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Effects of oral administration of titanium dioxide fine-sized particles on plasma glucose in mice

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ABSTRACT

Titanium dioxide (TiO_2) is an authorized additive used as a food colorant, is composed of nano-sized particles (NP) and fine-sized particles (FP). Previous study reported that oral administration of TiO_2 NPs triggers an increase in plasma glucose of mice. However, no previous studies have focused on toxic effects of TiO_2 FPs on plasma glucose homeostasis following oral administration. In the current study, mice were orally administered TiO_2 FPs greater than 100 nm in size (64 mg/kg body weight per day), and effects on plasma glucose levels examined. Our results showed that titanium levels was not changed in mouse blood, livers and pancreases after mice were orally administered TiO_2 FPs. Biochemical analyzes showed that plasma glucose and ROS levels were not affected by TiO_2 FPs. Histopathological results showed that TiO_2 FPs did not induce pathology changes in organs, especially plasma glucose homeostasis regulation organs, such as pancreas and liver. Western blotting showed that oral administration of TiO_2 FPs did not induce insulin resistance (IR) in mouse liver. These results showed that, TiO_2 FPs cannot be absorbed via oral administration and affect plasma glucose levels in mice.

1. Introduction

Titanium dioxide (TiO_2) is the second most commonly used material in consumer products (Brun et al., 2014; Sha et al., 2013). Conventional TiO_2 is an authorized additive used as a food colorant (Dorier et al., 2015; Tassinari et al., 2014). Daily human intake of TiO_2 from food is in the range of 15–37.5 mg per day for a 75 kg adult. Electron microscopy and stability testing of food-grade TiO_2 suggests that 36% comprises nano-sized particles (NPs) less than 100 nm in at least one dimension, and the compound readily disperses in water as fairly stable colloids (Weir et al., 2012). Orally administered TiO_2 NPs are reported to exert toxic effects in humans and animals (Shi et al., 2013; Smolkova et al., 2015). Repeated oral administration of TiO_2 NPs to CD-1 (ICR) mice causes inflammation and impairs the functions of the liver, kidneys and reproductive system (Shi et al., 2013; Zhao et al., 2013). Recently, our group showed that repeated oral administration of TiO_2 NPs leads to deterioration of plasma glucose homeostasis and increased plasma glucose levels in mice (Hu et al., 2015).

A large proportion (64%) of TiO_2 particles in food additives are fine-sized particles (FPs), larger than 100 nm (Weir et al., 2012). Biological response following deposition has been shown to be dependent on particle size (He et al., 2015; Li et al., 2008). Materials that were high in toxicity in NPs could be low in toxicity in FPs (Brun et al., 2014; Singh et al., 2007). Upon treatment of A549 human lung epithelial cells with different sizes of TiO_2 particles in the inhalation experiment, TiO_2 NPs induced significant reactive oxygen species (ROS) and IL-8 release, but not TiO_2 FPs, indicating that FPs induce a less toxic biological response (Singh et al., 2007). However, TiO_2 FPs are not absolute security. Inhalation exposure study with asthmatic mice showed that exposure to TiO_2 FPs induced 1.7-fold increasing of macrophages and exacerbation of asthmatic symptoms (Rossi et al., 2010). Pulmonary instillation study with rats showed that tracheobronchial cell proliferation rates significantly increased after rats exposed to 1 mg/kg and 5 mg/kg body weight dose TiO_2 FPs for 1 week, and this increased

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alveolar epithelial thickness (Warheit et al., 2006). Besides, the in-vitro genotoxicity of TiO_2 FPs in human bronchial epithelial BEAS 2B cells showed that TiO_2 FPs reduced cell viability at lower dose than TiO_2 NPs, for instance, 1 μg/cm^2 TiO_2 FPs induced DNA damage that 10 μg/cm^2 TiO_2 NPs induced (Falck et al., 2009). Thus, TiO_2 FPs can also cause toxicology effects in pulmonary toxicological experiments, but no detailed studies have focused on toxic effects of TiO_2 FPs on plasma glucose homeostasis following oral administration.

In the current study, we orally administered TiO_2 FPs to mice, with the aim of clarifying the toxicity effects of the fine-sized particle population on plasma glucose homeostasis. We also investigated the mechanism of effects of TiO_2 FPs on plasma glucose homeostasis.

2. Materials and methods

2.1. Ethics statement of the study

This study was done in strict accordance with recommendations of the Guide for the Care and Use of Experimental Animals approved by the Heilongjiang Province People's Congress (http://www.nicpbp.org.cn/sydw/CL0249/2730.html). The Ethics Research Committee of the School of Life Science and Technology of Harbin Institute of Technology approved the study protocol. All efforts were made to minimize suffering.

