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Molecular mechanism of titanium dioxide nanoparticles-induced oxidative injury in the brain of mice

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HIGHLIGHTS

- Exposure to TiO_2 NPs resulted in pathological changes in brain of mice.
- Exposure to TiO_2 NPs led to ROS production, peroxidation of lipid, protein and DNA in brain.
- Exposure to TiO_2 NPs caused the increases of p38, JNK, NF-κB, Nrf-2 and HO-1 expression in brain.
- Activation of HO-1 through the p38-Nrf-2 signaling pathway may modulate TiO_2 NPs-induced oxidative stress in brain.

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ABSTRACT

Numerous studies have demonstrated that the brain is one of the target organs in acute or chronic titanium dioxide (TiO_2) nanoparticles (NPs) toxicity, and oxidative stress plays an important role in this process. However, whether brain oxidative injury responds to TiO_2 NPs by activating the P38-nuclear factor-E2-related factor-2 (Nrf-2) pathway is not fully understood. The present study aimed to examine activation of the P38-Nrf-2 signaling pathway associated with oxidative stress in the mouse brain induced by intranasal administration of TiO_2 NPs for 90 consecutive days. Our findings indicate that TiO_2 NPs caused overproliferation of spongiocytes and hemorrhage in the mouse brain. Furthermore, TiO_2 NPs significantly activated p38, c-Jun N-terminal kinase, nuclear factor kappa B, Nrf-2 and heme oxygenase-1 expression in the brain, which in turn, led to increased production of reactive oxygen species, as well as lipid, protein and DNA peroxidation. These findings suggest that TiO_2 NPs-induced oxidative damage in the mouse brain may occur via the p38-Nrf-2 signaling pathway. Therefore, application of TiO_2 NPs in the environment should be performed with caution.

1. Introduction

Titanium dioxide nanoparticles (TiO_2 NPs) are widely used in a number of applications: as an additive, including as a white pigment in paint, as a food colorant, in sunscreens and in cosmetic creams as well as in the environmental decontamination of air, water, and soil by the destruction of pesticides (Fisher and Egerton, 2001; Kaida et al., 2004; Esterkin et al., 2005; Choi et al., 2006). With the rapid development of nanotechnology, the potential health hazards and environmental impact of manufactured TiO_2 NPs have gained increasing attention.

It has been demonstrated that oxidative stress is one of the most important toxicity mechanisms in the lung (Afaq et al., 1998; Oberdörster et al., 2005; Sun et al., 2012a, 2012b), gill (Federici et al., 2007; Zhang et al., 2007), liver (Wang et al., 2007a,b; Ma et al., 2009; Duan et al., 2010; Cui et al., 2010, 2011, 2012), spleen (Li et al., 2010; Sang et al., 2012), kidney (Chen et al., 2009; Scown et al., 2009; Zhao et al., 2010; Gui et al., 2011) and reproductive system (Zhu et al., 2010; Wang et al., 2011; Gao et al., 2012) in animals following exposure to TiO_2 NPs. Many studies have also shown that TiO_2 NPs can enter the central nervous system (CNS) via the olfactory pathway and damage the brain, i.e. vacuoles in neurons and fatty degeneration, obvious scattered Nissl bodies, large cell somata, irregular appearance of neurons and inflammatory responses (Wang et al., 2007a,b, 2008a,b; Wu et al., 2009). Furthermore, TiO_2 NPs-induced inflammatory responses in the mouse brain were associated with significant increases in tumor necrosis factor alpha, interleukin-1β, specific neurochemicals and lipid peroxidation (Wang et al., 2008b). TiO_2 NPs were also demonstrated to induce microglial activation, thus leading to inflammatory responses in the pre-inflamed brain of mice (Shin et al., 2010). TiO_2 NPs crossed the blood–brain

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barrier, accumulated in the mouse brain caused inflammatory cell infiltration, oxidative damage and hippocampal apoptosis, which in turn led to decreased cognitive function in the mouse brain (Hu et al., 2010, 2011; Ma et al., 2010). However, it is not known whether TiO_2 NPs cause activation of the upstream signaling pathway involved in oxidative stress in brain injury.

