Original Article

J Babol Univ Med Sci
Vol 18, Issu 1; Jan 2016. P:37-43

Comparison of the Effects of Pre-training Administration of Zinc Oxide and Zinc Oxide Nanoparticles on Long-term Memory of Adult Male Mice

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J Babol Univ Med Sci; 18(1); Jan 2016; PP:37-43

Received: Jun 30th 2015, Revised: Jul 29th 2015, Accepted: Sep 28th 2015.

ABSTRACT

BACKGROUND AND OBJECTIVE: Zinc oxide nanoparticles are one of the most widely used nanoparticles in fields of industry, medicine, pharmaceutical sciences, cosmetics, and nutrition. Multiple studies have demonstrated the negative effects of zinc oxide nanoparticles on the nervous system, while others have revealed their enhancing effects on the activity of nerve cells, involved in memory processes. The aim of this study was to compare the effects of zinc oxide nanoparticles and zinc oxide on long-term memory of mice.

METHODS: In this experimental study, 49 NMRI adult male mice, with the mean weight of 25±5 g, were randomly divided into seven groups, each consisting of seven mice: control group, three treatment groups receiving zinc oxide nanoparticles (1, 2.5, and 5 mg/kg of zinc oxide nanoparticles, respectively), and three treatment groups receiving zinc oxide (1, 2.5, and 5 mg/kg of zinc oxide, respectively). Intraperitoneal injections were performed before training (electric shock). Passive avoidance memory of mice was evaluated, using the Step-Down device. The latency time to descend the platform was regarded as an indicator of memory on days 1, 3, and 7 following training.

FINDINGS: Pre-training administration of zinc oxide nanoparticles and zinc oxide at a dose of 2.5 mg/kg yielded no effects on the motor activity of mice. However, a significant decline was reported in the latency time to descend the platform on days 1, 3, and 7 following training (58±17, 45±13, and 39±14 in the zinc oxide group and 93±18, 62±12, and 14±3 in the nano zinc oxide group, respectively) ($p<0.01$); however, the dosage of 5 mg/kg had less significant short-term effects (130±38, 49±14, and 68±10 in the zinc oxide group and 132±46, 41±13, and 58±24 in the nano zinc oxide group, respectively). Also, the dosage of 1 mg/kg was almost ineffective.

CONCLUSION: The results showed that weakened long-term memory, caused by zinc oxide administration, is not influenced by the size of particles.

KEY WORDS: *Memory, Nanoparticles, Zinc Oxide.*

Please cite this article as follows:

Eisapare N, Kasmati M, Mohammadi T. Comparison of the Effects of Pre-training Administration of Zinc Oxide and Zinc Oxide Nanoparticles on Long-term Memory of Adult Male Mice. J Babol Univ Med Sci. 2016;18(1):37-43.

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[DOR: 20.1001.1.15614107.1394.18.1.6.6]
[DOI: 10.22088/jbums.18.1.37]

Introduction

Zinc oxide is an inorganic compound with the formula ZnO. Considering the limited toxic characteristics of zinc oxide and its long-term use compared to other zinc compounds (e.g., sulfate and bicarbonate), food companies have extensively used it as a supplement (1). In recent years, use of nanoscale materials has increased rapidly, and oxidized compounds such as zinc oxide are produced as nanoparticles in various fields. The widespread use of nanoparticles has led to their entrance into the environment and human life (2).

Zinc oxide nanoparticles are among the most commonly used elements in industry, medicine, pharmacy, cosmetics, and nutrition (3). Due to their smaller size compared to zinc oxide, nanoparticles have a greater capacity to cross biological barriers and easily enter the brain (4,5). However, some studies have revealed a complete similarity between zinc oxide and zinc oxide nanoparticles in terms of acute toxicity (6). Damages caused by severe zinc deficiency in adult mice are similar to hippocampal lesions. Therefore, zinc deficiency affects hippocampal function, and memory disorders are widely associated with zinc deficiency (7). Moreover, young and old mice with zinc deficiency have been shown to have a weaker performance on T-maze, compared to control mice. Also, the effects of zinc deficiency on spatial working memory are more significant in younger mice, compared to older ones; this type of deficiency causes significant delays in the development of long-term memory (8).

