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Aggravated hepatotoxicity occurs in aged mice but not in young mice after oral exposure to zinc oxide nanoparticles

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ABSTRACT

The potential toxic effects of nanoparticles (NPs) might be more severe in susceptible populations such as the elderly. To confirm such effects, we built an aged mouse model and evaluated the effects after giving model mice oral doses of zinc oxide (ZnO) NPs. We observed enhanced ZnO NP absorption in aged mice compared with young mice most likely because of an age-related increase in intestinal permeability. The liver deposition of ZnO NPs was higher in aged mice compared with young mice. As a result, liver injuries were observed in aged mice, whereas young mice were not affected. ZnO NPs induced increases in oxidative stress and inflammation levels in both aged and young mice. Because aged mice have already endured age-related increases in oxidative stress and inflammation, ZnO NPs caused additional damage and resulted in acute liver injury. The above evidence suggests that the elderly are more vulnerable to NP exposure, and more caution should be taken with regard to the application of or accidental exposure to ZnO NPs among the elderly.

1. Introduction

The elderly population is increasing worldwide, and the proportion of the global population who is over 60 years old will increase from 10% in 2000 to 32% in 2100 (Wolfgang Lutz and Scherbov, 2008). Aging is a complex physiological process characterized by alterations to many physiological functions such as reduced metabolic activity (Vestal, 1997) and a state of chronic inflammation (Franceschi and Campisi, 2014). Oxidative stress is largely responsible for chronic inflammation (Yu and Yang, 1996) and aging. These physiological characteristics make the elderly more vulnerable to environmental pollutants such as particulate matter and more prone to certain diseases (Yu and Yang, 1996; Gouveia and Fletcher, 2000a, 2000b; Cakmak et al., 2007; Li et al., 2014).

With the rapid development of nanotechnology in industrial and consumer markets, the environmental accumulation of nanomaterials and human exposure to nanoparticles (NPs) are unavoidable. NPs perturb various physiological systems (Zhang et al., 2014) such as the respiratory system (Schinwald et al., 2012) and male reproductive system (Bai et al., 2010). Because of their efficient UV absorption and antimicrobial properties, zinc oxide (ZnO) NPs have been explored with regard to a variety of applications and products such as cosmetics, sunscreens, food additives, packaging materials (Schilling et al., 2010), fungicides (He et al., 2011), anticancer drugs and imaging agents (Han et al., 2015). The widespread application of ZnO NPs and their production, transportation, and disposal will increase their environmental accumulation (e.g., in groundwater) and human exposure (e.g., via oral exposure). After entering the gastrointestinal (GI) tract, ZnO NPs can be absorbed into the circulation and translocated to various organs (Pasupuleti et al., 2012; Ryu et al., 2014). The liver, an important organ of reticuloendothelial system, is a major target (Kao et al., 2012). A heavy build-up of ZnO NPs in the liver can generate excessive oxidative stress, cause DNA damage and cell apoptosis (Sharma et al., 2012a, 2012b; Chung et al., 2015; Yang et al., 2015; Pati et al., 2016), and perturb liver function (Fazilati, 2013; Filippi et al., 2015; Yang et al., 2015; Kaya et al., 2016).

Despite the adverse effects of ZnO NPs that have been reported, most in vivo studies have focused on the toxicity of ZnO NPs to healthy young animals. Because oxidative stress is involved in both nanotoxicity and the aging process, concern has been raised regarding possible aggravated nanotoxicity in the elderly. A recent report observed more severe neurotoxicity and hippocampal pathological changes following intraperitoneal exposure to ZnO NPs in 18-month-old C57BL/6J mice than in 6-month-old or younger mice (Tian et al., 2015).

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In this study, we established an aged mouse model and investigated biodistribution and nanotoxicity in aged and young mice following oral exposure to ZnO NPs. We found that the aged mice exhibited more liver deposition (35.74 μg/g) of the absorbed ZnO NPs than the younger mice (29.14 μg/g). ZnO NPs also caused more severe liver damage in aged mice, likely because of their reduced antioxidant activity and lower immunity.

2. Materials and methods

2.1. Characterization of ZnO NPs

ZnO NP dispersion at 50% by weight in distilled water was purchased from Sigma-Aldrich (St. Louis, MO, USA). The size and morphology of the ZnO NPs were determined using transmission electron microscopy (TEM), which was performed using a JEOL 1200 EX Transmission Electron Microscope (JEOL, Ltd. Tokyo, Japan) at 80 kV. The images were captured using an AMT 2k CCD camera. The number-based size distribution of the ZnO NPs was determined by counting 100 particles using a nanomeasurer.

For the hydrodynamic diameter and zeta potential analysis, ZnO NPs were diluted in various solutions with different pH values (pH 2.0, 7.0, and 8.0) or a medium. The hydrodynamic diameters of the ZnO NPs were measured using dynamic light scattering (DLS, Malvern Nano ZS, Malvern, UK). The zeta potential was measured using a laser particle size analyzer (Malvern Nano ZS, Malvern, UK).

For in vivo experiments, a ZnO NP solution was dispersed in distilled water (30 mg/mL) and sterilized at 121 °C for 30 min. The suspensions were sonicated for 15 min prior to use.

