Journal of Hazardous Materials 243 (2012) 19–27

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Ovarian dysfunction and gene-expressed characteristics of female mice caused by long-term exposure to titanium dioxide nanoparticles

Guodong Gao[a,b,1], Yuguan Ze[a,1], Bing Li[a,1], Xiaoyang Zhao[a,1], Ting Zhang[c,d,1], Lei Sheng[a], Ringhu Hu[a], Suxin Gui[a], Xuezi Sang[a], Qingqing Sun[a], Jie Cheng[a], Zhe Cheng[a], Ling Wang[a], Meng Tang[c,d,*], Fashui Hong[a,**]

[a] Medical College, Soochow University, Suzhou 215123, People's Republic of China
[b] Shangrao Branch of Jiangxi Medical College, People's Republic of China
[c] Key Laboratory of Environmental Medicine and Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing 210009, People's Republic of China
[d] Jiangsu key Laboratory for Biomaterials and Devices, Southeast University, Nanjing 210009, People's Republic of China

HIGHLIGHTS

- Exposure to TiO_2 NPs could be significantly accumulated in ovary.
- Exposure to TiO_2 NPs caused ovarian injury in mice.
- Exposure to TiO_2 NP decreased fertility or the pregnancy rate.
- Exposure to TiO_2 NP resulted in imbalance of sex hormones in mice.
- Exposure to TiO_2 NP caused alteration of 228 genes expression of known function in ovary.

ARTICLE INFO

Article history:
Received 11 February 2012
Received in revised form 8 July 2012
Accepted 21 August 2012
Available online 11 September 2012

Keywords:
Titanium dioxide nanoparticles
Ovary
Fertility
Sex hormones
Gene-expressed profile

ABSTRACT

Although numerous studies have described the accumulation of titanium dioxide nanoparticles (TiO_2 NPs) in the liver, kidneys, lung, spleen, and brain, and the corresponding damage, it is unclear whether or not TiO_2 NPs can be translocated to the ovary and cause ovarian injury, thus impairing fertility. In the current study, ovarian injury and gene-expressed characteristics in female mice induced by intragastric administration of TiO_2 NPs (10 mg/kg) for 90 consecutive days were investigated. Our findings indicated that TiO_2 NPs can accumulate in the ovary and result in ovarian damage, cause an imbalance of mineral element distribution and sex hormones, decrease fertility or the pregnancy rate and oxidative stress in mice. Microarray analysis showed that in ovaries from mice treated with TiO_2 NPs compared to controls, 223 genes of known function were up-regulated, while 65 ovarian genes were down-regulated. The increased expression of *Cyp17a1* following TiO_2 NPs treatment suggested that the increase in estradiol biosynthesis may be a consequence of increased TiO_2 NPs. In addition, the elevated expression of *Akr1c18* implied that progesterone metabolism was accelerated, thus causing a decrease in the progesterone concentration. Taken together, the apparent regulation of key ovarian genes supports the hypothesis that TiO_2 NPs directly affects ovarian function.

Crown Copyright © 2012 Published by Elsevier B.V. All rights reserved.

1. Introduction

Nanotechnology provides driving force to progress in life sciences and information technology. However, several studies have called attention to the potential hazards arising from nanotechnology [1–4]. For the past few years, titanium dioxide nanoparticles (TiO_2 NPs) have increasingly been used in cosmetics, food additives, drug delivery vehicles, bio-medical ceramics and implanted biomaterial paints, waste water treatment, and sterilization, largely due to their appropriate physicochemical properties. The toxicity of TiO_2 NPs is associated with their physicochemical properties (e.g., size, surface area, and crystal phase) [5]. Some studies have reported that TiO_2 NPs enter the systemic circulation, migrate to various organs and tissues, and exert adverse effects [6–9]. Our previous studies have also shown that TiO_2 NPs exposure results in accumulation and damage in the liver, spleen, and kidneys of female mice [10–19].

