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Effect of Zinc Oxide Nano-Particles on Motor Coordination in the Attendance of Vitamin C in Rats

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Abstract

Introduction: Zinc oxide is an inorganic compound with the formula ZnO. Oxide is widely used in various applications, especially in pharmaceutical applications, as well as technical and cosmetic products. The aim of this study is to evaluate the effect of zinc oxide nano-particles (ZnO NPs) on the motor coordination of male rats in the presence and absence of vitamin C as an antioxidant.

Methods: In this experimental study, 80 male Wistar rats with a weight range of 200-250 g were used. The rats were randomly divided into ten 8-memebr groups control group (saline receivers) and rats which received zinc nano-oxide with doses 1.25, 2.5 and 5 mg/kg, rats receiving vitamin C with doses 30, 60 and 120 mg/kg and rats which received both zinc nano-oxide with doses 1.25, 2.5 and 5 mg/kg and vitamin C with a dose of 30 mg/kg.while different groups received the above doses intraperitoneally *(i.p)* the rate of motor coordination was measured in the first day of study using rotarod machine. Motor coordination was considered as the duration a rat could stay on the rotarod.

Results: According to the results, dose dependent ZnO NPs with doses 1.25, 2.5 and 5 mg/kg and the simultaneous prescription of different doses of ZnO NPs and a neutral amount of vitamin C decrease this rotarod duration.

Conclusion: ZnO NPs disturb motor coordination. Probably, this is not due to the oxidative feature of zinc because vitamin C could not improve this effect. It seems that this effect is applied by another mechanism such as changing the activities of neurochemical system contributing to motor coordination.

Keywords: ZnO NPs, Motor Coordination, Vitamin C, Rat

Introduction

There are many reports on the effect of zinc (Zn) on motor behavior and learning. Decreased level of consciousness (LOC), loss of consciousness, decreased activity, attention deficit, mental disorders and movement disorders are outstanding symptoms of zinc deficiency (1, 2). Today, many food companies prefer to use salts of element oxides such as zinc oxide salt because oxidized salts are low reactive compared with sulfate salts and reduce the toxic effects generated by the long-term use of such compositions. In addition to its catalytic and structural function in many proteins, zinc ion (Zn^{2+}) is deemed to play a vital role in neural transmission Processes (3). In its nano-particle form, zinc can easily path through blood brain barrier (BBB), reach brain and cause unwanted effects. However, there are few known effects caused by zinc nano-oxide on central nervous system (CNS) (4). According to Some empirical evidence, the incidence and progress of neurodegenerative diseases such as Alzheimer, Parkinson and selective mutism, SM, are associated with oxidative stress as well as the accumulation of high concentrations of metals, such as copper, aluminum, zinc and especially iron, in those zones of brain which are responsible for function deficiency and cellular damage. Free

radicals can play a significant role in BBB degeneration because BBB is sensitive to oxidative damages to a large extent (5). Zinc can act as an antioxidant in the brain (6).The transmission of zinc from plasma to external cellular fluid (ECF) and cerebrospinal fluid is tightly adjusted by brain barrier system, for example by blood-brain barrier and CSF-blood. BBB system interfere zinc homeostasis in the brain (7, 8). Vitamin C acts as an electron donor and the remover of free radicals such as super-oxide radicals and hydroxyl radicals in in-vitro condition (9). In the body, vitamin C acts as an electron donor and the remover of free radicals such as super-oxide radicals and hydroxyl radicals in in-vitro condition and shows different behaviors ranging from exhibiting antioxidant properties to protecting living entity and its bio molecules from oxidative damages (10, 11). Studies on nano-particles are in their preliminary stage and there are limited, contradictory and challenging studies on the effect of nano-particles on CNS. Therefore, the identification of mechanisms inducing positive or negative effects of such substances on one of the most important functions of the brain, i.e. motor coordination, can promote the awareness of researchers, pharmacists and industrial men dealing with nano-particles, including zinc nano-particle. This, in turn, prohibits the occurrence the probable unwanted side effects of such substances. Therefore the aim of this study is to evaluate the effect of ZnO NPs on the motor coordination of male rats in the presence and absence of vitamin C as an antioxidant.

