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A mechanistic study to increase understanding of titanium dioxide nanoparticles-increased plasma glucose in mice

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ABSTRACT

Titanium dioxide nanoparticle (TiO_2 NP) is an authorized food additive. Previous studies determined oral administration of TiO_2 NPs increases plasma glucose in mice via inducing insulin resistance. An increase in reactive oxygen species (ROS) has been considered the possible mechanism of increasing plasma glucose. However, persistently high plasma glucose is also a mechanism of increasing ROS. This study aims to explore whether TiO_2 NPs increase plasma glucose via ROS. We found after oral administration of TiO_2 NPs, an increase in ROS preceded an increase in plasma glucose. Subsequently, mice were treated with two antioxidants (resveratrol and vitamin E) at the same time as oral administration of TiO_2 NPs. Results showed resveratrol and vitamin E reduced TiO_2 NPs-increased ROS. An increase in plasma glucose was also inhibited. Further research showed resveratrol and vitamin E inhibited the secretion of TNF-α and IL-6, and the phosphorylation of JNK and p38 MAPK, resulting in improved insulin resistance. These results suggest TiO_2 NPs increased ROS levels, and then ROS activated inflammatory cytokines and phosphokinases, and thus induced insulin resistance, resulting in an increase in plasma glucose. Resveratrol and vitamin E can reduce TiO_2 NPs-increased ROS and thereby inhibit an increase in plasma glucose in mice.

1. Introduction

Metallic and metal oxide nanoparticles are used in numerous products designed for direct human use and consumption, such as food colorants (Burello and Worth, 2011; Smolkova et al., 2015). These nanoparticles are widely used as food additives because of their effectiveness at killing microorganisms through photo-activation and the resulting free radical activity (Dorier et al., 2015; Tassinari et al., 2014). This property indicates that these nanoparticles could pose a risk to biological targets that are sensitive to reactive oxygen species (ROS) (Smolkova et al., 2015; Armand et al., 2016). Titanium dioxide nanoparticles (TiO_2 NPs) are the metal oxide nanoparticles which are the second most commonly used material in consumer products, and they are authorized additive used as a food colorant (Brun et al., 2014; Sha et al., 2013). Studies showed that TiO_2 NPs induced a series of biological toxicity effects to human and animals via ROS (Shi et al., 2013; Smolkova et al., 2015). In a previous study, we proved that repeat oral administration of TiO_2 NPs increased plasma glucose in mice (Hu et al., 2015).

Organs involved in the regulation of plasma glucose homeostasis, such as the pancreas and liver, are also the biological targets that are sensitive to ROS. The pancreas is responsible for insulin secretion and previous research has shown that ROS-induced apoptosis in pancreatic cells results in an absolute loss of endogenous insulin, leading to insulin deficiency and causing hyperglycemia (Hou et al., 2013; Yeo et al., 2013). In addition, the liver is an important organ for post-prandial systemic glucose disposal (Perry et al., 2014; Faghihzadeh et al., 2015). ROS-induced phosphorylation of serine/threonine in insulin receptor substrate 1 (IRS1) in liver cells results in a decrease in insulin sensitivity, leading to insulin resistance and causing hyperglycemia (Gurevitch et al., 2012; Lukic et al., 2014). Therefore, ROS are important factors which can

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cause high glucose concentrations.

However, a relatively larger amount of information has been published relating to how elevated glucose concentrations can generate excessive levels of ROS (Rochette et al., 2014; Graves and Kayal, 2008). Studies have shown that high levels of glucose increase glycation of proteins that lead to the formation of advanced glycation end products (AGEs), increasing production of ROS (Moseley, 2012; Yamagishi, 2011). In addition, as β-cells are exposed for periods of time, glucose saturates the normal route of glycolysis and is increasingly shunted to alternate pathways, such that ROS are generated from distinct metabolic processes within and outside mitochondria (Al-Kafaji et al., 2015; Sathanoori et al., 2015). Therefore, persistently high glucose concentrations in turn lead to increased generation of ROS.

