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Prenatal exposure to nanoparticulate titanium dioxide enhances depressive-like behaviors in adult rats

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HIGHLIGHTS

- Prenatal exposure to TiO_2 NPs decreased the level of CAT, GSH-PX and T-AOC.
- Prenatal exposure to TiO_2 NPs increased the level of MDA.
- Prenatal exposure to TiO_2 NPs induced oxidative damage to nucleic acids.
- Prenatal exposure to TiO_2 NPs enhanced the depressive-like behavior in adult rats.

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ABSTRACT

Titanium dioxide nanoparticles (TiO_2 NPs) have the potential to produce reactive oxygen species and can be transferred from the mother to the fetal brain. The central nervous system exhibits remarkable plasticity in early life and can be altered significantly by environmental stressors encountered during fetal period. Additionally, prenatal stressors are involved with emotional problems in adulthood. The purpose of the current study is to evaluate whether prenatal exposure to TiO_2 NPs could induce oxidative damage in the offspring brain and eventually affect the emotional behaviors in adulthood. The results showed that prenatal exposure to TiO_2 NPs impaired the antioxidant status, caused a significant oxidative damage to nucleic acids and lipids in the brain of newborn pups, and enhanced the depressive-like behaviors during adulthood in the force swimming test and the sucrose preference test. These results suggest that the stress during fetal life induced by prenatal exposure to TiO_2 NPs could be implicated in depressive-like behaviors in adulthood.

1. Introduction

Nanoparticles are particles with a maximum size of <100 nm in at least one dimension. Nanotechnology research and development is proceeding rapidly and the production of novel man-made nanoparticles is increasing worldwide. The small size of nanoparticles significantly increases the surface area and enhances the chemical reactivity compared to normal-sized particles of the same substance (Beydoun et al., 1999; Jang et al., 2001). On the other hand, there is increasing concern that substances previously considered biologically inert may become toxic in a nanoparticulate state due to their increased chemical reactivity and easier penetration into cells (Preining, 1998). Titanium dioxide nanoparticles (TiO_2 NPs) are a white pigment widely used in paints, plastics, ceramics, rubber, cosmetics and water purification. TiO_2 has the potential to produce reactive oxygen species (ROS) in its photocatalysis (Fujishima et al., 2008). It has been reported that the administration of high-dose TiO_2 NPs could induce an oxidative attack in the mouse liver (Ma et al., 2009) and damage the liver function (Wang et al., 2007).

The central nervous system exhibits remarkable plasticity in early life and can be altered significantly by environmental stressors encountered during fetal period. In addition, the development of the blood brain barrier in utero or at an early postnatal age is incomplete (Watson et al., 2006). It has been reported that TiO_2 NPs administrated subcutaneously to pregnant mice were transferred from the mother to the fetal brain, and induced apoptosis in the olfactory bulb mitral cell of the offspring brain (Takeda et al., 2009). Shimizu et al. showed that maternal exposure to TiO_2 NPs may affect the expression of genes related to the brain development and function in mice (Shimizu et al., 2009).

Additionally, an emerging shift toward studying the developmental basis of health and disease (or, the fetal basis of adult

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disease (FeBAD)) is based on the finding that susceptibility to certain diseases can be established by environmental stressors encountered early in life (Heindel, 2006; Barlow et al., 2007). Animal studies have shown that prenatal stressors are involved with increased levels of anxiety and depression-like behavior in adulthood (Maccari and Morley-Fletcher, 2007; Mairesse et al., 2012). Clinical studies have also shown that gestational stressors in humans are more likely to cause cognitive and emotional problems in the offspring (Van den Bergh et al., 2005; Guilarte, 2009).

In light of the above information, the aims of the present study were to evaluate whether prenatal exposure to TiO_2 NPs could induce oxidative damage in the offspring brain and eventually affect the emotional behaviors in adulthood.

2. Methods

2.1. Subjects

The experiments utilized Sprague–Dawley rats (male and female) purchased from the Animal Center of Soochow University. The animals were maintained in an animal colony room with ad libitum access to food and water. The air-handling system provided the temperature at 23 ± 1 °C and the relative humidity at 55 ± 5%. Overhead fluorescent lights were turned on at 12 h light/12 h dark cycle (light on at 8:00 a.m.). All animals were handled in accordance with the procedures approved by the Animal Experimental Committee, Soochow University, and with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Guidelines).