* Corresponding author at: Jiangsu key Laboratory for Biomaterials and Devices, Southeast University, Nanjing 210009, People's Republic of China.
** Corresponding author. Tel.: +86 0512 61117563; fax: +86 0512 65880103.
E-mail address: Hongfsh_cn@sina.com (F. Hong).
[1] Guodong Gao, Yuguan Ze, Bing Li, Xiaoyang Zhao, and Ting Zhang contributed equally to this work.

0304-3894/$ – see front matter. Crown Copyright © 2012 Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jhazmat.2012.08.049

We also demonstrated that TiO_2 NPs were translocated to the brain and caused injury in female mice [20–22], which may have effects on hormone secretion in the pituitary gland and female reproduction; however, whether or not TiO_2 NPs can be translocated to the ovaries of mammals and affect reproduction is unknown. Recently, the toxicologic characteristics of some nanomaterials in ovarian cells *in vitro* have been reported. For instance, gold nanoparticles were shown to enter rat ovarian granulosa cells and subcellular organelles and alter estrogen accumulation *in vitro* [23], and were able to traverse the cell membrane and enter Chinese hamster ovary cells by the endocytic pathway [24]. In addition, calcium phosphate nanoparticles entered granulosa cells, and were distributed in the membranous compartments, including lysosome, mitochondria and intracellular vesicles, affected hormone production, and caused apoptosis in human granulosa cells [25]. Silicon carbide nanowires induced activation of mitogen-activated protein kinase cellular signal transduction pathways in mammalian cells [26], and confocal laser scanning microscopy observations showed that the TiO_2 NPs easily moved to the cytoplasm of cultured Chinese hamster ovary cells, and not to the nucleus [27]. TiO_2 or Al_2O_3 NPs agglomerated on both the surface and inside of Chinese hamster ovary cells and induced genotoxicity and cytotoxicity in these cells [28]. The widespread occurrence of TiO_2 NPs, albeit in minimal amounts, in the environment and in cosmetics makes the general population vulnerable to exposure. Exposure to TiO_2 NPs can occur via inhalation, transdermal absorption, and ingestion, therefore, we speculate that the ovary might also be one of the target organs of toxicity in mammals exposed to TiO_2 NPs.

The aim of the present study was to evaluate ovarian dysfunction and its gene-expressed profile following TiO_2 NPs exposure as a possible mechanism of ovarian injury. The toxicogenomic approach was used to determine the effects of TiO_2 NPs exposure on ovarian gene expression by microarray analysis. The examination of ovarian gene expression provides a full understanding of the effects of TiO_2 NPs on the mouse ovary.

2. Materials and methods

2.1. Preparation and characterization of TiO_2 NPs

Nanoparticulated anatase TiO_2 was prepared via controlled hydrolysis of titanium tetrabutoxide. The details of the synthesis and characterization of TiO_2 NP have been previously described by our group [20,29]. TiO_2 powder was dispersed on the surface of 0.5% (w/v) hydroxypropylmethylcellulose (HPMC) K4M solution, and the solutions containing TiO_2 particles were treated ultrasonically for 15–20 min and then mechanically vibrated for 2 or 3 min. X-ray-diffraction (XRD) patterns of TiO_2 NPs were obtained at room temperature with a MERCURY CCD diffractometer (MERCURY CCD Co., Japan) using Ni-filtered Cu Kα radiation. The particle sizes of both the powder and the nanoparticles suspended in 0.5% (w/v) HPMC solution after incubation for 12 h and 24 h (5 mg/ml) were determined using a TecnaiG220 transmission electron microscope (TEM) (FEI Co., USA) operating at 100 kV, respectively. Mean particle size was determined by measuring more than 100 individual particles which were randomly sampled. XRD measurements showed that TiO_2 NPs exhibit the anatase structure, and the average grain size calculated from the broadening of the (1 0 1) XRD peak of anatase was approximately 6 nm using Scherrer's equation. TEM demonstrated that the average particle size of the powder suspended in HPMC solvent after 12 h and 24 h incubation ranged from 5 to 6 nm. The surface area of the sample was 174.8 m^2/g. The mean hydrodynamic diameter of TiO_2 NP in HPMC solvent ranged from 208 to 330 nm (mainly 294 nm), and the zeta potential after 12 and 24 h incubation was 7.57 and 9.28 mV, respectively [20].