Methods

This experimental study was conducted on mature Wistar male rats (weight=200-250gr) prepared from the Research Centre and Experimental Animal House of Ahvaz Jundishapur University of Medical Sciences. The animals were kept in individual cages in 12 h light and 12 h dark conditions, temperature of 21±2°C. Animal handling and experimental procedures were performed under the observance of the University and Institutional legislation, controlled by the Local Ethics Committee for the Purpose of Control and Supervision of Experiments on Laboratory Animals. Then, they were grouped into 10 groups (n=8) in random as follows: control group (saline receivers) and rats which received zinc nano-oxide with doses 1.25, 2.5 and 5 mg/kg, rats receiving vitamin C with doses 30, 60 and 120 mg/kg and rats which received both zinc nano-oxide with doses 1.25, 2.5 and 5 mg/kg and vitamin C with a dose of 30 mg/kg. For preparation of zinc oxide nano-particles, each day, before conducting any test a required amount of ZnO NPs (prepared by Lolitec Company, Germany) with a particle size of 50 to 80 nano meter was dissolved in saline 0.9% by ultrasonic bath device for 15 minutes. In addition, the mixture was shaken by a shaker for 1 minute before every injection (12). Vitamin C was dissolved in saline and injected to the studied groups with a pre-specified dose. All injections were in acute dose and in i.p. form. The control group was injected with saline 0.9% with a dose of 10 mg/kg and motor coordination was evaluated in all groups only in the first day after the injection. Motor coordination test was performed to measure motor performance and coordination. For this purpose, the animals were placed on the Rotarod (Figure 1) with variable speed. The initial rotation speed of the rod was 5 rpm, which was later gradually increased to 25 rpm within 300 sec (5 min) (Figure 1). The main criterion for the balance in all groups was 25 rpm. The animals were formerly familiarized with this test. Then, each rat was tested 3 times per day with 45 min interval between the sessions. Also, the mean time was calculated (13). The current study data have been presented in a Mean ± SEM format and then they were analyzed through the use of appropriate statistical tests in Excel and SPSS20 environments and by taking advantage of one-way ANOVA method and LSD Post hoc and the discrepancies obtained

in the results derived for various groups was considered to be statistically significant in $P<0.05$ level.

Results

The duration of staying on the rotarod (motor coordination) significantly decreased in the rats that received ZnO NPs with doses 2.5 and 5 mg/kg compared with the rat which received of saline ($p<0.05$ and $p<0.01$, respectively) (Table 1). The rat which received different doses of vitamin C showed no significant difference in the duration of staying on the rotarod compared with the group which received saline (Table 1). The rats which received both ZnO NPs with doses 1.25, 2.5 and 5 mg/kg and vitamin C showed a significant reduction in the duration of staying on the rotarod compared with the rats which received saline only ($p<0.05$ and $p<0.01$) (Table 1).The rats which received both ZnO NPs and vitamin C showed no significant difference in the duration of staying on the rotarod compared with the rats which received vitamin C only (Table 1).The rats which received both ZnO NPs with different doses and vitamin C with a dose of 30 mg/kg showed no significant difference in the duration of staying on the rotarod compared with rats which received only different doses of ZnO NPs (Figure 2). Considering that there was no significant difference between the experimental groups with each other.

Table 1. Comparison of the duration of staying on rotarod (motor coordination) saline group and receivers of ZnO NPs with doses 1.25, 2.5 and 5 mg/kg in the first day after injection and between saline group and receivers of different doses of vitamin C

Group	Mean	Standard Deviation
Saline	38.92	8.63
ZnOPs 1.25 mg/kg	25.17	3.27
ZnOPs 2.5 mg/kg	19.21 *	3.17
ZnOPs 5 mg/kg	12.04 **	4.08
Vitamin C 30 mg/kg	29.89	5.61
Vitamin C 60 mg/kg	31.76	7.72
Vitamin C 120 mg/kg	36.55	8.31
ZnOPs 1.25 Vitamin C 30 mg/kg	21.38 #	1.76
ZnOPs 2.5 + Vitamin C 30 mg/kg	18.9 #	1.49
ZnOPs 5 + Vitamin C 30 mg/kg	17.29 ##	2.79

*P<0.05, **P<0.01 and between the receivers of vitamin C only with a dose of 30 mg/kg and the receivers of different doses of ZnO NPs along with vitamin C with a dose of 30 mg/kg in the first day after injection. #P<0.05, ##P<0.01