2.2. Timed pregnancies

Timed pregnancies were produced by placing sexually mature females with single males and then examining the females for sperm plugs the following morning (8:00–10:00). Rats with sperm plugs were weighed and the day of plug detection was referred to as gestational day (GD) 0.

2.3. Treatments

The details of the characterization of TiO_2 NPs were previously described by Ma et al. (2009). According to a previous study (Shimizu et al., 2009), a 500 μL volume of TiO_2 NPs suspended at 1 μg/μL was injected subcutaneously into pregnant rats ($n = 8$) on GD 6, 9, 12, 15, and 18 for the exposure group, while 500 μL of vehicle alone was injected into pregnant rats ($n = 8$) as a control group (Fig. 1) (Shimizu et al., 2009).

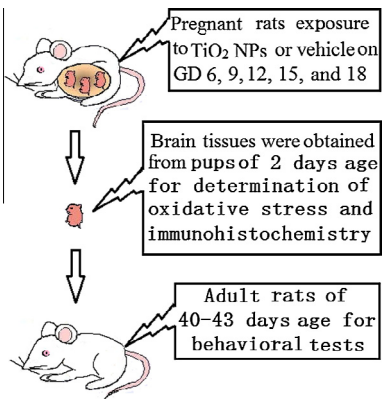

Fig. 1. An image of the experimental time-line indicating prenatal exposure and postnatal tests (Shimizu et al., 2009).

2.4. Rearing conditions

On postnatal day (PN) 2, the neonates were weighed and culled to 3 male pups per litter. The pups were reared by their biological mothers until weaned on PN 20. On PN 20, the animals were culled to one pup per litter, were ear punched for litter identification, and were grouped according to prenatal condition ($n = 8$/group). Separate cages contained rats from separate litters. On PN 40, the 16 rats ($n = 8$/group) were used in behavioral test.

2.5. Determination of oxidative stress

Brain tissues were obtained from pups ($n = 32$/group, from 8 different mothers) on PN 2 (Fig. 1). Rats were anesthetized with ether and the hippocampuses were removed. Rat hippocampuses were homogenized in 10:1 (vol/wt) ice-cold PBS. A quantity of the homogenate was used to determine the activities of catalase (CAT), glutathione peroxidase (GSH-PX), total antioxidant capability (T-AOC) and the concentration of malondialdehyde (MDA) in the hippocampus samples according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). These methods are described briefly below.

CAT is responsible for the detoxification of H_2O_2, a precursor for intracellular free radicals (Mittler, 2002). The activity of CAT in the samples was measured by the decrease in the H_2O_2 concentration. The H_2O_2 decomposition reaction catalyzed by catalase was stopped by adding ammonium molybdate. The remaining H_2O_2 combined with ammonium molybdate to form a yellow compound, which absorbed maximally at 405 nm. One unit of catalase activity was defined as 1 mmol of decomposed H_2O_2 in one milligram of tissue for one minute and expressed as units per mg protein.

The T-AOC is a useful index for the capacity of tissue samples to modulate the damage associated with enhanced production of free radicals (Lissi et al., 1992). A spectrometric method was applied to evaluate the T-AOC. In the reaction mixture, ferric ions were reduced by antioxidant reducing agents and a blue complex Fe^{2+}-TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) was produced. One unit of T-AOC was equal to 0.01 increase in absorbance of the reaction mixture at 520 nm per milligram protein per minute under 37 °C incubation. The T-AOC activities were expressed as units per mg protein.

GSH-PX is responsible for breaking down peroxides (Mittler, 2002). The activity of GSH-PX was quantified by measuring the rate of glutathione oxidation by hydrogen peroxide ($2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$) as catalyzed by GSH-PX present in the sample. The assay was initiated with the addition of glutathione reductase and NADPH. Glutathione reductase converts oxidized glutathione (GSSG) to the reduced form, while oxidizing NADPH to NADP. The rate of GSSG formation was subsequently measured by following the decrease in absorbance of the reaction mixture at 340 nm as NADPH was converted to NADP. A GSH-PX unit was defined as the enzyme activity required to convert 1 nmol of NADPH to NADP per mg tissue protein. The GSH-PX activity was expressed as units per mg protein.

MDA is one of the most frequently used indicators of lipid peroxidation (Ljubuncic et al., 2000). The thiobarbituric acid reaction (TBAR) method was used to measure MDA. The method was used to obtain a spectrophotometric measurement of the color produced during the reaction of TBA with MDA at 535 nm. MDA content was expressed as nmol/mg protein.

Protein concentrations were determined according to the Lowry method (Lowry et al., 1951).

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