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Zinc oxide nanoparticles in predicted environmentally relevant concentrations leading to behavioral impairments in male swiss mice

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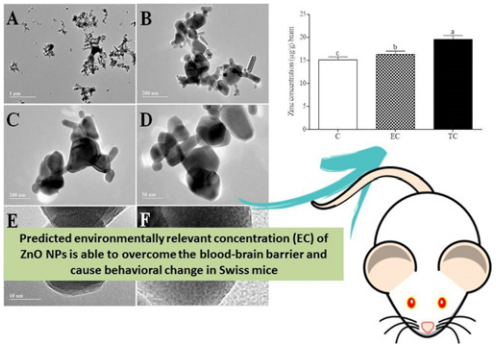
HIGHLIGHTS

- Predicted environmentally relevant concentration of ZnO NPs cause behavioral change.
- ZnO NPs causes anxiogenic effect in male Swiss mice.
- ZnO NPs are able to overcome the blood-brain barrier in mice.

GRAPHICAL ABSTRACT



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ABSTRACT

Although the potential neurotoxic effects from the exposure to zinc oxide nanoparticles (ZnO NPs) on humans and on experimental models have been reported in previous studies, the effects from the exposure to environmentally relevant concentrations of them remain unclear. Thus, the aim of the present study is to investigate the effects from the exposure to environmentally relevant concentrations of ZnO NPs on the behavior of male Swiss mice. The animals were daily exposed to environmentally relevant concentrations of ZnO NPs (5.625×10^{-5} mg kg^{-1}) at toxic level (300 mg kg^{-1}) through intraperitoneal injection for five days; a control group was set for comparison purposes. Positive control groups (clonazepam and fluoxetine) and a baseline group were included in the experimental design to help analyzing the behavioral tests (open field, elevated plus maze and forced swim tests). Although we did not observe any behavioral change in the animals subjected to the elevated plus maze and forced swim tests, our data evidence the anxiogenic behavior of animals exposed to the two herein tested ZnO NPs concentrations in the open field test. The animals stayed in the central part of the apparatus and presented lower locomotion ratio in the central quadrants/total of locomotion during this test. It indicates that the anxiogenic behavior was induced by ZnO NP exposure, because it leads to Zn accumulation in the brain. Thus, the current study is the first to demonstrate that the predicted environmentally relevant

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ZnO NPs concentration induces behavioral changes in mammalian experimental models. Our results corroborate previous studies that have indicated the biological risks related to the water surface contamination by metal-based nanomaterials.

1. Introduction

Nanoscience and nanotechnology involve processes applied to different segments such as food production, electronics, pharmacy, biotechnology, cosmetics, nanomedicine, agriculture and national security, as well as are related to materials and products ranging from 1 to 100 nm (Hu et al., 2016). There is no doubt nanotechnology is one of the science fields facing great development in the last decade due to high investments in research; the United States is the greatest investor in this technology, followed by Germany and Japan (Sant'Anna et al., 2013).

The estimated annual production of nanomaterials (NMs) jumped from 1000 tons in 2004 to 5000 tons in 2010; the estimate for the next decade is of approximately 100,000 tons (Paschoalino et al., 2010). This growing production indicates the inevitable exposure of the environment to NMs, and it means environmental risk depending on these materials' nanospecific properties such as size, surface area, hydrodynamic diameter, agglomeration/dispersion capacity and sedimentation rate. Such properties can facilitate NMs translocation to organic systems and lead to different toxic effects (Rocha et al., 2015, Rocha et al., 2017).

Currently, zinc oxide NPs (ZnO NPs) are some of the most widely used nanoparticles (McCall, 2011). They compose commercial products such as toothpastes, cosmetics, sunscreens, textile material, wall paints and building materials (Xia et al., 2008; Zvyagin et al., 2008; Smijs & Pavel, 2011; Vanderiel & Jong, 2012). Previous studies have already shown that ZnO NPs lead to different toxic effects on mammal systems (mainly in mice and rats). Therefore, ZnO NPs promote toxic effects at different biological organization levels due to their cyto- and genotoxicity (Han et al., 2017; Pati et al., 2016), neurotoxicity (Tian et al., 2015), reproductive toxicity (Jo et al., 2013; Talebi et al., 2013), immunotoxicity (Kim et al., 2014). Moreover, they cause behavioral (Xie et al., 2012; Torabi et al., 2013; Xiaoli et al., 2017; Sheida et al., 2017) histopathological (Yan et al., 2015; Almansour et al., 2017), metabolic and biochemical changes (Amara et al., 2014; Wang et al., 2016, 2017).