Zinc supplements have negative effects on newborns' cognitive function (9), while it has been shown that zinc deficiency has adverse impacts on short-term memory of young mice in a water maze (8). In fact, mice with no zinc deficiency spend more time in the dark room and show more activity (10). Today, despite the beneficial effects of zinc, a great deal of attention has been paid to the side-effects of this compound, which may be caused by high zinc concentrations (11).

Some studies have suggested the harmful effects of zinc oxide nanoparticles on the body (12-14). In fact, zinc oxide nanoparticles cause cytotoxicity by the production of reactive oxygen species and lead to oxidative damage, inflammatory stimulation, and cell death (15). Moreover, exposure to zinc vapor may have negative effects on memory and cognition, which may be associated with age and sex (16). Also, several studies have demonstrated the negative effects of zinc oxide nanoparticles on learning and memory in Wistar rats (17, 18).

Reports on the effects of zinc oxide nanoparticles on cognitive processes involved in memory and learning have been contradictory in behavioral and laboratory experiments, and effects of these compounds on the central nervous system remain controversial and inconsistent. Therefore, identifying the positive or negative effects of these compounds on important brain functions such as memory can increase the awareness of researchers, pharmacists, and manufacturers, who use nanoparticles for various causes. Such studies can help develop preventive approaches against the adverse side-effects of nanoparticles. The aim of this study was to compare the effects of zinc oxide nanoparticles and zinc oxide on long-term memory of mice.

Methods

In this experimental study, we examined 49 NMRI adult male mice (mean weight= 25±5 g), purchased from the animal house of Veterinary Faculty at Shahid Chamran University of Ahvaz, Iran. The animals were divided in seven groups (seven mice each) and maintained in Plexiglass cages under laboratory conditions at a temperature of 23±2 °C with adequate ventilation (12 hours of light and 12 hours of darkness).

Zinc oxide nanoparticles smaller than 70 nm (LoLiTec Company, Germany were dissolved in 0.9% saline for daily administration before the experiment and were dispersed, using the Ultrasonic Bath 2600S (Pars Nahand Company, Iran) for 22 minutes. Prior to each injection, the compound was dispersed by a shaker for 1 minute. Zinc oxide (Merck Company, Germany) was also dispersed prior to the examination for 5 minutes in 0.9% saline by the shaker. Three groups received 1, 2.5, and 5 mg/kg of zinc oxide nanoparticles, three groups were administrated 1, 2.5, and 5 mg/kg of zinc oxide, and the control group received 0.9% saline through intraperitoneal injections.

The behavioral test was carried out, using the Step-Down device (Model ST-5500, Industrial Tower Company, Tehran, Iran) for 30 minutes after the injection. This device was used for the assessment of passive avoidance behavior in mice. The device was 30×30×40 cm^3 in dimension and had three dark-colored Plexiglas walls; the front wall was transparent

[DOR: 20.1001.1.15614107.1394.18.1.6.6]
[DOI: 10.22088/jbums.18.1.37]

for monitoring animal behaviors. The device had a stainless steel bottom and a wooden platform (4×4×4 cm^3), which was placed in the center of the bottom. During training, the mice were placed on the wooden platform and the latency time to descend the platform was recorded. Immediately after stepping down the wooden platform, the mice received electric shock (15 V) for 15 seconds (training), and then, they were returned to the cages. The trained mice (electric shock) were again placed on the wooden platform the next day, 3 and 7 days (i.e., day of the test) the latency time was recorded. Electric shock was not employed on the day of the test.

The mice would receive the maximum score (300 seconds) provided that they could remain on the platform for 5 minutes during the test. Similar to the first day, latency time to descend the platform was recorded on days three and seven after training (electric shock) to analyze the memory of mice. Open-Field device was employed for motor activity evaluation. Motor activity test was used to assess the possible impact of zinc oxide on motor activity, which can interfere with memory evaluation. 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 animal.

