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Mitochondrial dysfunction, perturbations of mitochondrial dynamics and biogenesis involved in endothelial injury induced by silica nanoparticles☆

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ABSTRACT

As silica nanoparticles (SiNPs) pervade the global economy, however, the followed emissions during the manufacturing, use, and disposal stages inevitably bring an environmental release, potentially result in harmful impacts. Endothelial dysfunction precedes cardiovascular disease, and is often accompanied by mitochondrial impairment and dysfunction. We had reported endothelial dysfunction induced by SiNPs, however, the related mechanisms by which SiNPs interact with mitochondria are not well understood. In the present study, we examined SiNPs-induced mitochondrial dysfunction, and further demonstrated their adverse effects on mitochondrial dynamics and biogenesis in endothelial cells (HUVECs). Consequently, SiNPs entered mitochondria, caused mitochondrial swelling, cristae disruption and even disappearance. Further analyses revealed SiNPs increased the intracellular level of mitochondrial reactive oxygen species, eventually resulting in the collapse of mitochondrial membrane potential, impairments in ATP synthesis, cellular respiration and the activities of three ATP-dependent enzymes (including Na^+/K^+-ATPase, Ca^{2+}-ATPase and Ca^{2+}/Mg^{2+}-ATPase), as well as an elevated intracellular calcium level. Furthermore, mitochondria in SiNPs-treated HUVECs displayed a fission phenotype. Accordingly, dysregulation of the key gene expressions (FIS1, DRP1, OPA1, Mfn1 and Mfn2) involved in fission/fusion event further certified the SiNPs-induced perturbation of mitochondrial dynamics. Meanwhile, SiNPs-treated HUVECs displayed declined levels of mitochondrial DNA copy number, PGC-1α, NRF1 and also TFAM, indicating an inhibition of mitochondrial biogenesis triggered by SiNPs via PGC-1α-NRF1-TFAM signaling. Overall, SiNPs triggered endothelial toxicity through mitochondria as target, including the induction of mitochondrial dysfunction, as well as the perturbations of their dynamics and biogenesis.

1. Introduction

Silica nanoparticles (SiNPs) are among the nanomaterials most frequently utilized in products on the global market. It is widely used as additives for food, cosmetics, drugs, varnishes and printer toners. It was estimated that a consumer intake of nanoscale silica from food was about 1.8 $mg \cdot kg\ bw^{-1} \cdot d^{-1}$ (Dekkers et al., 2011). Furthermore, these NPs are being applied in biomedical and biotechnological fields, e.g., drug or DNA delivery, enzyme immobilization, bioimaging, diagnosis, and even cancer therapy. Consequently, the anticipated increases in the production and application of SiNPs in the industrial, commercial, and biomedical fields inevitably result in an increase in their environmental presence (Keller et al., 2013). Currently, SiNPs is on the lists for toxicity evaluation by the Organization for Economic Cooperation and Development (OECD) and the National Institute of Environmental Health Sciences (NIEHS). Reportedly, ultrafine particles (UFP) in which silica is inorganic component are widely spread in the atmosphere through sandstorm, construction and combustion processes (Asweto et al., 2017). Epidemiological evidences have confirmed the correlation

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between the nano-scale UFP and cardiovascular disease (Aguilera et al., 2016). Furthermore, the increasing evidence points to the specific adverse effects of SiNPs on cardiovascular events (Yu et al., 2016). The endothelium is critical to the maintenance of vascular homeostasis. Endothelial dysfunction precedes cardiovascular disease (Rocha et al., 2010). Endothelial injury is an important mechanistic event by which the inhaled particles have effects on the prevalence of cardiovascular disease (Mills et al., 2009). It is well-documented that SiNPs exposure was associated with endothelial dysfunction (Duan et al., 2014b; Guo et al., 2015; Guo et al., 2016), impaired vascular homeostasis (Corbalan et al., 2011; Duan et al., 2014a; Nemmar et al., 2014), and consequently cardiovascular diseases. However, the underlying mechanisms by which SiNPs interact with, and affect the functions of cellular components in endothelium, are still unclear and not well understood.

The mitochondrion serves essential roles in cell metabolism, ROS generation, redox regulation, calcium (Ca^{2+}) homeostasis, cell proliferation, cell cycle progression and cell death, as well as cellular energy transduction and adenosine triphosphate (ATP) production. A series of studies have shown mitochondria as the potentially relevant target organelles for nanoparticles (NPs) toxicity (Fujioka et al., 2014). We ever observed SiNPs deposited inside mitochondria, caused mitochondrial damage, collapse of mitochondrial membrane potential ($\Delta\Psi m$), resulting in the mitochondrial-mediated apoptosis in HepG2 cells (Sun et al., 2011). Similarly, SiNPs did impairments to mitochondrial dehydrogenase activity, $\Delta\Psi m$ and respiratory chain complexes activities in hepatocytes (Xue et al., 2014). Endothelial mitochondria are essential to the vascular pathophysiology, considering as “frontline against vascular disease” (Davidson and Duchen, 2007), while its dysfunction would inevitably result in a collapse of endothelial homeostasis. However, how SiNPs interact with mitochondria (e.g. mitochondrial dynamics, biogenesis) and thus affect endothelial function still remains unknown.

