n in doses of 5 mg/kg and 1 mg/kg in the presence of an effective dose of atropine (1 mg/kg) could significantly return memory impairments caused by atropine (Figure 5). This indicates an interaction between the cholinergic system and the physiological effects of magnesium on memory-forming processes. The physiological effect of magnesium on memory is achieved through a balancing of the glutamate NMDA receptor, which is able to block channels of this receptor in a

voltage-dependent condition (21). NMDA receptors are important for learning and memory (21).

Some studies have emphasized the importance of the interference of muscarinic receptors type 1 and NMDA receptors on memory. For example, central injections of scopolamine and MK801 (an antagonist of NMDA receptors) before training disrupts memory recall. However, the simultaneous prescription of an ineffective dose of MK801 and an effective dose of scopolamine inhibits scopolamine's disruptive effect on memory (22, 23).

According to the above evidence, it seems that NMDA receptor agonists and antagonists inhibit the memory disruption caused by anticholinergic drugs. Furthermore, in our study, magnesium was considered a blocker of this receptor and thus, it could prevent the memory disruption induced by atropine. Memantine, an antagonist of NMDA receptors, is also used to improve memory in Alzheimer's patients (24). This contradiction may be due to the NMDA receptor's interactions in various situations.

There is no strong evidence concerning memory improving mechanisms of NMDA receptor antagonists. One way in which these antagonists may be able to enhance cognitive acts through selective inhibition is by a pathological activation, while protecting the physiological activation of the NMDA receptor. This principle has been shown when magnesium was removed from the simple slice leads to premature activation of NMDA and inhibition of LTP. Under these conditions, the addition of the specific competitive NMDA receptor antagonist, AP5, was able to fully restore the ability to induce LTP (25, 26).

Our data suggest that the potentiating effect of MgO-n as modulating the voltage dependent block of NMDA receptors on memory is due to an interference with the cholinergic pathway.

However, due to the complex structure of the NMDA receptor and the ability to be modulated in different conditions and there are many uncertainties to clear the dark angles of them, further studies are needed.

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Footnotes

Authors' Contribution: Zeinab Sargholi Nootarki, performed behavioral experiments and took part in partnership and supervision; Mahnaz Kesmati, wrote the manuscript; Mahdi Poormehdi Borujeni, performed the statistical calculations.

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