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Maternal exposure to titanium dioxide nanoparticles during pregnancy; impaired memory and decreased hippocampal cell proliferation in rat offspring

Abbas Mohammadipour[a], *Alireza Fazel*[a], *Hossein Haghir*[a,b], *Fatemeh Motejaded*[a], *Houshang Rafatpanah*[c], *Hoda Zabihi*[d], *Mahmoud Hosseini*[e], *Alireza Ebrahimzadeh Bideskan*[a,*]

[a] *Department of Anatomy and Cell Biology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
[b] *Medical Genetic Research Center (MGRC), School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
[c] *Immunology Research Center, Buali Institute, Mashhad University of Medical Sciences, Mashhad, Iran*
[d] *Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
[e] *Neurocognitive Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*

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ABSTRACT

Titanium dioxide nanoparticles (TiO_2-NPs) are massively produced in the environment, and because of their wide usage, they are a potential risk of damage to human health. TiO_2-NPs are often used as additives for paints, papers, and foods. The central nervous system (CNS), including hippocampal regions, is potentially susceptible targets for TiO_2-NPs. This study aimed to determine the effects of exposure to TiO_2-NPs during pregnancy on hippocampal cell proliferation and the learning and memory of offspring. Pregnant Wistar rats received intragastric TiO_2-NPs (100 mg/kg body weight) daily from gestational day (GD) 2 to (GD) 21. Animals in the control group received the same volume of distilled water via gavage. After delivery, the one-day-old neonates were deeply anesthetized and weighed. They were then killed and the brains of each group were collected. Sections of the brains from the rat offspring were stained using Ki-67 immunolabeling and the immunohistochemistry technique. Some of the male offspring ($n = 12$ for each group) were weaned at postnatal day (PND21), and housed until adulthood (PND60). Then the learning and memory in animals of each group were evaluated using passive avoidance and Morris water maze tests. The immunolabeling of Ki-67 protein as a proliferating cell marker showed that TiO_2-NPs significantly reduced cell proliferation in the hippocampus of the offspring ($P < 0.05$). Moreover, both the Morris water maze test and the passive avoidance test showed that exposure to TiO_2-NPs significantly impaired learning and memory in offspring ($P < 0.05$). These results may provide basic experimental evidence for a better understanding of the neurotoxic effects of TiO_2-NPs on neonatal and adult brains.

* *Corresponding author at*: Department of Anatomy and Cell Biology, School of Medicine, Mashhad University of Medical Sciences, Azadi Sq., Vakilabad Blvd., P.O. Box 91779-48564 Mashhad, Iran. Tel.: +98 511 8002486; fax: +98 511 8002487.
E-mail address: EbrahimzadehBA@mums.ac.ir (A.E. Bideskan).
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1. Introduction

Titanium dioxide nanoparticles (TiO_2-NPs) are often used as additives in papers, paints, ceramics, plastics, and foods (Lomer et al., 2004; Oberdorster et al., 2005). The properties of absorption and reflection with ultra violet (UV) light also prompt the wide use of TiO_2-NPs in a variety of cosmetics. Moreover, they are used in the environmental decontamination of air, soil, and water (Wang et al., 2011). As ultrafine-sized materials, TiO_2-NPs can enter the human body through various routes such as inhalation, ingestion, and skin (Oberdorster et al., 2005; Jin et al., 2008). In recent years, studies have shown that after entering the body, TiO_2-NPs accumulate in the liver, kidneys, spleen, lungs, heart, and brain (Wang et al., 2007; Liu et al., 2009; Ma et al., 2010). Many studies have also shown that exposure to TiO_2-NPs may damage the central nervous system (CNS) (Wang et al., 2007; Hu et al., 2011). Still other studies have found that TiO_2-NPs promote the production of reactive oxygen species (ROS) (Long et al., 2006, 2007).

Wang et al. found that TiO_2-NPs that migrated into the hippocampus led to oxidative stress and inflammation (Wang et al., 2008). The hippocampus is highly vulnerable to oxidative stress due to its high metabolic rate (Cui et al., 2004).