The number of disconnected lines during 5 minutes of the experiment depicted the motor activity of the animal. To analyze the data, ANOVA and Tukey's test were performed. InStat Version 3 was employed, and Microsoft Excel 2013 was used to draw the charts and $p<0.05$ was considered statistically significant.

Results

Evaluation of the effects of different doses of zinc oxide and zinc oxide nanoparticles on memory: The groups receiving 1 mg/kg of zinc oxide and zinc oxide nanoparticles were not significantly different from the control group in terms of latency time at 1, 3, and 7 days after training. However, a considerable difference was detected between the control group and groups receiving 2.5 mg/kg of zinc oxide and zinc oxide nanoparticles in terms of latency time at 1, 3, and 7 days after training ($p<0.05$ and $p<0.01$, respectively). However, the effects of zinc oxide and zinc oxide nanoparticles were not significantly different at these intervals. Within three days of the experiment, the two groups receiving 5 mg/kg of zinc oxide and zinc oxide nanoparticles were significantly different from the control group ($p<0.05$ and $p<0.01$, respectively); also, a descending trend was observed on days 1 and 7 after training. Based on the findings, there was no significant difference between zinc oxide and zinc oxide nanoparticles at different intervals. The comparison of 2.5 and 5 mg/kg doses of zinc oxide and zinc oxide nanoparticles showed a significant difference on days 1 and 7 after training ($p<0.05$). Therefore, 5 mg/kg of zinc oxide and zinc oxide nanoparticles could cause memory impairment, although these effects were less significant than 2.5 mg/kg (fig 1).

Evaluation of the effects of different doses of zinc oxide and zinc oxide nanoparticles on motor activity: The motor activity test was not performed at a dose of 1 mg/kg for either types of zinc oxide (injected before training). According to previous studies, 5, 10, and even 25 mg/kg of zinc oxide and zinc oxide nanoparticles have no effects on motor activity (12, 16). However, in order to confirm previous studies, the motor activity test was carried out with 2.5 and 5 mg/kg of oxide and zinc oxide nanoparticles (injected before training), using the Open-Field device. Based on the statistical analysis, the groups receiving 2.5 and 5 mg/kg of zinc oxide and zinc oxide nanoparticles were not significantly different from the control group; this might indicate the ineffectiveness of these compounds in motor activity (fig 2).

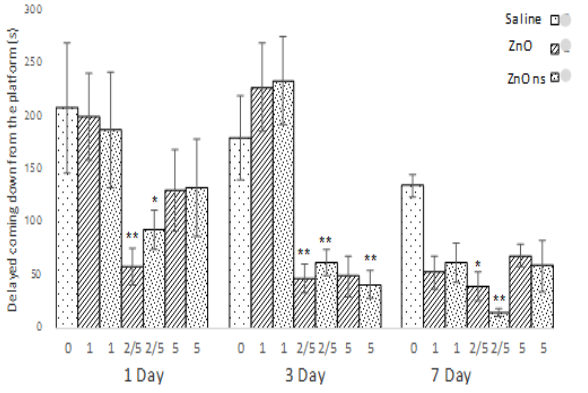

Figure 1. Effects of zinc oxide and zinc oxide nanoparticles (1, 2.5, and 5 mg/kg) on avoidance memory (during a week), using the Step-Down device. Each column represents Mean±SEM values. *p<0.05 and **p<0.01 in comparison with the control group

[DOR: 20.1001.1.15614107.1394.18.1.6.6]
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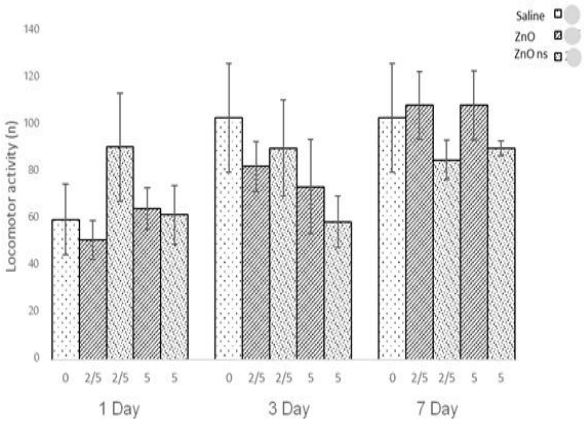

Figure 2. Effects of zinc oxide and zinc oxide nanoparticles (2.5 and 5 mg/kg) on locomotor activity (during a week), using the Open-Field device. Each column represents Mean±SEM values.