Mitochondria continually undergo fusion and fission - two apparently opposite and highly regulated processes. Mitochondrial fusion and fission, termed as mitochondrial dynamics, is crucial to the maintenance of mitochondrial shape, size, number, and even function. In brief, mitochondrial fission is regulated by dynamin-related protein-1 (DRP1) and Fission-1 (FIS1), while fusion by mitofusins (Mfn1, Mfn2) and optic atrophy 1 (OPA1). Furthermore, mitochondrial biogenesis in cooperation with mitophagy determines mitochondrial content, structure, and function, and participates in cell metabolism, oxidative stress, and signal transduction. Damage to mitochondrial dynamics and biogenesis is vital in cardiovascular disease (Dorn et al., 2015). In this study, the adverse effects of SiNPs on endothelial mitochondrion were investigated, including its structure, function, dynamics and also biogenesis. Additionally, the master regulator of mitochondrial biogenesis peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α), and its downstream targets - nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (TFAM), were determined. To our best knowledge, we report for the first time that SiNPs induce perturbations of mitochondrial dynamic and biogenesis via the PGC-1α-NRF1-TFAM signaling, resulting in mitochondrial dysfunction in endothelial cells. Our research reveals the details of those previously observed effects referred to SiNPs, which is vital for the understanding of NP-related potential human health issues.

2. Materials and methods

2.1. Materials

The preparation and characterization of SiNPs have already been described in our previous study (Guo et al., 2015). Endothelial cell line, HUVECs (human umbilical vein endothelial cells) was purchased from the Cell Resource Center, Shanghai Institutes for Biological Sciences, China. Both MTT and JC-1, a fluorescent probe for $\Delta\Psi m$ determination, were obtained from Sigma-Aldrich, USA. Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), and MitoSOX™ were acquired from Thermo Fisher Sientific, USA. MitoTracker Green and Fluo-3 AM were purchased from Beyotime, China. The Na^+/K^+-ATPase, Ca^{2+}-ATPase and Ca^{2+}/Mg^{2+}-ATPase activity assay kits were supplied by Nanjing Jianchen Bioengineering Institute, China.

2.2. Cell culture and treatment

HUVECs were cultured in DMEM supplemented with 10% FBS at 37 °C in a 5% CO_2 humidified incubator. Cells were incubated up to about 24 h and grown to about 80% confluence before experiments. SiNPs were dispersed in deionized water, and diluted to appropriate concentrations by DMEM culture medium. After SiNPs treatment, cells were collected for a series of analyses according to the experiment schedule.

2.3. SiNP uptake and cellular ultrastructure observation

After 50 $\mu g \cdot mL^{-1}$ SiNPs treatment for 24 h, HUVECs cells were collected for the observation of SiNPs uptake and cellular ultrastructure by using TEM as previously described (Guo et al., 2016).

2.4. Mitochondrial activity analysis

The reduction of MTT to formazan was dependent on the activity of mitochondrial succinode hydrogenase. Thus mitochondrial activity of HUVECs was analyzed using the MTT assay (Souza et al., 2014; Tvrda et al., 2016). Briefly, 1×10^4 HUVEC cells were placed in each of 96 wells. On the next day, after twice rinse by phosphate buffered saline (PBS), the cells were incubated with SiNPs (dose: 0, 12.5, 25, 50 and 100 $\mu g \cdot mL^{-1}$ respectively) for 6, 12, 24, 48 or 72 h at 37 °C. At last, the absorbance (A_{490}) was measured by a microplate reader (SpectraMax M5; Molecular Devices, USA).

2.5. Mitochondrial ROS (mtROS) measurement

The mtROS production was measured using MitoSOX™, a mitochondrial superoxide indicator. MitoTracker Green, a selective mitochondrial fluorescent probe that is not affected by $\Delta\Psi m$, was used for the specific staining of endothelial mitochondria. HUVECs were treated with 50 $\mu g \cdot mL^{-1}$ SiNPs for 24 h, after that, cells were co-stained with 5 μM MitoSOX™ and 100 nM MitoTracker Green in serum free culture medium at 37 °C for 30 min in the darkness. The cells were extensive washed with phosphate buffered saline (PBS) and then observed under a laser scanning confocal microscope, LSCM (LSM 710; Zeiss, Germany). Moreover, the MitoSox™ fluorescence intensity was also determined using a flow cytometer (Becton-Dickinson, USA).

2.6. $\Delta\Psi m$ assay

JC-1 probe can selectively enter into mitochondria, and its red/green ratio is frequently used to reflect the change of $\Delta\Psi m$. After SiNPs treatment for 24 h, cells were washed with PBS for three times, and then incubated with JC-1 working solution, 10 $mg \cdot L^{-1}$ for 20 min. At last, the cells were observed under LSCM. Meanwhile, the red/green ratio was analyzed by Leica QWin image analysis software (Leica Microsystems, Wetzlar, Germany). At least 20 fields were picked in each group for the measurement of mean values of

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