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Oral administration of nano-titanium dioxide particle disrupts hepatic metabolic functions in a mouse model

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ABSTRACT

TiO_2 nano-particle (TiO_2 NP) is widely used in industrial, household necessities, as well as medicinal products. However, the effect of TiO_2 NP on liver metabolic function has not been reported. In this study, after mice were orally administered TiO_2 NP (21 nm) for 14 days, the serum and liver tissues were assayed by biochemical analysis, real time quantitative polymerase chain reaction, western blot and transmission electron microscopy. The serum bilirubin was increased in a dose dependent manner. Deposition of TiO_2 NP in hepatocytes and the abnormality of microstructures was observed. Expression of metabolic genes involved in the endogenous and exogenous metabolism was modified, supporting the toxic phenotype. Collectively, oral administration of TiO_2 NP (21 nm) led to deposition of particles in hepatocytes, mitochondrial edema, and the disturbance of liver metabolism function. These data suggested oral administration disrupts liver metabolic functions, which was more sensitive than regular approaches to detect material hepatotoxicity. This study provided useful information for risk analysis and regulation of TiO_2 NPs by administration agencies.

1. Introduction

TiO_2 is used in many household necessities including paint, coating, plastic, paper, ink, medicine, food product, cosmetic and toothpaste (Kaida et al., 2004; Wang et al., 2007a,b; Wolf et al., 2003). It is also a component of articulating prosthetic implants for the hip and knee (Sul, 2010). In recent years, nano-titanium dioxide has been widely used in industrial and consumer products based on their strong catalytic activity which is attributed to the small size. Concerns have been raised that Nano-TiO_2 may present risks to human health (Fabian et al., 2008; Tsuji et al., 2006).

TiO_2 nano-particle (TiO_2 NP) is produced and used in a variety of particle sizes, from fine-size (0.1–2.5 μm) to nano-size (<0.1 μm) (Dankovic et al., 2007). Many studies reported that TiO_2 NPs are more toxic than fine-particles (Fabian et al., 2008; Oberdorster 2001; Zhao et al., 2009). TiO_2 NP of diameter 21 nm caused a greater pulmonary inflammatory response than TiO_2, where more TiO_2 NP entered the alveolar interstitium in the lungs (Oberdorster et al., 1994). Sager reported similar results after intra-tracheal instillation of well-dispersed of TiO_2 NP (80/20 anatase/rutile, 21 nm) and TiO_2 fine-particle (100% rutile, 1 μm) in rats. An equivalent dose of TiO_2 NPs was supposed to be 40 times more potent than TiO_2 fine-particles (Sager et al., 2008).

In medication, nano-particles have been developed to carry drugs and vaccines to improve their bioavailablity (Hillyer and Albrecht 2001). Intravenous or subcutaneous injection was considered as a unique approach to deliver TiO_2 NP into the human body (Zhao and Castranova, 2011). However, TiO_2 NPs are also widely used for toothpaste, food colorants and nutritional supplements. According to a recent study, candies, sweets and chewing gums contained a big amount of TiO_2 in less than 100 nm (Weir et al., 2012). As a food additive, TiO_2 was approved with the stipulation that the additive should not exceed 1% by body weight. So

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CYP, cytochrome P450; DBIL, direct bilirubin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IBIL, indirect bilirubin; IL-6, interleukin 6; LDH, lactate dehydrogenase; Mrp, multidrug resistance-related protein; OATP, bile salt export pump; OSTB, organic solute-transporter-β; Q-PCR, Quantitative polymerase chain reaction; TBA, total bile acid; TiO_2 NP, TiO_2 nano-particle; TNFα, tumour necrosis factor alpha-like; UGT, UDP glucuronosyltransferase.

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the gastro-intestinal tract is an important absorption route for TiO_2 NPs, besides the known inhalation and dermal exposure (Shi et al., 2013). Considering exposure related to the oral ingestion and biological activity of TiO_2 NPs, toxicity information is needed to set exposure limits for TiO_2 NPs.

In a sub-acute toxicity investigation in Wistar rats orally treated with TiO_2 NPs (160, 400, and 1000 mg/kg), the disturbance of amino acid metabolism and gut microflora environment was revealed to be results of liver and heart injury (Bu et al., 2010). In a 60-day toxicity study, ICR mice ingesting TiO_2 NPs (5 nm anatase; 5, 10, and 50 mg/kg/day) altered of Ca, Mg, Na, K, Fe, and Zn contents in the brain. Additionally, the activity of Na^+/K^+-ATPase, Ca^{2+}-ATPase, Ca^{2+}/Mg^{2+}-ATPase, acetylcholine esterase, and nitric oxide synthase was modified (Hu et al., 2010). In ICR mice exposed to TiO_2 NP (5–6 nm, intragastric administration; 2.5, 5, and 10 mg/kg/day) for 90 days, inflammation, apoptosis and spleen injury were observed (Sang et al., 2012). However, the effect of TiO_2 NPs on metabolic function of liver tissues has not been reported.

In the present study, mice were orally administered two doses of TiO_2 NP for 14 days, the metabolic function and microstructures of liver tissues were analyzed, as well as biochemical reactions. The oral challenge of TiO_2 NP led to the deposition of particles in liver tissues and alteration of microstructure. Metabolic genes involved in the endogenous and exogenous metabolism were modified, supporting the disruption of bilirubin homeostasis. These data suggest a risk of metabolism disruption by TiO_2 NP exposure, as well as an useful strategy for risk analysis of nano-materials.