Discussion

This study indicated that 2.5 and 5 mg/kg of zinc oxide and zinc oxide nanoparticles could deteriorate the learning capacity and memory in mice. The findings on the effects of zinc oxide nanoparticles on memory were in line with the results of a study by Valipour and colleagues. According to their study, acute administration of zinc oxide nanoparticles deteriorates memory in Wistar rats; however, it was revealed that this effect is not related to oxidative stress by nanoparticles (18).

Han and colleagues also reported that long-term exposure to zinc oxide nanoparticles may destroy zinc homeostasis and result in increased long-term potentiation (LTP). Imbalance between these two mechanisms would postpone the acquisition process and cause memory impairment (17). Moreover, this study indicated no significant difference between zinc oxide and zinc oxide nanoparticles at similar doses. However, zinc oxide nanoparticles could enter the cells directly through cell membranes or membrane channels and proteins; some might also enter the cells via endocytosis (19).

Some studies have shown that zinc oxide nanoparticles, zinc oxide, and Zn^{2+} are quite similar in terms of severe toxicity (6). Therefore, it seems that Zn^{2+} ions, which are released from zinc oxide and zinc oxide nanoparticles, enter the cells and destroy synaptic homeostasis, which may cause an excessive increase in LTP via intracellular processes and cause insufficient LTP (17), resulting in memory impairment. On the other hand, high concentration of zinc can inhibit the excessive proliferation of NMDA receptors in the hippocampus. Free Zn^{2+} ions mainly exist in synaptic vesicles and a specific set of glutamatergic neurons in the brain, especially granule cells in the dentate gyrus of hippocampus (20, 21). Mossy fibers, containing zinc, can be found in presynaptic vacuoles. Also, Zn^{2+}, which exists in 45% of the Schaffer collateral system, may be used as an endogenous neurotransmitter to impose inhibitory effects on NMDA receptors. LTP induction in the Schaffer collateral system is dependent on the activity of these receptors (21, 22).

The primary role of Zn^{2+} is to reduce the NMDA receptor currents. The inhibitory effects of zinc on NMDA receptors are of grave significance, given the essential role of these receptors in transmission and synaptic plasticity (23). These receptors are glutamate-gated channels, which are responsible for excitatory postsynaptic currents in central synapses (23).

NMDA receptors are diverse and some of them are sensitive to zinc ions as endogenous blockers (20). The extracellular portion of each NMDA subunit would bind active allosteric ligands such as Zn^{2+}. There are two types of Zn^{2+} binding sites in separate subunits, which inhibit NMDA receptors through different mechanisms (23).

Pre-training injection of zinc oxide and zinc oxide nanoparticles may increase the amount of Zn^{2+} in glutamatergic synapses and inhibit NMDA receptors; this would ultimately reduce the incidence of LTP and impair acquisition and learning. Also, LTP inhibition by zinc may be explained by calcium channel blockers in nerve terminals, which reduce the excitatory post-synaptic potential (22).

In this study, 5 mg/kg of zinc oxide and zinc oxide nanoparticles caused a relative impairment in long-term memory of mice on the first and seventh days of the experiment; however, on the whole, these effects were less significant, compared to the dose of 2.5 mg/kg. It appears that the number of nanoparticles is higher at the dose of 5 mg/kg. Therefore, the mobility of nanoparticles is higher and there is a possibility of binding at higher suspensions; this in fact can lead to the binding of particles (24, 25).

