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Effects of the interaction of TiO_2 nanoparticles with bisphenol A on their physicochemical properties and in vitro toxicity

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ABSTRACT

In this paper we evaluated the effects of the interaction of TiO_2 nanoparticles (nano-TiO_2) with bisphenol A (BPA) on their physicochemical properties and in vitro toxicity in human embryo L-02 hepatocytes. Different concentrations of BPA (0, 0.1, 1, 10 μmol/L) and nano-TiO_2 (0, 0.1, 1, 10 mg/L) were mixed to analyze the size distribution, zeta potential, adsorption capacity and uptake of nano-TiO_2, and the toxicity of nano-TiO_2 and BPA in L-02 cells. The addition of BPA to nano-TiO_2 dispersions increased the aggregation level and zeta potential of nano-TiO_2 in all media. Nano-TiO_2 had a similar adsorption capacity in different media, although a higher aggregation level was observed in cell culture medium. Nano-TiO_2, with or without BPA, could enter L-02 cells after 24 h exposure. Nano-TiO_2 alone did not induce significant DNA and chromosome damage, but the mixture of nano-TiO_2 and BPA increased toxicity via increasing oxidative stress, DNA double strand breaks and micronuclei formation. The aggregated nano-TiO_2 can enrich BPA effectively. The BPA-bound nano-TiO_2 are proven to be uptaken into nuclei of exposed cells, which may increase intracellular BPA and nano-TiO_2 levels and thus lead to synergistic toxicity. However only small synergic effects were observed at the concentrations of BPA and nano-TiO_2 used in this study.

1. Introduction

Titanium dioxide nanoparticles (nano-TiO_2) have been widely used as additives in cosmetics, pharmaceuticals, food colorants, sunscreens and coatings for self-cleaning windows. Nano-TiO_2 may react with a wide range of organic and biological molecules and then exhibit toxic effects in various cell lines, with or without photoactivation [1–3]. Several physicochemical characteristics, such as particle shape and size, surface area, agglomeration state, surface potential (zeta-potential) and surface chemistry are reported to be connected with the toxicity of nanoparticles [4]. Furthermore, these properties are dependent on environmental conditions. Particle concentrations, pH, the presence of electrolytes and other chemicals can influence the agglomeration, size distribution, zeta potential and stability of colloidal nano-TiO_2 [5,6]. It was reported that at higher concentrations, nano-TiO_2 showed increased agglomeration. Agglomeration levels of nano-TiO_2 in water and in cell culture medium were quite different [7].

Extensive usage of nano-TiO_2 increases the risk of combined exposure of nano-TiO_2 with other environmental pollutants. Bisphenol A (4,4-isopropylidine diphenol, BPA) is a monomer widely used for the production of polycarbonate plastics and epoxy resins, such as baby bottles, foodstuff containers and dental sealants. Regular consumption of cold beverages from polycarbonate bottles is associated with a substantial increase in urinary BPA concentrations [8]. BPA is an environmental estrogen disruptor that can cause adverse health effects on human beings by affecting growth, development, and reproduction. A daily exposure dose of 50 μg/kg body weight/day was stated to be safe for humans by the U.S. Food and Drug Administration and the U.S. Environmental

Abbreviations: Nano-TiO_2, titanium dioxide nanoparticles; BPA, bisphenol A; DMEM, Dulbecco's minimum essential eagle's medium; TEM, transmission electron microscopy; FT-IR, Fourier transform infrared spectrophotometer; DCFH-DA, 2,7-dichlorofluorescin diacetate; MN, micronucleus; LOQ, limit of quantification; LOD, limit of detection; MDA, malonaldehyde; ROS, reactive oxygen species; OTM, The Olive tail moment.

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Protection Agency in the 1980s. Several in vivo and in vitro studies reported that significant adverse effects were observed at concentrations well below this predicted safe dose. Meanwhile other studies showed that no significant adverse effects were observed on rats at concentrations higher (200 μg/kg/day) than this predicted safe dose [9,10]. The extensive use of both BPA and nano-TiO_2 causes the serious possibility that BPA can interact easily with nano-TiO_2 in the environment. For example, the nano-TiO_2 photocatalytic degradation of BPA may result in an increased exposure risk of combined nano-TiO_2 and BPA if the degradation is not fully complete. The interaction may influence the agglomeration, colloidal stability and adsorption capacity of nano-TiO_2, which could affect adsorption, distribution, fate, intracellular exposure level and toxicity of both BPA and nano-TiO_2. According to the best of our knowledge, however, no study that focused on the interaction of nano-TiO_2 and BPA was reported.

The mixing of different compounds may induce unexpected toxic effects, even if the toxicities of the individual compounds are well known. The complexity of mixture toxicity lies in the potential for interaction between the mix constituents [11]. In our previous study, trace nano-TiO_2 that has no significant toxicity to human embryo hepatic L-02 cells enhanced the toxicity of trace p,p′-DDT synergistically. The effective adsorption of p,p′-DDT by nano-TiO_2 may contribute to this increase of toxicity [12]. Interactions of nanoparticles with organic chemicals were reported to affect physicochemical properties and adsorption of nanoparticles [13,14]. Therefore, exceptional physicochemical properties, adsorption kinetics and distribution of nano-TiO_2 should be considered together with the in vitro toxicity of nano-TiO_2 and BPA mixture at the same time.

