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Risk assessment of silica nanoparticles on liver injury in metabolic syndrome mice induced by fructose

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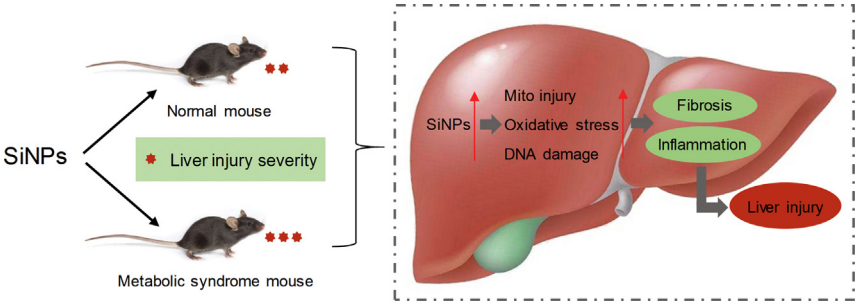
HIGHLIGHTS

- Silica nanoparticles (SiNPs) aggravate liver injury in metabolic syndrome mice.
- SiNPs lead to mitochondrial injury in liver.
- SiNPs stimulate hepatic ROS generation.
- SiNPs lead to hepatic DNA damage.

GRAPHICAL ABSTRACT



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ABSTRACT

This study aims to assess the effects and the mechanisms of silica nanoparticles (SiNPs) on hepatotoxicity in both normal and metabolic syndrome mouse models induced by fructose. Here, we found that SiNPs exposure lead to improved insulin resistance in metabolic syndrome mice, but markedly worsened hepatic ballooning, inflammation infiltration, and fibrosis. Moreover, SiNPs exposure aggravated liver injury in metabolic syndrome mice by causing serious DNA damage. Following SiNPs exposure, liver superoxide dismutase and catalase activities in metabolic syndrome mice were stimulated, which is accompanied by significantly increased malondialdehyde and 8-hydroxy-2-deoxyguanosine levels as compared to normal mice. Scanning electron microscope (SEM) revealed that SiNPs were more readily deposited in the liver mitochondria of metabolic syndrome mice, resulting in more severe mitochondrial injury as compared to normal mice. We speculated that SiNPs-induced mitochondrial injury might be the cause of hepatic oxidative stress, which further lead to a series of liver lesions as observed in mice following SiNPs exposure. Based on these results, it is likely that SiNPs will increase the risk and severity of liver disease in individuals with metabolic syndrome. Therefore, SiNPs should be used cautiously in food additives and clinical settings.

1. Introduction

Due to their unique characteristics, including large surface area, high structural stability, easy surface functionalization, low cost of production, excellent biocompatibility, and protracted circulation properties, silica nanoparticles (SiNPs) have been widely applied in manufacturing as food additives and in biomedicine for drug delivery, imaging, cell tracking, and photothermal therapy (Benezra et al., 2011; Go et al., 2017; Tang and Cheng, 2013). As a consequence, human exposure to this nanomaterial has highly increased.

Although generally considered safe, the wide applications of SiNPs have prompted investigators to perform in vitro studies on the toxicity

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of this nanomaterial. A series of studies have shown that SiNPs are able to penetrate cytomembrane due to the small size and unique surface activity. After internalized by the cells, SiNPs interact with plasma membrane, intracellular structures and organelles and induce their cytotoxicity through the release of reactive oxygen species and proinflammatory cytokines, leading to DNA and cell membrane damage, and even apoptosis (Jose et al., 2011; Kasper et al., 2011; Napierska et al., 2009; Nemmar et al., 2014; Yamashita et al., 2011). However, conflicting results have also been reported by others, showing no cytotoxic or genotoxic effects of SiNPs (Kim et al., 2017; Park et al., 2016; Yim et al., 2017).

One of the main entry pathways of SiNPs into the body is the digestive tract. Previous in vivo studies demonstrated that orally administrated SiNPs could surpass the gut barrier and thereby gain access to various peripheral organs (Barua and Mitragotri, 2014; Florek et al., 2017; Yoshida et al., 2014). Liver is among the several target organs that suffer from SiNPs-induced impairments (Lee et al., 2014). SiNPs have been shown to accumulate and be retained in the liver for over 30 days (Nishimori et al., 2009; Xie et al., 2010). Although information on hepatotoxicity of orally administered SiNPs are limited, numerous animal studies have reported increased acute liver injury in animals following exposure to SiNPs through intravenous injection (Hasezaki et al., 2011; Isoda et al., 2013; Nishimori et al., 2009). Among these studies, hepatic oxidative stress and inflammation were commonly observed in SiNPs exposed animals. Moreover, hepatic fibrosis, a worsened form of liver injury, has also been reported (Yu et al., 2017). Given the ever-increasing exposure level of human individuals to SiNPs of various sources, concerns must be raised on SiNPs exposure in the induction of liver disease in human population.