The hippocampus is one of the important regions of the brain that has been implicated in learning and memory (Kim et al., 2009). Recent studies also confirmed that TiO_2-NPs can be transferred from mother to offspring via breastmilk and by passing through the placenta (Gao et al., 2011; Shimizu et al., 2009). Shimizu et al. also reported that maternal exposure to nano-particulate titanium dioxide during the prenatal period affects the expression of genes related to brain development (Shimizu et al., 2009). Moreover, Gao et al. reported that 100 mg/kg TiO_2 exposure during development decreased hippocampal synaptogenesis in offspring (Gao et al., 2011).

Thus, it seems that exposure to TiO_2-NPs during pregnancy may possibly affect the offspring hippocampus. For the study of hippocampal proliferation, the Ki-67 protein (as a proliferating cell marker) immunolabeling method is well-established (Seolhwa et al., 2011; Kim et al., 2009; Scholzen and Gerdes, 2000). This protein is expressed during mitosis. The formation of new neurons in the hippocampus occurs mainly during the gestational period (Scholzen and Gerdes, 2000).

In the present study, the effect of maternal exposure to TiO_2-NPs during pregnancy on offspring hippocampal proliferation was investigated by Ki-67 immunolabeling. The Morris water maze and passive avoidance tests were used to study memory changes in pups.

2. Materials and methods

2.1. Chemicals and preparation

The TiO_2 nanoparticles used in this study were a kind of nanopowder, anatase, with a particle size of 10 nm, surface area >150 m^2/g, purity +99%, and density 3.9 g/m^3, and were purchased from Nano Lima, Co. (Iran).

TiO_2-NPs were suspended in distilled water. Quantitative suspensions (100 mg/kg) were prepared fresh every day and fed to the rats with gavage.

2.2. Nanoparticle characterization by transmission electron microscopy

The sizes of TiO_2-NPs were determined using transmission electron microscopy (TEM), which showed that particles diameters were in the nano-size range. An image of the sample is shown in Fig. 1.

2.3. Animals and treatment

Male and female Wistar rats aged 3–4 months and weighing 250–300 g were purchased from the Animal Center of Mashhad University of Medical Sciences. The rats were housed under controlled conditions of temperature (20 ± 2 °C) and lighting (12 h light:12 h dark photoperiod) and were permitted free access to food and water. The female rats were caged with male rats at a ratio of 3:1. The onset of pregnancy was confirmed by the presence of sperm in vaginal smears, and the pregnant dams were immediately housed in individual cages with free access to water and food until delivery. Experimental procedures were carried out in accordance with Mashhad University of Medical Sciences, Ethical Committee Acts.

The pregnant rats were randomly divided into two groups of control and TiO_2.

Animals in the TiO_2 group ($n = 6$) received 100 mg/kg TiO_2 orally (gavage) from prenatal day 2 to day 21. The animals in control group ($n = 6$) were administered distilled water (Gao et al., 2011). After delivery, two male pups from each litter were randomly sampled, weighed, and assigned to an experimental group. One pup was used for the determination of hippocampal titanium content, and another was used for an histological examination. Some of the male offspring ($n = 12$ for each group) were weaned at postnatal day (PND) 21, and housed until adulthood (PND 60). Then the learning and memory in animals of each group were evaluated using passive avoidance and Morris water maze tests.

2.4. Hippocampus titanium contents determination

The whole hippocampus of each day-1 neonate was weighed, digested, and analyzed for titanium content. Briefly, prior to elemental analysis, the tissues of interest were digested in nitric acid overnight. After adding 0.5 ml H_2O_2, the mixed solutions were heated at about 160 °C using a high pressure reaction container in an oven chamber until the samples were completely digested. Then, the solutions were heated to 120 °C to remove the remaining nitric acid until the solutions were colorless and clear. Next, 3 ml of 2% nitric acid was added to the remaining solutions. Inductively coupled plasma-mass spectrometry (ICP-MS, HP4500, USA and Japan) was used to analyze the titanium concentration in the samples. Detected data were expressed as nanograms per gram of hippocampal tissue (Gao et al., 2011; Zhang et al., 2010).