In this study, effects of the interaction of nano-TiO_2 with BPA on the physicochemical properties (including particle size, zeta potential and adsorption kinetics), uptake of nano-TiO_2 by L-02 cells and in vitro toxicity of nano-TiO_2 and BPA were studied synchronously. The liver is the major organ to metabolite BPA [9], and the location in which nano-TiO_2 accumulates mainly following either oral administration or intravenous administration [15,16]. Therefore, we used human embryo hepatic L-02 cell as a model to study the uptake and toxicity of BPA and nano-TiO_2 exposure. Furthermore, nano-TiO_2 induced oxidative stress, DNA damage and micronuclei formation [1–3]. BPA produced oxidative stress and DNA damage in cultured cells and in rodents [9,17,18]. For these reasons, we used intracellular oxidative stress, DNA breaks, chromosome damage and cell viability as indexes to evaluate the toxicity of nano-TiO_2 and BPA exposure in L-02 cells.

2. Materials and methods

2.1. Nano-TiO_2 particle

Nano-TiO_2 (Degussa P25) was obtained from Degussa (Hanau, Germany). The crystalline anatase/rutile ratio is 8:2 with a primary particle diameter of approximately 25–50 nm (Fig. S1, supporting material) and BET surface area of 50 m^2/g. Nano-TiO_2 was sterilized in an autoclave and freshly suspended in distilled water immediately before use. Stock solution of BPA (20 mmol/L) was prepared in DMSO and stored at 4 °C.

2.2. Dispersion stability of nano-TiO_2

Size distribution and zeta potential of autoclaved nano-TiO_2, with or without BPA in distilled water, 0.2 g/L $CaCl_2$ solution or serum-free Dulbecco's minimum essential Eagle's medium (DMEM, containing 100 units/mL penicillin G and 100 μg/mL streptomycin, ph 7.7) were measured by dynamic light scattering using Zeta Potential & Photon Correlation Spectroscopy (Beckman Coulter, USA). Different concentrations of BPA and autoclaved nano-TiO_2 were mixed, vortexed for 2 min, ultrasonicated for 10 min, and then measured immediately. Time-dependent changes (0–60 min) in dispersion stability were analyzed in the mixture of 10 μmol/L BPA and 10 mg/L nano-TiO_2.

2.3. Uptake of nano-TiO_2 by L-02 cells

After L-02 cells were exposed to 10 mg/L nano-TiO_2 alone, 10 mg/L nano-TiO_2 and 10 μmol/L BPA for 24 h, the cells were collected, washed and fixed in 2.5% glutaraldehyde, post-fixed with OsO_4 and dehydrated in graded concentrations of ethanol and then embedded in Eponate-12 overnight. Ultra-thin sections were cut (80 nm), counterstained with lead citrate and uranyl acetate, and then observed with Tecnai G2 20 TWIN transmission electron microscopy (Fei, The Netherlands) at 200 kV.

2.4. Adsorption kinetics

Nano-TiO_2 suspension (10 mg/L) was mixed with 10 μmol/L BPA solutions (in distilled water, 0.2 g/L $CaCl_2$ or serum free DMEM at pH 7.7). The solutions were sealed and stirred at room temperature. At different intervals, samples were taken out. The supernatants were used for analysis after the solid particles were precipitated. BPA solutions at different concentrations without nano-TiO_2 were used as references. To prevent photodegradation of BPA by nano-TiO_2, the samples were covered with aluminum foil during the entire adsorption test. BPA in the supernatants was determined by Waters 1512 HPLC system with a 2487 dual λ absorbance detector (Waters, USA) at 281 nm on a Waters Symmetry reversed-phase ODS column (5 μm, 150 mm × 4.6 mm i.d.). The mobile phase was water–acetonitrile 5:5 (v/v). The calibration graph for BPA was linear in the range from 0.1 to 10 μmol/L with R^2 = 0.9996. The limit of detection (a signal-to-noise ratio of 3) was 0.01 μmol/L. The limit of quantification (a signal-to-noise ratio of 10) was 0.1 μmol/L.

The binding of BPA to nano-TiO_2 was also detected on a Bruker VERTEX 70 Fourier transform infrared spectrophotometer (FT-IR) equipped with ATR accessory. Considering the detection sensitivity and requirements of FT-IR, high concentration of BPA (20 μmol/L) was mixed with high level (486.7 mg/L) of nano-TiO_2 in distilled water at pH 7.7 for 24 h. Then nano-TiO_2 was separated and washed 4 times with distilled water. After being precipitated, the samples were dried at 25 °C in a vacuum oven for 24 h prior to FT-IR analysis. Scans of the region between 500 and 4000 cm^{-1} were collected for each FT-IR spectrum.

2.5. Cell culture and treatment

A human embryo hepatic L-02 cell line was purchased from the China Center for Type Culture Collection (Wuhan University, China). L-02 cells were grown in DMEM containing 10% fetal bovine serum, 100 units/mL penicillin G and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were seeded in 24-well plates at a density of 1×10^5 cells/well. After seeding for 24 h, the cells were washed and treated with a series of nano-TiO_2 (0, 1, 5, 10 mg/L) and/or BPA (0, 0.1, 1, 10 μmol/L), respectively, in DMEM without fetal bovine serum in the dark. As BPA was prepared in DMSO, control cells were treated with an equal volume of DMEM containing 0.1% DMSO.

2.6. Cell viability

Intracellular ATP level was used to determine the viability of exposed L-02 cells. In brief, following either 6, 12, or 24 h of exposure, DMEM medium was removed and 2% trichloroacetic acid was