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Silica nanoparticles induced endothelial apoptosis via endoplasmic reticulum stress-mitochondrial apoptotic signaling pathway

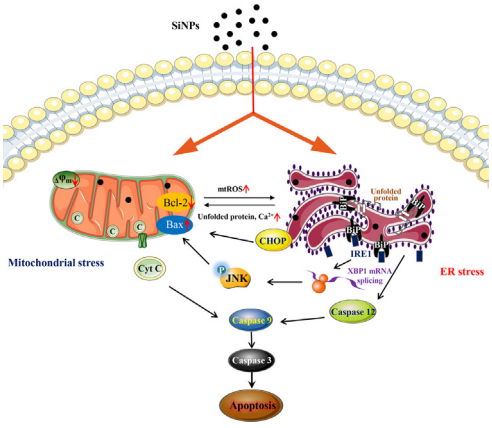
Caixia Guo [a, b], Ru Ma [a, b], Xiaoying Liu [b, c], Yinye Xia [a, b], Piye Niu [a, b], Junxiang Ma [a, b], Xianqing Zhou [b, c], Yanbo Li [b, c, *], Zhiwei Sun [b, c]

[a] Department of Occupational Health and Environmental Health, School of Public Health, Capital Medical University, Beijing, 100069, China
[b] Beijing Key Laboratory of Environmental Toxicology, Capital Medical University, Beijing, 100069, China
[c] Department of Toxicology and Sanitary Chemistry, School of Public Health, Capital Medical University, Beijing, 100069, China

HIGHLIGHTS

- The internalized SiNPs deposited in mitochondrial and ER regions with ultra-structure changes.
- SiNPs induced mitochondrial oxidative stress, ΔΨm collapse and cytosolic Ca^{2+} overload.
- SiNPs induced ER stress confirmed by enhanced ER staining, GRP78/BiP transcription and XBP1 splicing.
- ER stress-mitochondria cascade-mediated apoptotic signaling was mediated the endothelial apoptosis induced by SiNPs.

GRAPHICAL ABSTRACT



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ABSTRACT

Along with their extensively application, human exposure to amorphous silica nanoparticles (SiNPs) has highly increased. Accumulative toxicological researches have provided the scientific correlation between SiNPs exposure and cardiovascular diseases. Endothelial apoptosis is vital in the initiation and progression of atherosclerosis. However, molecular details between SiNPs and endothelial apoptosis remain unidentified. Here, we investigated the uptake and toxic mechanism of SiNPs using HUVECs (Human umbilical vein endothelial cells). Consequently, at 24-h exposure, SiNPs were located freely or within membrane-bound agglomerates in the cytosol, especially in mitochondrial and endoplasmic reticulum (ER) regions with swelled mitochondria, cristae rupture or aggregated ER. Further, we demonstrated that SiNPs induced endothelial apoptosis as evidenced by the Annexin V/PI staining and flow cytometry determination. In line with the ultrastructure alterations, SiNPs triggered mitochondrial ROS generation, ΔΨm collapse, cytosolic Ca^{2+} overload, as well as ER stress confirmed by enhanced ER staining, up-regulated GRP78/BiP and XBP1 splicing. More notably, in line with the induction of apoptosis, SiNPs-induced ER stress-associated activation of CHOP, caspase-12, and IRE1α/JNK pathways, which may regulate the BCL2 family member as evidenced by a increased proapoptotic BAX while a decline of anti-

* Corresponding author. Address: Department of Toxicology and Sanitary Chemistry, School of Public Health, Capital Medical University. No.10 Xitoutiao, You An Men, Beijing, 100069, China.
E-mail address: ybli@ccmu.edu.cn (Y. Li).

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apoptotic Bcl-2, ultimately facilitate the mitochondria-mediated apoptotic caspase cascade as confirmed by the upregulated expressions of cytochrome *c*, Caspase-9 and -3. Altogether, our results indicated the activation of ER stress-mitochondria cascade-mediated apoptotic pathways may be a key mechanism among the SiNPs-induced endothelial apoptosis.

1. Introduction

Nanotechnology is currently one of the fastest growing areas of science across the globe. Alongside nanotechnology advances, synthetic amorphous silica nanoparticles (SiNPs) are popularly used in a broad range of disciplines owing to their unique physic-chemical properties, low cost and relatively easy production. SiNPs have been extensively researched and developed for medical and biomedical applications, such as cell tracking, image, diagnosis, and treatment of diseases. Specifically, SiNPs have been designed for gene or drug delivery, cancer therapy via intravenous injection (Xu et al., 2016). Nowadays, SiNPs are on the priority lists for toxicological evaluation by the Organization for Economic Cooperation and Development, OECD. Endothelium, a single layer of endothelial cells (ECs), lines the blood vessel lumen, and acts as a key regulator of vascular homeostasis in that it regulates the transendothelial transport of various molecules to arterial intima, but also produces different molecules to modulate the vascular function. It is well recognized that SiNPs could translocate to the circulation system via inhalation, ingestion, skin contact, and even vein injection (Du et al., 2013; Matsuo et al., 2016; Nemmar et al., 2016). Because uptake of nanoparticles can lead to endothelial dysfunction (Zhang et al., 2016; Yan et al., 2017); therefore, it is pivotal to deeply understand their interaction with vascular endothelia in terms of the particle-induced toxicological adverse effects and the underlying biological mechanisms.