However, most of these studies worked with ZnO NPs doses or concentrations from 1 mg kg^{-1} to 300 mg kg^{-1} (Vandebriel & Jong, 2012). These concentrations are higher than the ones often found in the environment (water surface: 1.5 ng L^{-1} to 360 ng L^{-1}) (Dumont et al., 2015), fact that implies lack of realistic results about the ecotoxicological impact from these NPs. According to Fabrega et al. (2012), ZnO NPs can enter the water surface from multiple non-point sources. The aquatic or land animals feed on the contaminated water and accumulate ZnO NPs in their bodies, fact that reinforces the environmental and health concerns.

Previous studies have shown that several microscopic, spectroscopic and separation techniques have been employed to detect NMs such as gold, silver, zinc oxide NPs and quantum dots (Segets et al., 2009; Howard, 2010; Weinberg et al., 2011; Silva et al., 2011; Majedi et al., 2012). However, these methodologies are insufficient to monitor the current environmental concentrations of engineered NMs (Farré et al., 2011). Thus, unfortunately, we are far from having methods to collect data about occurrence levels, fate and engineered NMs transportation in the environment. Before developing a solid analytical approach, it is worth fully understanding the NMs domain, but such understanding requires assessing the material-source matrices, these materials' transformation in the natural aquatic environment, and their specific physical/chemical behavior in water medium (Weinberg et al., 2011).

Accordingly, some studies have used spatially and temporally explicit methods to model/estimate ZnO NPs concentrations in the environment (Boxall et al., 2007; Gottschalk et al., 2009; Gottschalk et al., 2011; Sun et al., 2014; Dumont et al., 2015; Markus et al., 2016). According to Hassellov et al. (2008), it is obvious that the experimental validation of these model/estimate is highly desirable, although it is still impossible assessing and quantifying the nano-sized fraction of a certain material in the environment at trace concentrations. Nevertheless, the results shown in these studies provide ways to develop analytical methods able to improve estimates on predicted environmental concentrations. A method to be successful must provide important knowledge concerning on-going investigations about the possible effects from the environmentally relevant NMs concentrations on different organisms.

Despite their important contributions, the focus of many of the aforementioned studies on ZnO NPs toxicity has definitely not taken into account these materials' potential effects when they are available at environmentally relevant concentrations (Vanderiel & Jong, 2012; Saptarshi et al., 2015). Accordingly, the aim of the present study was to analyze the effects of the exposure to ZnO NPs at environmentally relevant (5.625×10^{-5} mg kg^{-1}) and high concentrations (300 ng L^{-1}) on the behavior of male Swiss mice, as well as to set the Zn concentration in their brain tissue. Mice exposed to ZnO NPs are expected to suffer the neurotoxic effects possibly resulting in neurobehavioral disorders, if one considers the evidence of ZnO NPs accumulation in the central nervous system (CNS) of experimental mammalian models (Feng et al., 2015). Thus, the present study emerges as an incremental step within a series of studies focused on the toxicity of ZnO NP.

2. Materials and methods

2.1. Characterizing the ZnO NPs

The ZnO NPs were purchased at Sigma Aldrich (Saint Louis, MO, USA; CAS number 544906). The morphology and individual diameter distributions were measured through transmission electron microscopy (TEM). The NPs were suspended in 100 mg L^{-1} distilled water, stirred and then sonicated for 1 min; 3-mL aliquots were pipetted and deposited on formvar-coated 200 mesh copper grids immediately after the last procedure, then the water excess was gently blotted on filter paper. The grids were directly inserted into a Jeol-JEM1220 TEM operating at 100 kV after they were dried. The images were taken at 50 k magnification in a dedicated CCD camera. Approximately 180 NPs were measured and the mean diameter (±SD) of each isolated particle was determined. The purity of the ZnO NPs was also analyzed through image analysis in the Scandium software of the Olympus Soft Imaging Solutions GmbH and ImageJ (National Institute of Health, USA).

The stock solution of ZnO NPs (18 mg L^{-1}) was prepared in distilled water and dispersed for 10 min in a sonicator to prevent aggregation. It was kept at 4 °C and used in the experiment after 5 days. The stock solution was sonicated for 20 min before each experiment and subsequently diluted in distilled water.

2.2. Animals and experimental design

Sixty-five (65) adult male Swiss mice (2.5–3 months old – nulliparous) were kept in the bioterium of the Biological Research Laboratory at *Instituto Federal Goiano – Campus Urutaí* (Urutaí, Goiás State, Brazil).