Since it is unclear whether ROS plays an important role in increasing plasma glucose after mice are orally administered TiO_2 NPs, the current study aims to explore this by orally administering mice with TiO_2 NPs and then observing the sequence of the increases in ROS levels and plasma glucose levels. Additionally, antioxidants such as resveratrol (Res) and vitamin E (Ve) were orally administered to mice in this study, to reduce ROS levels and to observe whether there was a reduction in the high plasma glucose levels. For further research, we aimed to detect the molecular mechanism through which TiO_2 NPs increased plasma glucose and the molecular mechanism by which Res and Ve reduced TiO_2 NPs-increased plasma glucose.

2. Materials and methods

2.1. Preparation and physicochemical characterization of the TiO_2 NPs reagent administered

Powder-form TiO_2 NPs (P25, Product number 718467, Sigma Co., Ltd., Germany) were obtained from commercial sources and used without any coating throughout this study. Before given mice, TiO_2 NPs were suspended in PBS and vigorously stirred by using ultrasonic wave at 300 W for 72 cycles (each cycle consisted of 10 s ON and 15 s OFF). The concentration of the test reagent administered was referenced to the previous study (Hu et al., 2015) and it was 64 mg/kg body weight for each mouse per day. At the first time given mice, the size, shape and distribution of the reagent in the doses used to administered mice were measured using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (Quanta, FEI Co., Ltd., Hillsboro, USA). The hydrodynamic size in PBS was measured using a dynamic light scattering (DLS, Brookhaven Instruments Corporation, Brookhaven, USA).

2.2. Animals and treatments

All animal experiments were reviewed and approved by Ethics Research Committee of the School of Life Science and Technology of Harbin Institute of Technology, and carried out according to guidelines 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). Six-week-old CD-1 mice of 40 males (average body weight: 24.82 ± 2.27 g, average plasma glucose: 4.12 ± 0.70 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 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 four groups: control, NP, NP + Res and NP + Ve. After vigorous stirring, TiO_2 NPs suspension (64 mg/kg body weight per day) was given to mice by a syringe via the oral administration. NP + Res and NP + Ve group mice were given resveratrol (Res) (100 mg/kg body weight per day) and vitamin E (Ve) (300 mg/kg body weight per day) by the oral administration at the same time as oral administration of TiO_2 NPs. Control group mice were given equal volume PBS.

2.3. Blood collection and analysis

Blood was collected from the tail vein of mice every two weeks. Before the collection, mice were fasted for 16 h. After determined that plasma glucose increased in blood collected from tail vein, mice plasma glucose were followed up for eight weeks to test whether the increase of plasma glucose was stable. At the end of week 18, 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). Heart blood triglycerides (TG), free fatty acid (FFA), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and total cholesterol (TC) were measured using each kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Tumor necrosis factor (TNF)-α and interleukin (IL)-6 were measured using TNF-α kit and IL-6 kit (R&D Systems, MN, USA).

2.4. Oral glucose tolerance test (OGTT)

At week 18, mice were fasted for 16 h and then orally administered glucose (1.5 g/kg BW). Blood was collected for plasma glucose and insulin level measurement from tail veins 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).

2.5. 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 solution was 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).

2.6. ROS levels assessment

ROS levels were assessed by using levels of total superoxide dismutase (T-SOD), glutathione (GSH) and methane dicarboxylic aldehyde (MDA). Blood was centrifuged at 750 g for 10 min at 4 °C to separate sera and cell debris. Liver was homogenized in 9 vol (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 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) and Enzyme standard instrument (Nanoquant Infinite 200, Tecan Co., Ltd., Swiss).

2.7. TUNEL staining

For pathological studies, the pancreas and livers were fixed in 4% paraformaldehyde solution for 24 h, embedded in paraffin, cut into