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Interaction of titanium dioxide nanoparticles with glucose on young rats after oral administration

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Abstract

Titanium dioxide nanoparticles (TiO_2 NPs) have a broad application prospect in replace with TiO_2 used as a food additive, especially used in sweets. Understanding the interaction of TiO_2 NPs with sugar is meaningful for health promotion. We used a young animal model to study the toxicological effect of orally administrated TiO_2 NPs at doses of 0, 2, 10 and 50 mg/kg per day with or without daily consumption of 1.8 g/kg glucose for 30 days and 90 days. The results showed that oral exposure to TiO_2 NPs and TiO_2 NPs + glucose both induced liver, kidney, and heart injuries as well as changes in the count of white and red blood cells in a dose, time and gender-dependent manner. The toxicological interactions between orally-administrated TiO_2 NPs and glucose were evident, but differed among target organs. These results suggest that it is necessary to limit dietary co-exposure to TiO_2 NPs and sugar.

From the Clinical Editor: Nanotechnology has gained entrance in the food industry, with the presence of nanoparticles now in many food items. Despite this increasing trend, the potential toxic effects of these nanoparticles to human remain unknown. In this article, the authors studied titanium dioxide nanoparticles (TiO2 NPs), which are commonly used as food additive, together with glucose. The findings of possible adverse effects on liver, kidney, and heart might point to a rethink of using glucose and TiO2 NPs combination.

Key words: Combined toxic effects; Interaction; Titanium dioxide nanoparticles; Glucose; Antagonistic effect

Nanofood is defined as food that is manufactured using nanotechnology techniques or tools during cultivation, production, processing, or packaging. Nanofood has gained strong interest in food industry, as nanotechnology helps improve food quality and food packaging as well as enhance nutrient absorption.[1] However there is emerging concern on the possible adverse health effects of nanofood, particularly the risk of oral uptake of food-related nanoparticles (NPs) (defined as having at least one dimension <100 nm).[2] NPs may have higher toxic potential than particles of larger sizes due to their larger surface area to volume ratio, improved penetration to cells, enhanced catalytic activity and increased reactivity.[3-6] This increase in reactivity can also result in harmful interactions between NPs and nutrients in food, but no studies have addressed the toxicological effect of co-exposure to NPs and nutritional contents via oral consumption.

Titanium dioxide (TiO_2) is a commonly used food additive for whitening and brightening food products, especially candies, white sauces, dressings and certain powdered foods.[7] The foods with the highest content of TiO_2 are sweets or candies, and children liking to eat sweets were identified as having the highest exposures to TiO_2.[8] In order to achieve better taste and quality, many manufacturers are inclined to use TiO_2 with smaller primary particle sizes, which leads to an exponential increase in the percentage of nanosized TiO_2 used among food-grade TiO_2 particles.[9,10] A testing of food-grade TiO_2 found that approximately 36% particles was in the form of NPs,[8,11] and a study showed that over 40% of TiO_2 in gum is TiO_2 NPs.[12] Titanium dioxide nanoparticles (TiO_2 NPs) show the broad

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application prospect in replace with TiO_2 used as a food additive, especially used in sweets or candies.

Although TiO_2 was considered as an inert and safe substance, many recent studies have suggested that TiO_2 NPs may be more toxic than traditional larger particles of TiO_2,[13-15] Compared with fine TiO_2 particles (125 nm), TiO_2 NPs (25 and 80 nm) can cause more serious injury in mice liver and kidney after a single dose of 5 g kg^{-1} body weight (BW) via oral gavage.[16] Upon oral exposure, TiO_2 NPs can significantly impact on the liver,[16-18] kidney,[19] heart,[17] and reproductive system,[20] as well as hemostasis[18] and immune responses.[18] Our previous study showed that young rats may be more susceptible to the adverse effects of oral intake of TiO_2 NPs.[17] However, these studies focused on the health impact of short-term and high-dose oral exposure to TiO_2 NPs. More investigations are needed on the toxicological effect of long-term and low-dose oral exposure to TiO_2 NPs, which is a better approximation of human exposure. Furthermore, considering that TiO_2 NPs are mostly used in sweets or candies, intake of TiO_2 NPs will be associated with the intake of sugar.[8] Meanwhile, excessive intake of sugar is harmful to human health.[21,22] It is scientifically meaningful with real-world implications to study about the toxicological interaction between orally administrated TiO_2 NPs and sugar.