2.2. The release of Zn^{2+} by ZnO NPs in water and artificial gastric fluid (AGF)

To determine the stability of the ZnO NPs under gastric conditions and in the blood circulation, ZnO NPs were suspended in AGF (0.20% sodium chloride and 0.32% proteolytic enzymes, pH 1.5) and water (pH 7.4) at a concentration of 30 mg/mL. ZnO NP suspension in water or AGF was allocated to incubate at 37 °C for 0.5, 1.0, 2.0, 6.0, 12.0, and 24.0 h. Then, the suspensions were centrifuged at 5000*g* for 30 min, and the supernatant was collected for Zn concentration determination using an inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7700, Santa Clara CA, USA). The mean of three determinations was used to calculate the dissolution of Zn^{2+}.

2.3. D-Galactose-induced aging mice model

Male C57BL/6J mice (9 weeks old, 23–25 g) were obtained from the Chinese Academy of Medical Sciences (Beijing, China). The animals were housed in clean polypropylene cages and maintained in an air-conditioned animal house at 20 ± 2 °C with 50–70% relative humidity and a 12-h light/dark cycle. All animal studies were approved by the Animal Care and Utilization Committee of Shandong University. All experimental procedures were performed in accordance with institutional guidelines.

The D-galactose-induced aging mice model has been widely recognized worldwide because the changes caused by D-galactose administration are consistent with natural aging (Ho et al., 2003). This aging model is stable, convenient and inexpensive compared with other models such as the natural aging model, the ozone-induced aging model and the senescence-accelerated mouse-prone model (Ho et al., 2003). To establish the D-galactose-induced aging mice model, mice (50 in each group) were given daily subcutaneous injections of 5% D-galactose solution (Sigma Aldrich, MO, USA) at a dose of 0.5 g/kg or saline (young control) for six weeks (Santos et al., 2012). The body weight of the mice was recorded daily. At the end of the 6th week, the serum, brains and livers of 10 mice in each group were collected to determine immunological status and the oxidative stress level.

To study the immunological status of the mice, IL-2 and IFN-γ in serum were detected with mouse IL-2 and IFN-γ ELISA kits (Boster Biological Technology Co., Ltd., Wuhan, China), respectively. To investigate oxidative stress level, the malondialdehyde (MDA) and superoxide dismutase (SOD) in mice serum were determined using an MDA assay kit and an SOD assay kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China), respectively. To determine the antioxidant capacity, the total antioxidative capacity (T-AOC; U/mL) in serum was calculated (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). The formation of advanced glycation end products (AGEs) in mice serum was measured using an AGE ELISA kit (R&D Systems Inc., MN, USA).

2.4. ZnO NP administration

Oral gavage was selected as the route of exposure for the mice in this study because ZnO NPs are used in food packaging and heavily released into ground water, making it highly possible that these particles enter the body through the digestive system.

The doses at which humans might be exposed to ZnO NPs have not been established. According to the Organization for Economic Cooperation and Development (OECD), the suggested doses for investigating the toxicity of new substances are 5, 50, 300, 2000, and 5000 mg/kg (Guideline, 2001). Given the difficulty of creating ZnO NP suspensions at high concentrations, the maximum dose selected in this study was 300 mg/kg (Sharma et al., 2012a, 2012b). To evaluate the effects induced by lower doses, a dose of 50 mg/kg was also selected.

D-Galactose-induced aging mice and young control mice were given a daily oral dose of ZnO NPs (50 and 300 mg/kg) for two weeks. To determine the possible effects of the Zn^{2+} ions released from the ZnO NPs during the experiments, a supernatant of ZnO NP suspension (300 mg/kg) was used as a control in addition to the vehicle control group. Each group consisted of 10 mice.

All mice were weighed and observed for mortality and clinical signs of toxicity daily. At the end of the second week, the mice were euthanized under anesthesia, and their blood and organs (heart, liver, spleen, lungs, kidneys and intestines) were collected and analyzed. The coefficient of organ to body weight was calculated as the ratio of organ wet weight (g) to body weight (g).

2.5. Deposition of ZnO NPs in organs

To investigate the biodistribution of the ZnO NPs after oral exposure, the Zn content in the organ tissues was analyzed. The mouse organs (heart, liver, spleen, lungs, kidneys and small intestine) were collected, freeze-dried, and ground into powder. A fraction of the powder (approximately 0.04 g) was weighed and soaked in 3 mL of aqua regia at 90 °C for 12 h, then in 3 mL of $HNO_4/HClO_4$ (v/v 4:1) at 120 °C for 12 h. The digestion solutions were diluted and analyzed for concentrations of Zn using ICP-MS.

2.6. TEM examination of ZnO NPs in the liver and intestines

Liver or intestinal tissue were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) and rinsed. Then, the tissues were fixed for 1 h in 2% osmium tetroxide with 3% potassium ferrocyanide and rinsed. Next, they were treated with en bloc staining with a 2% aqueous uranyl acetate solution and dehydrated. They were then placed into propylene oxide, a series of propylene/epon dilutions, and embedded. Thin (70-nm) sections were cut on a Leica UC6 ultramicrotome, and TEM images were taken using a JEOL 1200 EX transmission electron microscope (JEOL, Ltd. Tokyo, Japan) equipped with an AMT 2k digital camera.