Cardiovascular diseases (CVD), a major cause of death worldwide, are an important public health issue. Endothelial dysfunction and injury account for a large portion of CVD because it is a key early step in the initiation and progression of atherosclerosis, and also involved in the occurrence of atherosclerotic complications (Bonetti et al., 2003; Davignon and Ganz, 2004). Accumulating evidence suggested the adverse effects of SiNPs exposure on endothelial cells (Yu et al., 2016). Likewise, we have demonstrated that SiNPs disturbed endothelial cell homeostasis, and impaired endothelial function and angiogenesis *in vivo*and *in vitro* (Duan et al., 2014; Guo et al., 2015, 2016, 2018). Moreover, Apoptosis in endothelial cells (a condition termed endothelial apoptosis) is a fundamental pathogenic feature of vascular diseases, such as atherosclerosis, diabetes, and even cancer. Experimental evidence suggested that the vascular regions prone to atherosclerotic lesion are characterized by a high endothelial cell turnover, attributing to the increased endothelial apoptosis (Riwanto et al., 2013). Endothelial apoptosis are generally found in advanced atherosclerosis, and it is critical to the progression of atherosclerotic lesion (Davignon and Ganz, 2004; Hopkins, 2013). The increased endothelial apoptosis in atherosclerotic plaque could promote secondary necrosis, which subsequently lead to the expansion of plaque lesion and even plaque rupture. A series of studies indicated SiNPs had a capability to induce apoptosis in cultured cells, including endothelial cells (Liu and Sun, 2010; Guo et al., 2016; Zou et al., 2016; Wang et al., 2017). However, the detailed molecular mechanisms underlain in this pathophysiological process are still unclear.

Human umbilical vein endothelial cells (HUVECs) expressing typical endothelial phenotype, are one of the well-characterized vascular endothelial cells, and widely used in the nanotoxicological study (Cao et al., 2017). Here, we investigated the uptake and the subsequent apoptotic induction of SiNPs in HUVECs, and further demonstrated the possible molecular mechanisms of endothelial apoptosis induced by SiNPs. The results revealed that besides oxidative stress, the internalized SiNPs could induce endothelial apoptosis through ER stress-mitochondria cascade-mediated signaling. Our findings provide a comprehensive understanding of the SiNPs-induced endothelial toxicity, and also provide important insight into the theoretical basis for the prevention and treatment of CVD.

2. Material and methods

2.1. Materials and reagents

The fluorescent probes including Fluo-3 AM (a intracellular calcium indicator), and mitochondrial or ER-specific MitoTracker Green/ER-Tracker Red were purchased from Beyotime, China, whereas JC-1 for $\Delta\Psi m$ determination was obtained from Sigma-Aldrich, USA, and MitoSOX™ Red for mitochondrial reactive oxygen species (mtROS) assessment from Thermo Fisher Scientific, USA. The kits for Annexin V-FITC Apoptosis Detection, Protein Rapid Extraction and bicinchoninic acid (BCA) protein assay were all acquired from Keygen, China, while the ECL chemiluminescence reagent from Pierce, USA. The reagents for quantitative real-time PCR measurement were all purchased from Takara, Japan.

2.2. Characterization of SiNPs

The morphology of synthesized SiNPs using Stöber method was observed under a scanning electron microscope (SEM; Hitachi S4800, Japan) and also a transmission electron microscope (TEM; JEM2100, Japan). Then at least five hundred particles in TEM images were randomly selected and the diameter of particles was analyzed through using Image J software, after which the average diameter of observed particles was calculated. The hydrodynamic sizes and zeta potential of SiNPs were examined by a Zetasizer (Malvern Nano-ZS90, UK). Before experiment use, SiNPs in the stock suspension were dispersed through using a sonicator (160 W, 20 kHz, 5 min; Bioruptor UCD-200, Belgium).

2.3. Cell culture and treatment

Endothelial cell line, HUVECs was acquired from the Cell Resource Center, Shanghai Institutes for Biological Sciences, China, and cultured in the cell culture medium DMEM (Thermo Fisher Scientific, USA) supplemented with 10% FBS (Thermo Fisher Scientific, USA) at 37 °C in a 5% CO_2 humidified incubator. Before experiments, cells were incubated up to about 24 h and grown to about 80% confluence. Then cells were exposed to 24-h SiNPs diluted by DMEM (final concentration: 12.5, 25, 50 and 100 μg/mL, respectively). Cells in control group were only treated with an equivalent volume of DMEM. Additionally, the dosage of SiNPs was selected according to our previous results acquired by MTT assay