Because most ingested sugar is converted into glucose during digestion and glucose is the primary form of sugar that is transported around the bodies of animals in the bloodstream,[23] the present study chose glucose to study the combined toxicological effects of TiO_2 NPs and sugar through oral administration. TiO_2 NPs, glucose and TiO_2 NPs + glucose were orally delivered among 3-week-old healthy Sprague–Dawley rats for 30 and 90 days, at the end of which organ coefficient, pathological findings, hematological parameters and biochemical indicators were assessed to characterize the toxicological effects.

Methods

Nanoparticle characterization

Titanium dioxide nanoparticles (TiO_2 NPs) were purchased from Shanghai Aladdin Reagent Co. Ltd, China. The size and shape of the particles were 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). X-ray powder diffractometry (XRD, PANalytical's X'Pert PRO, X'Celerator) was used to characterize the crystal structure of the particles. The surface functional group of the particles was determined using a Fourier transform infrared spectrometer (FTIR, Nexus 470, Thermo Nicolet, USA). The specific surface area (SSA) of the particles was measured according to the Brunauer–Emmett–Teller (BET) method (Quantachrome, Autosorb 1, Boynton, FL, USA). The particle hydrodynamic diameters and zeta potentials were tested using the ZetaSizerNano ZS90 (Malvern Instruments Ltd, Malvern, UK).

Animal and experimental design

Three-week-old healthy Sprague–Dawley rats were bred and supplied by the Department of Laboratory Animal Science, 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 5 male and 5 female rats in each treatment group.

All experimental rats were provided humane care of the animals. The study was 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, and received approval from the Peking University Institutional Review Board.

The TiO_2 NPs, glucose and TiO_2 NPs + glucose were dispersed in ultrapure water and sonicated for 15 min. In order to obtain homogenized suspension, the particle dispersion solution was vortexed before every use. The intragastric doses of TiO_2 NPs for three-week old young rats were selected based on the oral intake TiO_2 NPs for children under the age of 10 years in the US,[8] which was estimated to be about 1-2 mg TiO_2 kg^{-1} BW per day.[8] As roughly 36% of the particles in food-grade TiO_2 (referred to as E171) are in the nano range, the daily oral intake of TiO_2 NPs was estimated to be 0.5 mg kg^{-1} BW per day.[8] In this study, we considered safety factor and set it to 100 when doing the calculation of exposure dose. Safety factor, also known as extrapolation coefficient, is often presented in toxicological studies for uncertainty in the extrapolation of the results from animal experiments to humans. Some toxicologists suggested that it is available to design exposure doses of rats to be in multiples of human exposure, multiple of which is typically 100 in sub-chronic toxicity tests of the food hygiene work.[24,25] The 100 times dose of the estimated exposure of children under 10-years-old (50 mg kg^{-1} BW) was selected as the highest dose of TiO_2 NPs administrated in rats. The intragastric doses of glucose were selected based on the amount of added sugar consumption among U.S. children and adolescents,[26] which has been estimated to be about 360 kcal per day, equivalent to approximately 1.8 g glucose kg^{-1} BW per day as glucose produces 4 kcal g^{-1} energy and assuming average body weight at 50 kg.

Suspensions of TiO_2 NPs (0, 2, 10, 50 mg kg^{-1} BW), glucose (1.8 g kg^{-1} BW), and TiO_2 NPs (0, 2, 10, 50 mg kg^{-1} BW) + glucose (1.8 g kg^{-1} BW) were administrated to rats via oral gavage. Chemicals were given orally in a volume of 1 mL on each rat 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 young rats were found (supplemental data, Figure S1, S2) and no mortality was observed.

After 30 days or 90 days, animals were weighed and sacrificed. The blood samples were collected from the abdominal aortic vascular. Serum was harvested by centrifuging blood at 3000 rpm (1500 g) for 10 min. The tissues and organs including the liver, kidney, spleen, testicle, ovary, and heart were harvested and weighed.