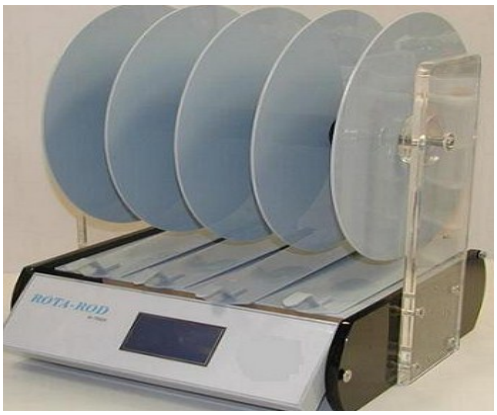

Figure1. Rotarod Device

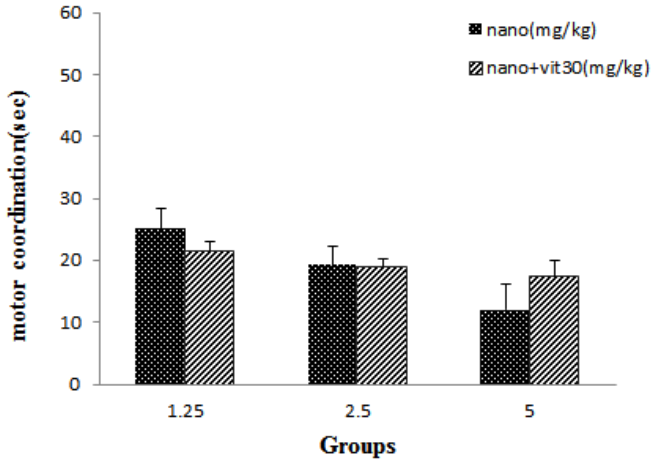

Figure 2. Comparison of the duration of staying on rotarod (motor coordination) between the rat receiving different of different doses of ZnO NPs and the different doses of zinc nano-oxide along with vitamin C with a dose of 30 mg/kg in the first day after injection.

Discussion

According to the findings of this study, *i.p* injection of ZnO NPs can significantly weaken the motor coordination of rats in the first day after receiving drug. The results show that ZnO NPs can directly and indirectly affect neural centers and in turn affect motor coordination and disturb motor function. In this study, it was found that low levels of inactive vitamin C (30 mg/kg) was not able to prevent the loss of motor coordination caused by ZnO NPs, although with low levels of ZnO NPs (1.25 mg), it seems to indicate a relative inhibition. So that the level of equilibrium degradation in co-administration of ZnO NPs and vitamin C is lower than that of nano-oxide alone. But it is not statistically significant. Perhaps if the amount of ZnO NPs is reduced, this effect will be more pronounced. However, the study showed that vitamin C cannot compensate for the effect of attenuating zinc in the amounts used. In this regard, it is likely that the effect of attenuation of ZnO NPs on equilibrium is not just related to the oxidative stress activity resulting from the production of ROS from this nanoparticle and may be part of the defect caused by ZnO NPs by receptors and neurotransmitters. Vitamin C cannot prevent them from interacting with them. More evidence is needed to prove these probabilities. Studies on the effect of different doses of zinc chloride on the motor coordination and motor behavior of male young rats showed that the rats which received 30 or 50 mg/kg zinc chloride for two weeks showed higher motor coordination than control group and could stay more on rotarod machine and there was a significant difference between the groups. On the other hand, prescribing higher doses of zinc chloride had no effect on motor activity, motor coordination and degenerative activities at first but, it significantly reduced them later (14). This implies that in special conditions, such as higher doses, zinc can show a weakening effect on motor activity and coordination. Since materials show different behavior at nano scale, it can be suggested that the size of zinc oxide nano-particle may serve as one of the probable degenerative effects of it in the motor coordination so that according to some studies, nano-scaled compounds can reach brain and may be associated with neurodegenerative diseases (2). According to studies, increased concentration of zinc in substantia nigra destructs substantia nigra cells and decreases the secretion of dopamine In turn, the decreased secretion of dopamine from the two nuclei controls the neural γ-Amino

butyric-substantia nigra path. On the other hand, increased levels of zinc directly controls neural γ-Amino butyric path and stimulates neural glutamatergic path (14). Decreased controlling signals such as GABA and dopamine and increased stimulating signals such as glutamate trigger the continuous output of stimulating signals towards the cortex-spinal motor control system. The signals can definitely over-stimulate a large number or whole muscles of body and result in tonic spasm. Akinesia problem which is seen in Parkinson disease is generally due to the fact that following the decreased secretion of dopamine in basal ganglia, its secretion decreases in the limbic system too. This may reduce mental stimulation for motor activity to such an extensive extent that can result in akinesia. On the other hand, motor plans need frequent switches between stimulation and control. Therefore, the lack of the controlling effect of dopamine inhibits the initialization and progress of successive plans demanding stimulation stages in addition to controlling stages. This is exactly what happens in akinesia (15).

Conclusion

According to the results of this study, zinc oxide nano-particles have a weakening effect on motor coordination. However, this effect is not likely due to the oxidative stress factors of the nano-particles and discovering its accurate mechanism demands more studies.

Ethical issues

No applicable.

Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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