On the other hand, some studies have shown that nanoparticles have a greater tendency towards accumulation over time (24). Another theory explaining the less significant effect of higher doses is that Zn^{2+} adhesion to cells is controlled by specific receptors such as metal-binding proteins (26). Probably, the amount of Zn^{2+} ions, released from zinc

[DOR: 20.1001.1.15614107.1394.18.1.6.6]
[DOI: 10.22088/jbums.18.1.37]

oxide and zinc oxide nanoparticles at 2.5 mg/kg is sufficient for the receptors; hence, due to the accumulation of these receptors, the adhesion of Zn^{2+} to cells does not improve by increasing the concentration, but remains unchanged. In this regard, Izumi and colleagues showed that at low micromolar concentrations, zinc has no effects on LTP in the CA1 hippocampal slices, while LTP is weakened at high micromolar concentrations (27). In our study, pre-training injection of zinc oxide and zinc oxide nanoparticles at a dose of 1 mg/kg did not change the learning process, which confirms the mentioned results. Considering the role of glutamatergic receptors such as NMDA in cognition and learning and the relation between these receptors and zinc ions, it is suggested that the effects of agonists and antagonists of receptors, zinc oxide, and zinc oxide nanoparticles on cognitive behavior, be evaluated. According to the results, it seems that long-term memory impairment by zinc oxide is not affected by the size of particles.

Acknowledgments

Hereby, we would like to thank the deputy of research and technology at Shahid Chamran University of Ahvaz for their financial support and cooperation.

[DOR: 20.1001.1.15614107.1394.18.1.6.6]
[DOI: 10.22088/jbums.18.1.37]

References

1.Kesmati M, Torabi M, Ghandizadeh-Dezfuli M. Nanoparticles of zinc oxide reduces acute somatic pain in adult female wistar rats. Zahedan J Res Med Sci. 2014;16(2):24-8.[In Persian]

2.Handy RD, Von Der Kammer F, Lead JR, Hassellov M, Owen R, Crane M. The eco toxicity and chemistry of manufactured nanoparticles. Ecotoxicology. 2008;17(4):287-314.

3.Emamifar A, Kadivar M, Shahedi M, Soleimanian-Zad S. Evaluation of nanocomposite packaging containing Ag and ZnO on shelf life of freshorange juice. Innov Food Sci Emerg Technol. 2010;11(4):742-8.

4.Schrand AM, Rahman MF, Hussain SM, Schlager JJ, Smith DA, Syed AF. Metal-based nanoparticles and their toxicity assessment, WIREs Nanomed Nanobiotechnol. 2010;2(5):544-68.

5.Win Shwe TT, Fujimaki H. Nanoparticles and neurotoxicity. Int J Mol Sci. 2011;12(9):6267-80.

6.XiongD, Fang T, Yu L, Sima X, Zhu W. Effects of nano-scale TiO2, ZnO and their bulk counterparts on zebrafish: Acute toxicity, oxidative stress and oxidative damage. Sci Total Environ. 2011;409(8):1444-52.

7.Golub MS, Keen CL, Gershwin ME, Hendrickx AG. Developmental zinc deficiency and behavior. J Nutr. 1995;125(8 Suppl):2263-71.

8.Smith M, Harris P. The effects of severe zinc deficiency on the spatial working memory of young and adult female rats laurendevito and Leanne nassar. Colgate University Journal of the Sciences, 2000; 155-162.

9.Hamadani JD, Fuchs GJ, Osendarp SJ, Huda SN, Grantham-McGregor SM. Zinc supplementation during pregnancy and effects on mental development and behaviour of infants: a follow-up study. Lancet. 2002; 360(9329):290-4..

10.Ebuehi OAT, Akande GA. Effect of zinc deficiency on memory, oxidative stress and blood chemistry in rats. Int J Bio Chem Sci. 2009;3(3):513-23.

11.Bardosono S, Dewi L.E, Sukmaniah S, Permadhi I. D, Eka A, Lestarina L. Effect of a sixmonth iron-zinc fortified milk supplementation on nutritional status, physical capacity and speed learning process in Indonesian underweight schoolchildren: randomized, placebo-controlled, Medical J. Indonesia, 2009; 18: 191-200.