Metabolic syndrome is now endemic, and many societies are grappling with the rising prevalence of metabolic syndrome-associated diseases, including nonalcoholic fatty liver disease (NAFLD), type 2 diabetes (T2D), and atherosclerotic heart disease (Samuel and Shulman, 2012). It is estimated that ~1 billion individuals currently have NAFLD, a hepatic manifestation of metabolic syndrome, around the world (Loomba and Sanyal, 2013). Clinically, SiNPs have been used as carriers for drug delivery in treating metabolic syndrome-associated diseases (Heleg-Shabtai et al., 2016; Tang et al., 2017; Yang et al., 2017). This greatly raises the exposure of SiNPs to these people in addition to various environmentally relevant exposures, for example food additives. It has been shown that metabolic syndrome-associated liver diseases often manifest in excessive oxidative stress and inflammation (Farrell et al., 2012; Rolo et al., 2012; Tilg and Moschen, 2008), which shares common features with SiNPs-induced liver injuries. Determining the extent to which SiNPs exposure and metabolic syndrome have synergistic effects on liver damage has important implications environmental significance regarding the risk assessment of SiNPs on certain population. However, studies of relevance are currently scarce.

Therefore, the objective of this study was to determine whether oral exposure to SiNPs promotes hepatotoxicity in a metabolic syndrome mice model induced by high fructose intake. Investigations on metabolic syndrome features, hepatic histopathology, oxidative stress, DNA damage, as well as mitochondrial injury were conducted in both normal and fructose-induced metabolic syndrome mice following SiNPs exposure. Data obtained from this work provide new insight into the health hazards assessment of SiNPs in population with metabolic syndrome.

2. Materials and methods

2.1. Silica nanoparticles

SiNPs were obtained from Haitai Nanotech Port Co., Ltd. (Nanjing, China). The shape and size of SiNPs were verified using a transmission electron microscope (TEM) (JEOL JEM2100, Japan). The phase structure of SiNPs was characterized by X-ray diffraction (XRD) spectra (Bruker D8 Focus, Germany). SiNPs suspensions were dispersed using a sonicator (160 W, 20 kHz, 5 min) (Biosafer 900-92, China) prior to use to minimize their aggregation. The Zeta potential and polydispersity index of the SiNPs were measured by Zetasizer Nano ZS90 (Malver, USA).

2.2. Animal experiments

Male Kun-Ming mice (20 ± 2 g) were obtained from the Experimental Animal Center of Nanjing Medical University (Nanjing, China) and housed in a temperature- and humidity-controlled environment with a 12-h light-dark cycle and free access to standard pellet diet and tap water ad libitum. All animal use procedures were conducted in accordance with Chinese Legislation on the Use and Care of Laboratory Animals and were approved by the Institute for Experimental Animals of Nanjing University.

The metabolic syndrome model induced by high fructose intake was built according to a previously described method (Arindkar et al., 2013). Experimental mice were randomly divided into three groups, with 15 mice in each and fed by oral gavage. One group was fed with 10 mg/kg SiNPs, another with 30% (wt/vol) fructose (Fru), and the third was fed with both (10 mg/kg SiNPs and 30% fructose) (Fru + SiNP). This procedure was continual for 30 days. Mice fed normal drinking water were used as the control group. Exposure concentrations of SiNPs used in this study were selected based on our preliminary experimental data and on previous studies (Hasezaki et al., 2011; Isoda et al., 2013).

Following 30 days of exposure, all mice were sacrificed by cervical dislocation. Blood was collected by eyeball removal with a heparinized syringe and placed in ice-cooled tubes. Serum was centrifuged at 3000 rpm for 15 min at 4 °C and stored at −80 °C. Livers were removed and weighed before frozen for further analyses. The liver index was calculated as the ratio of liver weight to body weight. For the measurement of Si content in the liver, part of liver tissues was weighed and digested with ultrapure nitric acid overnight. The solutions were then heated at 120 °C to remove the remaining nitric acid. The Si concentrations in sample solutions were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (Perkin Elmer Corporation, USA). The Si concentrations in the liver were expressed as μg/g wet weight.

2.3. Histopathological analysis

Liver slices of the different groups and control mice were taken and fixed in 10% formalin solution and 2.5% glutaraldehyde solution. After 24–28 h, the formalin fixed organ samples were dehydrated in a grade alcohol series and embedded in paraffin wax. Sections of 4–5 μm thickness were subjected to hematoxylin-eosin (H&E) staining and Masson staining and examined by light microscopy for histopathologic assessment. Hepatic inflammation and fibrosis were examined and scored (0–3) according to Brunt's method (EM et al., 1999) with some modification. The 0–3 scoring includes: 0, none; 1, mild; 2, moderate; and 3, severe. Four liver tissue samples were randomly chosen from each group and three slices were examined for each tissue.

Glutaraldehyde fixed liver tissues were rinses with phosphate buffer and postfixed in aqueous osmium tetroxide. After rinsing with phosphate buffer again, the liver tissues were dehydrated in ascending ethanol series, and embedded in Araldite 6005. Thin sections (60–80 nm thick) were contrasted with uranyl acetate and lead citrate and analyzed in a Zeiss EM10 electron microscope for ultrastructural observation of mitochondria.

2.4. Oxidative stress analysis

The hepatic oxidative stress of mice was determined by measurement of superoxide dismutase (SOD) and catalase (CAT) activity as well as lipid peroxidation product malondialdehyde (MDA) and 8-