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Effect of titanium dioxide nanoparticles on the cardiovascular system after oral administration

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HIGHLIGHTS

- This is the first research systematically evaluating the cardiovascular effects of oral intake of TiO_2 NPs at doses simulating potential human exposure.
- Our research found that long-term ingestion of TiO_2 NPs could lead to a slight cardiovascular risk.
- Cardiac damage and inflammatory response caused by TiO_2 NPs were suggested as the possible reasons for cardiovascular adverse effects.

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ABSTRACT

Titanium dioxide nanoparticles (TiO_2 NPs) have been widely used in various consumer products, especially food and personal care products. Compared to the well-characterized adverse cardiovascular effect of inhaled ambient ultrafine particles, research on the health response to orally administrated TiO_2 NPs is still limited. In our study, we performed an *in vivo* study in Sprague-Dawley rats to understand the cardiovascular effect of TiO_2 NPs after oral intake. After daily gastrointestinal administration of TiO_2 NPs at 0, 2, 10, 50 mg/kg for 30 and 90 days, heart rate (HR), blood pressure, blood biochemical parameters and histopathology of cardiac tissues was assessed to quantify cardiovascular damage. Mild and temporary reduction of HR and systolic blood pressure as well as an increase of diastolic blood pressure was observed after daily oral administration of TiO_2 NPs for 30 days. Injury of cardiac function was observed after daily oral administration of TiO_2 NPs for 90 days as reflected in decreased activities of lactate dehydrogenase (LDH), alpha-hydroxybutyrate dehydrogenase (HBDH) and creatine kinase (CK). Increased white blood cells count (WBC) and granulocytes (GRN) in blood as well as increased concentrations of tumor necrosis factor α (TNF α) and interleukin 6 (IL-6) in the serum indicated inflammatory response initiated by TiO_2 NPs exposure. It was hypothesize that cardiac damage and inflammatory response are the possible mechanisms of the adverse cardiovascular effects induced by orally administrated TiO_2 NPs. Data from our study suggested that even at low dose of TiO_2 NPs can induce adverse cardiovascular effects after 30 days or 90 days of oral exposure, thus warranting concern for the dietary intake of TiO_2 NPs for consumers.

1. Introduction

Nanotechnology has gained strong interest in food industry and is widely utilized in processes including food cultivation, production, processing and packaging with the goal of improving product quality, facilitating nutrient absorption and enhancing visual presentation (Chen et al., 2014). Concerns on its safety have been raised among both the scientific community and the general public, among which the potential adverse health effects of orally consumed food-related nanoparticles (NPs) is a critical one

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(Bouwmeester et al., 2009). As NPs possess smaller sizes, larger surface-to-volume ratio compared with particles of larger sizes of the same chemical composition, they exhibit improved penetration to cells, catalytic activity, higher biological activity (Johnston et al., 2013; Oberdorster, 2010; Oberdorster et al., 2005; Savolainen et al., 2010). In recent years, epidemiological and toxicological studies have demonstrated a positive association between airborne particulate matter (PM) and adverse cardiovascular effects (Jia et al., 2013; Nelin et al., 2012; Polichetti et al., 2009), especially for fine ($\leq$2.5 μm) (Weichenthal et al., 2014) and ultrafine ($\leq$100 nm) particles (Karottki et al., 2014). Toxicity evaluation of engineered nanomaterials also showed that inhaled NPs can directly or indirectly pose adverse health consequences for the cardiovascular system (Du et al., 2013; Duan et al., 2013; Simeonova and Erdely, 2009). However, few studies have examined the adverse cardiovascular effects of orally ingested NPs.

Titanium dioxide (TiO_2) is a common food additive used for the whitening and brightening of foods, especially candies, sauces, dressings, and certain powdered foods. The US Food and Drug Administration (FDA) claims that the food-grade TiO_2 (referred to as E171) is an inert and safe material that can be used as a color additive in quantities up to 1% by weight of the food. As the goal of using TiO_2 as a food additive can be better achieved by using primary TiO_2 particles of smaller sizes, the use of food-grade TiO_2 NPs has been projected to increase exponentially (Hendren et al., 2011; Robichaud et al., 2009). Two recent studies characterized food-grade TiO_2 and suggested that up to approximately 36% of the TiO_2 particles were nanoparticles with less than 100 nm in at least one dimension (Weir et al., 2012; Yang et al., 2014). Based on this data, the typical amount of TiO_2 NP consumed by a US adult was estimated to be 0.5 mg per kilogram body weight per day (Weir et al., 2012). Although TiO_2 was considered as an inert and safe substance, existing studies have suggested TiO_2 NPs may be more toxic than traditional larger particles of TiO_2 (Blank et al., 2013; Oberdorster, 2001; Sager et al., 2008). The National Institute for Occupational Safety and Health (NIOSH) also supports this distinction by setting two separate occupational exposure limits for fine TiO_2 particles and ultrafine TiO_2. Thus, existing knowledge on the safety of orally consumed TiO_2 of larger sizes cannot be readily applied to TiO_2 NPs, and the potential adverse health effect of oral ingestion of TiO_2 NPs in food products merits separate evaluations.