To determine whether TiO_2 NPs cause activation of the upstream signaling pathway involved in oxidative stress in brain injury, investigations into the P38-nuclear factor-E2-related factor-2 (Nrf-2) pathway are required. It is well known that the mitogen-activated protein (MAP) kinase cascades (i.e. p38 and c-Jun N-terminal kinase (JNK)) are associated with the upstream signaling mechanism responsible for regulating oxidative stress (Kyriakis and Avruch, 2001), and oxidative stress can activate JNKs and p38 MAP kinases involving MAP kinase cascades (Hagemann and Blank, 2001; Takeda et al., 2003; Qadri et al., 2004). Redox-sensitive transcription factors, especially, nuclear factor kappa B (NF-κB) and Nrf-2 have been identified as target transcription factors of TiO_2 NPs toxicity (Ma et al., 2009; Cui et al., 2011). Under oxidative stress, activated NF-κB can initiate the transcription of a variety of genes and the expression of their proteins that function in the immunological and cellular detoxifying defense systems (Janssen et al., 1995; Pinkus et al., 1996), and has been shown to be a transcription factor regulated by intracellular redox status (Sen and Packer, 1996). It has been demonstrated that Nrf2 binds to antioxidant response elements (AREs) and regulates genes involved in protecting cells from oxidative damage (Zhang, 2006; Kensler et al., 2007). For example, the expression of Nrf2-regulated antioxidant genes was decreased in the lungs of mice with cigarette-smoke induced emphysema (Rangasamy et al., 2009). The products of these cytoprotective genes, such as glutathione S-transferase and NAD(P)H: quinine oxidoreductase 1 (Korashy and El-Kadi, 2006), heme oxygenase 1 (HO-1) (Chen et al., 2005) and γ-glutamylcysteine synthetase (Yang et al., 2005), can prevent chemical toxicity and oxidative stress. It is considered to be one of the most important mechanisms by which cells neutralize the effects of various stresses and survive (Jaiswal, 2004). It is known that Nrf2 under normal conditions is sequestered in the cytoplasm by Kelch-like ECH-associated protein 1 (Keap1) which functions as a negative regulator and promotes the quick degradation of Nrf2 via the ubiquitin proteasome system (Motohashi and Yamamoto, 2004; Kobayashi et al., 2006). In response to oxidative stress, Nrf2 dissociates from Keap1 and translocates into the nucleus where it forms a heterodimer with macrophage activating factor (Maf), and ultimately activates ARE-dependent gene expression (Sen and Packer, 1996; Kimura et al., 2007). HO-1 catalyzes the first and rate-limited step in the oxidative degradation of heme to carbon monoxide, biliverdin, bilirubin as well as iron, and is highly induced by heme and oxidative stress, and its induction has been shown to increase antioxidant defenses in rats (Morse and Choi, 2002). Therefore, we hypothesized that brain oxidative damage caused by TiO_2 NPs would be associated with activation of Nrf2 and HO-1 in the mouse brain.

The main aim of the present study was to examine whether developmental TiO_2 NPs exposure caused activation of the P38-Nrf-2 signaling pathway associated with oxidative stress in the mouse brain. We also evaluated whether this pathway offered protection against TiO_2 NPs-induced oxidative brain injury.

2. Materials and methods

2.1. Chemicals and preparation

Nanoparticulate anatase TiO_2 was prepared via controlled hydrolysis of titanium tetrabutoxide. Details of the synthesis and characterization of TiO_2 NPs were described in our previous reports (Yang et al., 2002; Hu et al., 2011). The average particle size of powdered TiO_2 NPs suspended in 0.5% w/v hydroxypropylmethylcellulose (HPMC) K4 M solvent after 12 h and 24 h incubation ranged from 5 to 6 nm and the surface area of the sample was 174.8 $m^2 g^{-1}$. The mean hydrodynamic diameter of the TiO_2 NPs in HPMC solvent ranged from 208 to 330 nm (mainly 294 nm), and the zeta potential after 12 h and 24 h incubation was 7.57 mV and 9.28 mV, respectively (Hu et al., 2011). XRD, TEM and DLS findings are available on request.

2.2. Animals and treatment

80 CD-1 (ICR) male mice (24 ± 2 g) were purchased from the Animal Center of Soochow University (China). All mice were housed in stainless steel cages in a ventilated animal room. Room temperature of the housing facility was maintained at 24 ± 2 °C with a relative humidity of 60 ± 10% and a 12-h light/dark cycle. Distilled water and sterilized food were available *ad libitum*. Prior to dosing, the mice were acclimated to this environment for 5 d. All animals were handled in accordance with the guidelines and protocols approved by the Care and Use of Animals Committee of Soochow University (China). All procedures used in the animal experiments conformed to the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH, 1996).

For the experiments, the mice were randomly divided into four groups ($N = 20$), including a control group (treated with 0.5% w/v HPMC) and three experimental groups (2.5, 5, and 10 mg kg^{-1} body weight [BW] TiO_2 NPs). About the dose selection in this study, we consulted the report of World Health Organization in 1969. According to the report, LD50 of TiO_2 for rats is larger than 12 000 mg/kg BW after oral administration. In addition, the quantity of TiO_2 nanoparticles does not exceed 1% by weight of the food according to the Federal Regulations of US Government. In the present study, we selected 2.5, 5, and 10 mg kg^{-1} BW nano-TiO_2 exposed to mice by intranasally administration every day. They were equal to about 0.15–0.7 g nano-TiO_2 of 60–70 kg body weight for humans with such exposure, which were relatively safe doses. The mice were weighed, and the TiO_2 NPs suspensions were administered intranasally every day for 90 d. Symptoms and/or mortality were observed and carefully recorded each day during the 90-d period.

2.3. Preparation of brain

Following 90 d of TiO_2 NPs administration, all mice were weighed and then sacrificed after being anesthetized using ether. The brains were excised, rinsed in phosphate buffered saline (PBS), and quickly frozen at −80 °C.

2.4. Titanium content analysis

Brains were thawed, and approximately 0.1 g of brain was weighed, digested and analyzed for titanium content. Inductively coupled plasma-mass spectrometry (ICP-MS, Thermo Elemental X7, Thermo Electron Co., Finland) was used to analyze the titanium concentration in the samples. The data were expressed as nanograms per gram fresh tissue.

2.5. Histopathological examination of brain

For the pathologic studies, all histopathologic examinations were performed using standard laboratory procedures. The brains were embedded in paraffin blocks, then sliced (5 μm thick) and placed onto glass slides. After hematoxylin–eosin (HE) staining, the stained sections were evaluated by a histopathologist unaware

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