2.2. Animals and treatment

CD-1 (ICR) female mice were used in this study. One hundred fifty CD-1 (ICR) female mice (23 ± 2 g) were purchased from the Animal Center of Soochow University (China). All mice were housed in stainless steel cages in a ventilated animal room. The room temperature of the housing facility was maintained at 24 ± 2 °C with a relative humidity of 60 ± 10% and a 12-h light/dark cycle. Distilled water and sterilized food were available *ad libitum*. Prior to dosing, the mice were acclimated to the environment for 5 days. All procedures used in animal experiments conformed to the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals [30] Studies were approved by the Soochow University Institutional Animal Care and Use Committee.

In our preliminary experiments, TiO_2 NP suspensions at different concentrations (2.5, 5, and 10 mg/kg of body weight [BW]) were administered to mice by intragastric administration for 90 consecutive days. Treatment with 10 mg/kg BW TiO_2 NPs resulted in the most severe organ damage [22,31], which was used as the highest concentration for further experiments. The mice were randomly divided into 2 groups ($N = 30$), including the control group (treated with 0.5% (w/v) HPMC) and experimental group (10 mg/kg BW TiO_2 NPs). The mice were weighed, and the TiO_2 NP suspensions were administered to the mice by intragastric administration every day for 90 days. All symptoms and deaths were observed and carefully recorded every day during the 90 days. In addition, after 90 days, the fertility of treated females (10) was tested by caging with males (10) of proven fertility.

After the 90-day period, all mice were weighed and then sacrificed after being anesthetized with ether. Blood samples were collected from the eye vein by rapidly removing the eyeball. Serum was collected by centrifuging blood at 2500 rpm for 10 min. The ovaries were quickly removed and placed on ice and then dissected and frozen at −80 °C.

2.3. Elemental content analysis

The ovaries were removed from the freezer (80 °C) and thawed. Approximately 0.1 g of the ovary was weighed, digested, and analyzed for elemental content. Inductively coupled plasma-mass spectrometry ([ICP-MS] Thermo Elemental X7; Thermo Electron Co., USA) was used to analyze the titanium, sodium, magnesium, potassium, calcium, zinc, and iron concentrations in the samples.

2.4. Histopathologic evaluation of the ovaries

For pathologic studies, all histopathologic examinations were performed using standard laboratory procedures. The ovaries were embedded in paraffin blocks, then sliced (5 μm thickness) and placed onto glass slides. After hematoxylin–eosin (HE) staining, the stained sections were evaluated by a histopathologist unaware of the treatments, using an optical microscope (Nikon U-III Multipoint Sensor System, Japan).

2.5. Observation of ovarian ultrastructure by TEM

Ovaries were fixed in a fresh solution of 0.1 M sodium cacodylate buffer containing 2.5% glutaraldehyde and 2% formaldehyde followed by a 2 h fixation period at 4 °C with 1% osmium tetroxide in 50 mM sodium cacodylate (pH 7.2–7.4). Staining was performed overnight with 0.5% aqueous uranyl acetate. The specimens were dehydrated in a graded series of ethanol (75%, 85%, 95%, and 100%), and embedded in Epon 812. Ultrathin sections were obtained, contrasted with uranyl acetate and lead citrate, and observed with a Hitachi H600 TEM (Hitachi Co., Japan). Ovarian apoptosis was

Download English Version:

https://daneshyari.com/en/article/577580

Download Persian Version:

https://daneshyari.com/article/577580

Daneshyari.com