So far, few existing studies on health effects of orally administrated TiO_2 NPs examined the potential cardiovascular effects and its mechanisms. The majority of existing studies observed liver damage in mice and rats (Bu et al., 2010; Cui et al., 2010, 2011; Duan et al., 2010; Wang et al., 2007, 2013) and kidney has also been reported to be an important target organ of orally consumed TiO_2 NPs (Gui et al., 2011; Wang et al., 2007). As elevated expression of inflammatory cytokines such as TNF-α, INF-γ, and IL-8 in the blood after that oral intake of TiO_2 NPs was observed by previous studies (Gui et al., 2011; Trouiller et al., 2009), inflammatory response induced by TiO_2 NPs is considered to be one of the main mechanisms for a variety of adverse health effects. Our previous study demonstrated that oral intake of TiO_2 NPs at 10, 50 and 200 mg/kg body weight (BW) for 30 days resulted in decreased activities of alpha-hydroxybutyrate dehydrogenase (HBDH) and creatine kinase (CK) in young rats, indicating possible cardiac injury (Wang et al., 2013). Hence we hypothesize that there can be adverse cardiovascular effects of orally administrated TiO_2 NPs and its mechanism involves inflammatory responses to TiO_2 NPs and cardiac injuries.

In the present study we aim to understand effect of TiO_2 NPs on the cardiovascular system after oral administration using healthy Sprague-Dawley rats as a model for *in vivo* study. The doses were designed to mimic typical children exposure through consumption of sweets with TiO_2 NPs additive and daily oral administration was performed for 30 and 90 days. Heart rate, blood pressure, histopathology of cardiac tissues and a series of blood biochemical parameters were selected and monitored to assess cardiovascular effect, inflammatory response as well as potential cardiac damage.

2. Materials and methods

2.1. Nanoparticle characterization

The titanium dioxide nanoparticles (TiO_2 NPs) were purchased from Shanghai Aladdin Reagent Co., Ltd., China. The size and shape of the particles was characterized by transmission electron microscopy (TEM, JEOL JEM-200CX). The purity of the particles was analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES, IRIS Advantag, TJA, USA). The crystal structure of the particles was identified by X-ray powder diffractometry (XRD, PANalytical'sX'Pert PRO, X'Celerator). The surface functional group of the particles was determined using a fourier transform infrared spectrometer (FTIR, Nexus 470, Thermo Nicolet, USA). Brunauer–Emmett–Teller (BET) method (Quantachrome, Autosorb 1, Boynton, FL, USA) was used to measure the specific surface area (SSA) of the particles. The particle hydrodynamic diameters and Zeta potentials were tested using the ZetaSizer Nano ZS90 (Malvern Instruments Ltd., Malvern, UK).

2.2. Animal and experimental design

Three-week-old healthy Sprague-Dawley rats were bred and supplied by the Department of Laboratory Animal Science at Peking University Health Science Center. The rats were fed a commercial pellet diet and deionized water *ad libitum*, and kept in plastic cages at 20 ± 2 °C and 50–70% relative humidity with a 12:12 h light–dark cycle. After one week of acclimation, rats were weighed and randomized into experimental and control groups, with 10 female and 10 male rats in each treatment group.

All experimental rats were provided with humane care and all interactions were conducted in accordance with the Guiding Principles in the Use of Animals in Toxicology outlined by Society of Toxicology and the European Union Directive 2010/63/EU for animal experiments with approval from the Peking University Institutional Review Board.

TiO_2 NPs were dispersed in ultrapure water and sonicated for 15 min. In order to obtain homogenized suspension, the particle suspension was vortexed before every use. The intragastric doses of TiO_2 NPs were selected based on the oral intake TiO_2 NPs for children in the US, which was identified as having the highest exposures as the TiO_2 content of sweets is highest among all food products (Weir et al., 2012). A typical exposure was estimated to be about 1–2 mg TiO_2 kg^{-1} BW per day. As roughly 36% of food-grade TiO_2 particles (referred to as E171) are in the nanosizes, the daily oral intake of TiO_2 NPs was estimated to be 0.5 mg/kg BW per day (Weir et al., 2012). In this study, we applied a safety factor of 100 to take into account of interspecies extrapolation when calculating the exposure dose. Thus 50 mg/kg BW which is 100 times the dose of the estimated exposure among children was selected to serve as the highest dose of TiO_2 NPs administrated in rats.

Suspensions of TiO_2 NPs (0, 2, 10, 50 mg/kg BW) were administrated to rats *via* oral gavage in a volume of 1 mL daily for 30 or 90 consecutive days. The symptom and mortality were observed and recorded daily throughout the entire duration of exposure up to 90 days. The body weight of rats was assessed every 7 days and the food intake of rats was recorded every 3–4 days. During the experiments, no significant changes in the body weight and food intake of the exposed rats were found (Supplemental data, Fig. S1) and no mortality was observed. After 30 days or