2.2. Nanoparticles and physicochemical characterization

Powder-form TiO_2 FPs and NPs (FP: anatase, Sigma Co., Ltd., Germany; NP: anatase, Veking Co., Ltd, Hangzhou, China) were obtained from commercial sources and used without any coating throughout this study. Primary particle sizes and morphology were measured using scanning electron microscopy (SEM, Quanta, FEI Co., Ltd., Hillsboro, USA). The hydrodynamic size and zeta potential of particles in phosphate buffer saline (PBS) were measured using a dynamic light scattering (DLS, Brookhaven Instruments Corporation, Brookhaven, USA).

2.3. Animals and treatments

Six-week-old CD-1 mice of 30 males (average body weight: 28.03 ± 1.99 g, average plasma glucose: 5.22 ± 0.27 mmol/l) were obtained from Harbin Veterinary Research Institute (Harbin, China) and acclimated for 7 days after arrival at the study facility. Mice were housed in an animal room at a controlled temperature (21–24 °C) and light cycle (12 h light/dark). Autoclaved water and rodent diets (Keao Co., Ltd., Beijing, China) were provided *ad libitum*. Mice were randomly divided into three groups: blank, FP and NP. After vigorous stirring, TiO_2 FPs or NPs suspension (64 mg/kg body weight per day) was given to mice by a syringe via the daily oral administration. Blank group mice were given equal volume PBS.

2.4. Blood collection and analysis

Blood was collected from the tail vein of mice every 2 weeks. Before the collection, mice were fasted for 16 h. After determined that plasma glucose increased in tail vein blood, mice plasma glucose levels were followed up for 16 weeks to test whether the increase of plasma glucose was stable. At the end of week 28, mice were fasted for 16 h, and then blood was collected from mouse hearts. Plasma glucose was measured using glucose assay kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma insulin was measured using mouse insulin ELISA kit (Shibayagi Co., Ltd., Gunma, Japan). Tumor necrosis factor (TNF)-α and interleukin (IL)-6 were measured using TNF-α kit and IL-6 kit (R&D Systems, MN, USA).

2.5. Oral glucose tolerance test (OGTT)

At week 28, mice were fasted for 16 h and then orally administered glucose (1.5 g/kg body weight). Blood was collected for plasma glucose and insulin level measurement from the tail vein into capillary tubes each 10 μl at baseline and 0, 30, 60, 120 min after administration of glucose. Plasma glucose was measured using glucose assay kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma insulin was measured using mouse insulin ELISA kit (Shibayagi Co., Ltd., Gunma, Japan).

2.6. Titanium content analysis

Titanium content analysis was performed as previously described (Hu et al., 2015). Briefly, 0.1 g of each tissues were digested in nitric acid (ultrapure grade). The remaining solutions were measured titanium concentration using inductively coupled plasma-optical emission spectrometry (ICP-OES, Optima 5300 DV, Perkin Elmer Inc., CA, USA). Then 0.1 g of each tissues were homogenized in RIPA lysis buffer. The precipitation was observed by SEM. Surface element was analyzed by energy dispersive X-ray analysis (EDXA).

2.7. Histopathological analysis

Mice body weights were weighted every two weeks after mice were oral administered with TiO2 FPs and NPs. After 28 weeks, mice were after mice were euthanized, their tissues were collected and weighted. Organ coefficient was the ratio of weight of each tissues (mg) to mouse body weight (g). Then tissues were immersion-fixed with 4% paraformaldehyde in 0.4 M phosphate buffer, pH 7.6. Tissues were dehydrated and embedded in paraffin, and then cut into 5 μm sections. The sections were stained with hematoxilin and eosin (H&E) and subsequently processed for histopathological examination under a light microscope.

2.8. ROS levels assessment

ROS levels were assessed by using the levels of total superoxide dismutase (T-SOD), glutathione (GSH) and methane dicarboxylic aldehyde (MDA). Heart blood was centrifuged at 750 *g* for 10 min at 4 °C to separate sera and cell debris. Liver was homogenized in 9 volumes (1:10 w/v) of PBS. Homogenates were centrifuged at 750 *g* for 10 min at 4 °C to discard cell debris. The pellet was discarded and the supernatant was separated and used to measure ROS levels. The T-SOD, GSH and MDA of sera and liver supernatant were measured using each kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.9. Western blot

Western blot was performed as previously described (Hu et al., 2015). Briefly, 0.1 g of liver tissues were resuspended in RIPA lysis buffer. Lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred to PVDF membranes purchased from Bio-Rad Laboratories (CA, USA). PVDF membranes were incubated with antibodies against phospho-c-Jun amino-terminal kinase 1 (JNK1), phospho-p38 mitogen-activated protein kinase (MAPK), phospho-IRS1 (Ser307), phospho-Akt (Ser473), JNK1, p38 MAPK, IRS1 and Akt (Cell Signaling Technology, MA, USA). All primary antibodies were used 1:1000 final