The effects of acute administration of zinc oxidenanoparticles on long term memory in the presence and absence of vitamin C in adult male rat

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Background and Objectives: Despite the wide spread use of zinc oxide nanoparticles (Nano zinc oxide) in the medical and industry, a comprehensive study has been done on the effects of neuropsychological and cognitive its related processes. Considering the oxidative effects of nanoparticles, there has been a great challenge on the way to their medicinal use. The purpose of this study was to investigate the effect of acute application of Nanozinc oxide on memory processes in the presence and absence of vitamin C as an antioxidant in the adult male rats. Methods: Adult male wistar rats (200-250g) were divided into groups with 7 in each including saline, three groups received Nano zinc oxide (1.25, 2.5 and 5mg/kg), three groups received vitamin C (30, 60, 120mg/kg) alone and three groups received vitamin C and Nano zinc oxide in combination. Memory was assessed by Step Down device after teaching using passive avoidance learning. Results: It was revealed that in all three doses of Nano zinc oxide there was a significant loss of memory (P <0.001 and P <0.01) while no change in locomotor activity. Vitamin C as antioxidants, at doses of 60 and 120 mg impaired memory and in 30 mg was ineffective. Ineffective dose of vitamin C could not improve memory deficit resulting from the Nano zinc oxide. Conclusion: Considering the results of this study, acute application of Nano zinc oxide causes significant impairment in long-term memory, and this effect is probably not just due to its oxidative effect. This action mechanism may be effect through change in receptors activity or neurotransmitters releasing which is associated with memory that needs to be investigate further.

INTRODUCTION

Because of the widespread use of metal nanoparticles, these compounds are rapidly entering the environment and human systems [1] and the various forms enter living bodies. Despite properties that make them useful in various areas, they also have caused concern [2]. Nanoparticles of Zinc oxide are one of the most widely used nanoparticles in industry, medicine, and nutrition [3]. These nanoparticles could well pass the blood and brain barrier and may cause unwanted effects. So far, few of the effects were created by Nano zinc oxide on the CNS are known [4]. Some studies have suggested beneficial effects of these compounds as acute administration of Nano zinc oxide has show so considerable anxiolytic in animal models compared to conventional ones [5]. The chronic use of Nano zinc oxide compared to ordinary type exerts analgesic effect substantially in the presence and absence of aerobic exercise in rats [6] so it would provide a new approach in the treatment of related diseases. It is also worth noting that chronic infusion of Nano zinc oxide in rats is increase LTP in the hippocampus [7]. However, the results of in vitro studies on hippocampal pyramidal neurons isolated from ratshow a significant increase in intracellular ROS as a potential mechanism of apoptosis induced by Nano zinc oxide. It has been suggested that Nano zinc oxide can increase cytosolic calcium levels, which would be the production of intracellular ROS, and can consequently increase the progression of neuronal apoptosis [8]. There are controversies regarding the beneficial effects of zinc oxide nanoparticles on the relieving pain and anxiety on the one hand and the effects of stress oxidative induced toxicity on the other, so
the research is necessary to prevent adverse effects of Nanoparticles in the presence of an antioxidant. In many studies antioxidants and modulators of nerve effects of vitamin C (ascorbic acid) are well known [10, 11] and some studies have also shown the beneficial effects of this compound in protecting neurons [9, 10]. Since little research on the effects of antioxidants in preventing complications of zinc oxide nanoparticles has been observed especially in memory and cognitive processes, the aim of this study was to evaluate the effects of zinc oxide nanoparticles on long-term memory of the passive avoidance learning in the presence and absence of vitamin C in adult male rats.

MATERIALS AND METHODS

In this study, adult male wistar rats weighing 200-250 g obtained from the Center of Medical Sciences, Ahvaz breeding of laboratory animals were used. The animals were kept under standard conditions temperature (22 ± 2°C), humidity (55–60%) and illuminated 7:00 a.m. to 7:00 p.m., with enough food and water for animals in each cages. Prior to testing, each four rats were maintained in separate cages for 1 week. The animals were randomly divided into ten groups (n =7):

1) Group normal saline (sham) (n =7): Just a day after 10 ml/kg received saline injections after training (shock), a 24-hour memory test was performed.
2) Groups receiving zinc oxide nanoparticle (groups2, 3 and 4) with values of 1.25, 2.5 and 5 (mg / kg), after training (shock), like saline group, the 24h memory test was performed.
3) Groups receiving three doses of vitamin C (groups5, 6 and 7) as antioxidants, 30, 60 and 120 (mg / kg), after training, like saline group, the 24h memory test was performed.
4) Groups receiving of combination of vitamin C 30 mg/kg and zinc oxide nanoparticles at a dose of 1.25, 2.5 and 5 (mg / kg) one day after training a 24-hour memory test was performed (groups8, 9 and 10).

In order to assess locomotor activity the animals, all groups were tested by Open field immediately after assessing memory.