12.Gojova A, Guo B, Kota RS, Rutledge JC, Kennedy IM, Barakat AI. Induction of inflammation in vascular endothelial cells by metal oxide nanoparticles: effect of particle composition. Environ Health Perspect2007 ; 115(3):403-9.

13.Brunner TJ, Wick P, Manser P, Spohn P, Grass RN, Limbach LK, et al. In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility. Environ Sci Technol. 2006;40(14):4374-81.

14.Reddy KM, Feris K, Bell J, Wingett DG, Hanley C, Punnoose A. Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. Appl Phys Lett 2007; 90(213902): 213902-1–213902-3.

15.Deng X, Luan Q, Chen W, Wang Y, Wu M, Zhang H et al. Nanosized zinc oxide particles induce neural stem cell apoptosis. Nanotechnology 2009; 20(11): pp. 7.

16.Eizadi-Mood N, Pourabdian S, Fallah M. Effects of chronic Zinc Fume exposure on memory and cognition. Iran J Toxicol. 2010;3(3):317-23.

17.Han D, Tian Y, Zhang T, Ren G, Yang Zh. Nano-zinc oxide damages spatial cognition capability via over-enhanced long-term potentiation in hippocampus of Wistar rats. Int J Nanomedicine. 2011;6:1453-61.

18.Valipour S, Hoseini SE, Vahdati A, Kesmati M. The effects of acute administration of zinc oxidenanoparticles on long term memory in the presence and absence of vitamin C in adult male rat. Adv Environ Biol. 2014;8(13): 260-6.

19.Chang YN, Zhang M, Xia L, Zhang J, Xing G. The toxic effects and mechanisms of Cuo and Zno nanoparticles. Materials. 2012;5(12):2850-71.

20.Takeda A, Fuke S, Ando M, Oku N. Positive mudolation of long-term potentiation at hippocampal CA1 synapses by low micromolar concentrations of Zinc. Neuroscience. 2009;158(2): 585-91.

21.Talaei Zavareh SA, Hamidi G, Salami M. Long Term Potentiation as a Mechanism for Learning and Memory. Yakhteh Med J.2009;11(2):88-105.[In Persian]. Available from:http://www.sid.ir/fa/VEWSSID/J_pdf/54213884202.pdf

[DOR: 20.1001.1.15614107.1394.18.1.6.6]
[DOI: 10.22088/jbums.18.1.37]

22.Turner YT, Soliman RIM. Effects of zinc on spatial reference memory and brain dopamine (D1) receptor binding kinetics in rats. Prog Neuropsychopharmacol Biol Psychiatry. 2000;24(7):1203-17.
23.Amico-Ruvio S, Murthy ES, Smith PT, Popescu KG. Zinc Effects on NMDA receptor gating kinetics. Biophys J. 2011;100(8):1910-18.
24.Adams L, Lyon D, Alvarez P. Comparative eco-toxicity of nanoscale TiO2, SiO2, and ZnO water suspensions. Water Res. 2006; 40(19):3527-32.
25.Singh R, Pantarotto D, Lacerda L, Pastorin G, Klumpp C, Prato M, et al. Tissue bio-distribution and blood clearance rates of intravenously administered carbon nanotube radiotracers. Proc Natl AcadSci USA. 2006; 103(9):3357-62.
26.Baek M, Chung HE, Yu J, Lee JA, Kim TH, Oh JM, et al. Pharmacokinetics, tissue distribution and excretion of zinc oxide nanoparticles. Int J Nanomedicine. 2012; 7:3081-97.
27.Izumi Y, Auberson YP, Zorumski CF. Zinc modulates bidirectional hippocampal plasticity by effects on NMDA receptors. Neurosci. 2006; 26(27):7181-8.

[DOR: 20.1001.1.15614107.1394.18.1.6.6]
[DOI: 10.22088/jbums.18.1.37]