2. Materials and methods

2.1. Chemicals and reagents

TiO_2 nano-particle (21 nm, TiO_2 NP) was purchased from Sigma–Aldrich (Product number 718467, Lot MKBR0084V, MO, USA). According to the specification sheet, the surface area of the TiO_2 NP product was 35–65 m^2/g and the trace metals basis was above 99.5%, with primary particle size of 21 nm.

Alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bile acid (TBA), direct bilirubin (DBIL) and indirect bilirubin (IBIL) assay kits were purchased from Yonghe Sunshine Technology (Changsha, China). Antibodies against Cyp3a, Cyp2b, Cyp2c were provided by Cell Signaling (Danvers, USA). Antibody against GAPDH was a product by Abcam (MA, USA). Ultrapure water was freshly prepared using a Milli-Q50 SP Water System (MA, USA). All the other reagents were of the highest grade from commercial sources.

2.2. Animals and treatment

Fifteen male 5–7 week-old C57/BL6 mice (22–25 g) were maintained in the Experimental Animal Center of Ningbo University under a standard 12 h light/12 h dark cycle with free access to food and water. All procedures were in accordance with the National Institutes of Health guide for the care and use of Laboratory animals. The animal study protocols were approved by the Medical School of Ningbo University Animal Care and Use Committee.

TiO_2 NP powder was dispersed in 0.5% CMC-Na (w/v) solution, followed by ultrasonication for 30 min and vortex for 10 min. The 15 mice were divided into three groups (n = 5 for each group), including a control group (treated with vehicle 0.5% CMC-Na) and two experimental groups (treated with TiO_2 NP 250and 500 mg/kg respectively). The mice were weighed and orally administered TiO_2 NP suspension once daily for 14 days.

When the animal treatment finished, blood was collected by retro-obital bleeding in all the mice and centrifuged at 3000 *g* for 10 min at 4 °C. Then all the mice were killed by CO_2 asphyxiation, following which their livers were collected. The liver tissues were cut off and a slice was cut from the middle of the biggest lobe. Two tiny blocks of each liver slice from 3 mice in each group was trimmed and fixed. These blocks were subject to transmission electron microscope analysis. The sections left were fixed in 10% neutral buffered formalin and underwent procedures of hematoxylin and eosin. The remaining liver tissues were flash-frozen in liquid nitrogen, and then stored at −80 °C pending analysis.

2.3. Biochemical analysis

The IBIL, TBIL, TBA, ALP, AST and ALT in serum samples were assayed using Spectra Max M5 (CA, USA). Procedures required in the kits were followed in all the measurements.

2.4. Histopathology and ultrastructure analysis

Fixed liver tissues in the above experiments were dehydrated in a serial concentration of alcohol and xylene followed by paraffin embedding. Four-micrometer serial sections were cut and stained with hematoxylin and eosin. Histopathological examination was performed using an Olympus BX41 light microscope.

Liver tissues were prefixed in 2.5% glutaraldehyde, washed in a cacodylate buffer and postfixed in 1% osmium tetroxide. Tissues were then dehydrated in ascending grades of alcohols and embedded in an epon-araldite mixture. Ultrathin sections stained with uranyl-acetate and lead citrate were examined under a transmission electron microscope (Hitachi H-7650). Livers from 3 mice of each experimental group were randomly collected for these analyses. Three blocks of each group and 10 electron micrographs for each block were examined. All analyses used a descriptive method under supervision by a pathologist.

2.5. Quantitative polymerase chain reaction (Q-PCR) analysis

Total RNA from liver tissue (20 mg) was extracted using Trizol reagent according to the classic protocol. Specifically, the RNA was treated with chloroform, centrifuged (12,000 rpm, 20 min, 4 °C), and precipitated with 75% ethanol. Total RNA (1 μg) from each liver sample was subject to reverse transcription in a reaction volume of 20 μL using a Thermo Scientific RevertAid First Strand cDNA Synthesis Kit (CA, USA). The synthesized cDNA was stored at −20 °C until analysis.

The primer sequences listed in Table 1 were extracted from the public database (http://mouseprimerdepot.nci.nih.gov/). Q-PCR reactions contained 1 μL cDNA, 0.2 μL forward and reverse primers, and 5 μL SYBR Green PCR Master Mix (Roche, USA) in a total volume of 10 μL. Q-PCR was carried out on LightCycler® 480 System (Roche, USA). Amplification with 35–40 cycles was performed following the below steps: 95 °C 10 s for denaturation, 55 °C 10 s for renaturation, then 72 °C 15 s for elongation. The fluorescence signal was detected in the end of each cycle. 18S rRNA was used as an internal control. Melting curve was used to confirm the specificity of the primers. The mRNA levels were measured by Q-PCR and normalized by 18S rRNA. Messenger RNA levels in vehicle-treated control mice were arbitrarily set as 1 and results were expressed as mean ± S.D.

2.6. Western blot analysis

Expression of CYP3a, CYP2b and CYP2c was assayed by western blotting. Briefly, liver tissues were homogenized in RIPA buffer containing 1% PMSF (Shanghai, China). 30 μg protein from the liver homogenates was mixed with gel-loading buffer, boiled for 5 min,