Methods:

Nano zinc oxide with sizes below 70 nm, produced by Merck, Germany, the rate of required daily, 15 minutes before the start of the test dispersed by Ultrasonic devices in saline 0.9% then before each injection, again for 1 minute were distributed by the Shaker [6]. A single dose of Nano zinc oxide was intraperitoneally injected then all groups were training (shock) 30 min later of injection [11]. To the group receiving the combination of Nano zinc oxide and vitamin C was given 30 mg vitamin C, before Nano zinc oxide. To avoid the possible influence of motor impairment due to drugs, the mobility of animals weren’t clearly impaired, to assess spatial memory [12].

Evaluation of 24-hour memory in rats:

In this evaluation, a step down apparatus box with dimensions 40 × 30 × 30 cm was used. The height box was 15 cm from the level of ground, the floor was made of stainless steel rod with a diameter 1 mm and the distance 10 mm between them. A wooden platforms with dimension 2.5 × 7 × 27 cm was placed in the center of bottom bar. The experiment was conducted fairly in dark room. The box was lit by a 15 watt bulb during the test. In order to make rats familiar with the testing tool, they were put inside the box one day before the learning. During learning, the rat was placed inside a cylindrical plastic on the wooden platform 10 seconds after, the plastic cylinder was removed and low latency of the wooden platform was recorded. Soon after coming down all four legs from the wooden platform, an electric shock 0.5 mA was given for 3 seconds to get the bottom bar. The rats were then returned to the cage. The next day (remember the 24-hour period) after the rat on the platform was placed inside a plastic cylinder, plastic cylinder was removed after 10 seconds and low latency from the platform was recorded. Of course, the day reminding electric shock does not apply [13]. Rats that for more than 5 minutes during the test period stayed on the platform were given the maximum score of 300 seconds. The results of the recall score for each training session or memory, for each rat or a group from formula inflection ratio (IR) = (L1-L0/L0) was calculated. L0 show initial low latency from platform on the learning day. L1 shows low latency from platform on the memory test day [13].

Open field Test:

It was used to evaluate the motor activity of the animal. The test equipment consists of a rectangular plate made of wood; the floor is divided by lines into 16 squares. Initially, each rat was placed in the center and the passage frequency from lines as an index of locomotor activity was studied for 5 minutes. At the end of the test, each rat was removed from the test chamber and completely clean with a damp cloth and then was dried [14].
Statistical methods:
Results are expressed as mean ± SEM. Data was analyzed using SPSS (Version 21). To evaluate the results of tests in different groups ANOVA, one-way and Post hoc, LSD was used. In all cases, there is differences between the groups at P <0.05 is considered significant.

RESULTS AND DISCUSSION

Evaluation of long memory in passive avoidance learning by Step down Figure (1) shows the ratio of delay time coming down from the safe platform (step down latency) to 24 hours after administration Nano zinc oxide in quantities of 1.25, 2.5 and 5 (mg / kg) and the teaching compared to saline. Statistical analysis shows a significant decrease (P<0.001and P <0.01) in the above index. Thus, a single injection of Nano zinc oxide can interfere in passive avoidance learning (acquisition) and memory retrieval 24 hours.

Figure (2) shows the ratio of delay time coming down from the safe platform (step down latency) to 24 hours after administration of vitamin C in amounts 30,60, 120 (mg / kg) and the teaching compared with saline. Statistical analysis showed no effect in 30 mg and a significant reduction in above index in doses of 60 and 120 mg (P <0.001). Thus, vitamin C can impair learning and memory in high doses. Due to memory impairment resulting from vitamin C in doses of 60 and 120 mg, 30 mg was used for subsequent experiments.

Figure (3) shows the effect of various amounts of Nanozinc oxide (1.25, 2.5 and 5 mg / kg) in the presence of 30 mg/kg vitamin C (the amount of inert in memory test) compared with vitamin C alone. Statistical results show that groups receiving combination doses 2.5 and 5mg / kg (except 1.25) Nanozinc oxide with vitamin C demonstrate a significant decrease in coming down from the safe platform compared to the group receiving vitamin C alone, (P <0.05). The groups receiving different amounts of Nanozinc oxide didn’t show significant delay in time of coming down from the safe platform compared to the groups receiving the combination of different doses of Nanozinc oxide and vitamin C (30 mg/kg) (Figure 4.) Thus, the presence of vitamin C as an antioxidant cannot prevent from memory deficits due Nano zinc oxide.
Fig. 3: Comparison of the combined effect of Nanozinc oxide (1.25, 2.5 and 5 mg) and vitamin C (30 mg), on the delay time coming down safe platform than vitamin C (30 mg) alone.

Assessment of locomotor activity by open field test:

As shown in Figures 5, 6, 7, 8 count lines crossed in the open filed test as an index of locomotor activity are compared in rats saline group and groups of receiving different doses of Nanozinc oxide (Figure 5), saline group and groups of receiving different doses of vitamin C (Figure 6), as well as receiving group of 30 mg/kg vitamin C and groups of receiving combination of different doses of Nanozinc oxide and 30 mg / kg vitamin C (Figure 7) and groups receiving different doses of Nanozinc oxide with receiver Nanozinc oxide and combination of 30 mg/kg vitamin C (Figure 8).

Fig. 5: Comparison of the number of lines crossed in the open field test in rats receiving saline and groups of receiving different amounts of Nanozinc oxide.
Fig. 6: Comparison of the number of lines crossed in the open field test in rats receiving saline and groups of receiving different amounts of vitamin C (30, 60 and 120 mg)

Fig. 7: Comparison of the number of lines crossed in the open field test in rats receiving 30 mg/kg vitamin C and groups receiving combinations different amount of Nano zinc oxide with 30 mg of vitamin C

Fig. 8: Comparison of the number of lines crossed in the open filed test in rats receiving different doses of Nano zinc oxide and groups of receiving different doses of Nano zinc oxide in combination with 30 mg / kg vitamin C

Discussion:
The findings have shown that acute intraperitoneal administration of Nano zinc oxide can be weaken passive avoidance memory in the rat significantly. However, because of the results shown by the open filed test
performed on these groups, it can be concluded that this method of administration on locomotor activity in the rats of receiving different doses of Nanozinc oxide has influences even in the presence of vitamin C, and differences in delay time down from safe platform are not related to movement disorders. The results show that Nano zinc oxide can be direct or indirect effect on learning and memory in the brain and impairs memory formation. One of the possible destructive effects of Nano-zinc oxide on the memory can be related to its size. Also some studies have shown that Nano-sized compounds can reach the brain and may be associated with neurodegenerative diseases [15, 16]. Nano zinc oxide is produce reactive oxygen species (ROS) and the membrane eventual injury caused by it. Effects of zinc oxide nanoparticles on cultured neural stem cells have shown induction of apoptosis and so decrease of cell survival [17]. This features with large number of its toxic effects are consistent those found recently and the results of the memory impairment induced by Nano zinc oxide is also confirmed in this study. On the other hand, it has been shown that chronic infusion of Nano-zinc oxide in young rats enhances LTP in the hippocampus [7], which could be due to increased levels of cytosolic calcium [8]. This is one of the mechanisms of memory formation and is in consistent with disruption caused by zinc oxide nanoparticles in this study. Therefore, it seems to be one of the reasons why it could be related to the duration of drug injection, because the injection in this study is acute while in the above study it is chronic. It is noteworthy that the zinc ions are the glutamate NMDA receptor antagonist [18] and specified activities of these receptors in the formation of memory and LTP is essential [19]. Obviously the release of Nano zinc oxide can also decrease the activity of these receptors and impaired memory that is compatible with these results. The results of this study showed vitamin C as an antioxidant at low doses (30 mg) having no effect on memory, but in high doses it can cause memory impair. There is evidence that vitamin C, a potent antioxidant in humans [20, 21] and intracellular ascorbate in the CNS, performs numerous acts (May, 2012) and high concentrations in the brainplay potential role as a protective factor for brain [10, 22, 23]. However, a study on the expression of BDNF (factor derived neurotrophic brain) has shown that the use of high-dose vitamin C alone can act as a protective of oxidant and reduces its expression (Rai, et al., 2013). The high doses of vitamin C is cause memory corruption that returns the role of the vitamin as antagonist and inhibitorglutamate receptors. There is evidence that suggests vitamin C, apart from the effect of antioxidant, sits on the NMDA glutamate receptors and reduces its signaling [24-27]. These receptors are involved in learning and memory [19], and obviously reduction of their activity can disrupt memory formation, which is in agreement with the results of this study.

**Conclusion:**

In part of results, it was found that the ineffective dose of vitamin C (30 mg) is not able to avoid memory disrupt due to the low doses of Nano zinc oxide. Although low doses of Nano-zinc oxide (1.25 mg) show tended to inhibit relative. So the memory impairment in co-administration vitamin C and Nano zinc oxide compared to the degradation due to Nano zinc oxide alone is less (although not statistically significant (Figure 8). Perhaps reduced amounts of Nano zinc oxide is most effective. However, this study showed that vitamin C cannot compensate Nanozinc oxide attenuation effect in the values used. In this respect, it is likely that the suppressive effect of Nanozinc oxide on the memory is not relative only to the activity of stress oxidative due to production of reactive oxygen (ROS) of these nanoparticles and may be part of the memory impairment caused by the effect of zinc oxide nanoparticles has relative with learning and memory-related receptors such as glutamate NMDA receptors and neurotransmitters and vitamin C cannot prevent from Nanozinc oxide action due to interaction with them. More study is needed to confirm this possibility.

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**REFERENCES**


