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Size-dependent cytotoxicity of amorphous silica nanoparticles in human hepatoma HepG2 cells

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ABSTRACT

The purpose of this study is to compare the potential cytotoxicity induced by amorphous silica particles with different sizes. The effects of one fine particle (498 nm) and three nanoparticles (68, 43, and 19 nm) on cultured human hepatoma (HepG2) cells were investigated by detecting morphological changes, cell viability, cytomembrane integrity, DNA damage, cell cycle distribution, and apoptosis after the cells were treated with 100 μg/mL of four silica particles for 24 h. The results indicated that in HepG2 cells, the cytotoxicity generated by silica particles strongly depended on the particle size, and smaller silica particle possessed higher toxic effect. In order to further elucidate the possible mechanisms of cell injuries, intracellular reactive oxygen species (ROS) was measured. Increased ROS level was also observed in a size dependent way. However, the result showed the fine particle did not promote intracellular ROS level significantly, while cell injuries were detected in this treated group. Thus, our data demonstrated that exposure to different sizes of silica particles resulted in a size dependent cytotoxicity in cultured HepG2 cells, and ROS generation should be one possible damage pathway but might not be completely responsible for the toxic effect produced by silica particles.

1. Introduction

Nanomaterials refer to structures with at least one dimension in the range of 1–100 nm. Materials at this scale typically exhibit nanostructure-dependent properties, such as unique physical properties (optical, electrical, and magnetic) and highly chemical reactivity, which make them more attractive for commercial and medical applications (Oberdörster et al., 2005). As one of the important members of nanomaterials, silica nanoparticles are widely used in a variety of fields, such as chemical industry, cosmetics, agriculture, and foodstuffs. Due to their high stability, good biocompatibility, and minimal immunogenicity, recently silica nanomaterials are increasingly applied to biomedical science (Bottini et al., 2007; Kim et al., 2008). The widespread application of silica nanoparticles creates various sources for potential human exposure. It is possible for silica nanoparticles to enter human body through inhalation, ingestion, dermal penetration, and injection (Oberdörster et al., 2005). Therefore, information focuses on the safety and hazards of silica nanoparticles are urgently needed.

Investigations both in vivo and in vitro had been performed to assess the biological safety of silica nanoparticles. In several studies in vivo, investigators treated mice with Nano-SiO_2 and demonstrated that the particles could distribute in nearly all organs (Kim et al., 2006) and mainly accumulate, retain and induce adverse effects in lung, liver as well as spleen (Kaewamatawong et al., 2006; Nishimori et al., 2009; So et al., 2008; Xie et al., 2009). The relevant in vitro studies reported that exposure to nano-scale silica particles could result in cytotoxic effect and a decrease in cell viability in different cell lines (Di Pasqua et al., 2008; Napierska et al., 2009). However, detailed information on the molecular mechanisms of the injurious effects is still unclear.

In nanotoxicology research, it is well established that many physicochemical properties of nanomaterials could affect their biological activity. Particle size, as one of the important factors, plays an important role in determining the particular biological behavior of nanomaterials. Due to their extreme small size, nanoparticles possess specific large surface area, which makes the number of surface atoms or molecules increasing exponentially. Hence, particles at nano-range exhibit much higher chemical

Abbreviations: ROS, reactive oxygen species; TEM, transmission electron microscope; DLS, dynamic light scattering; CCK-8, cell counting kit-8; LDH, lactate dehydrogenase; DCFH-DA, 2′,7′-dichlorofluorescin diacetate; DCFH, 2′,7′-dichlorodihydrofluorescein; DCF, 2′,7′-dichlorofluorescein; SCGE, single cell gel electrophoresis; CP, cyclophosphamide; PI, propidium iodide; FCM, flow cytometry.

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and biological reactivity than fine particles (Nel et al., 2006). In recent years, size-dependent toxicity between micro- and nano-scale particles has been demonstrated (Brown et al., 2001; Midander et al., 2009; Oberdörster, 2000; Wang et al., 2006). However, with regard to the size-dependent toxicity of silica nanoparticles, results published were not consistent with each other (Kaewamatawong et al., 2005; Lin et al., 2006; Ye et al., 2009).

In this study, four sizes of silica particles, including three nano-scale particles and one fine particle as comparison, were investigated. The objective was to evaluate and compare the potential biological effects produced by different sizes of silica particles in HepG2 cells. Thus, cell viability and cytomembrane integrity were firstly detected to reflect the cytotoxic effect of silica particles. For the purpose to elucidate the relationship between ROS generation and cell damage, intracellular ROS level, DNA damage, cell cycle distribution and apoptosis of HepG2 cells were then measured.

2. Methods

2.1. Silica particles

Four sizes of amorphous silica particles (Si498, Nano-Si68, Nano-Si43, and Nano-Si19) with mass concentrations of 24.0, 23.6, 29.0, and 12.4 g/L respectively, were provided by School of Chemistry, Jilin University. The particle sizes and distribution of silica particles were measured by transmission electron microscope (TEM) (JEOL, Japan). Dynamic light scattering (DLS) technique was employed using zeta electric potential granulometer (Malvern, Britain) to examine the hydrodynamic sizes of silica particles in dispersion media.

2.2. Cell culture and exposure to silica particles

The human hepatoma cell line, HepG2, was purchased from Cell Resource Center, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum (Gibco, USA), 100 U/mL penicillin and 100 μg/mL streptomycin, and cultured at 37 °C in a 5% CO_2 humidified environment. For experiments, the cells were seeded in culture plates at a density of 1×10^5 cells/mL and allowed to attach for 24 h, then treated with silica particles suspended in DMEM of certain concentrations for another 24 h. Suspensions of silica particles were dispersed by sonicator (160 W, 20 kHz, 5 min) (Bioruptor UDC-200, Diagenode, Belgium) and diluted to various concentrations then added to HepG2 cells immediately. Cells maintained in DMEM without silica particles were used as control group. Each group had five replicate wells.

2.3. Cell viability (CCK-8 assay)

The effect of silica particles on cell viability was determined by WST-8 cell counting kit (CCK-8) according to the manufacture's instruction. HepG2 cells were exposed to 12.5, 25, 50, 100, and 200 μg/mL of silica particles for 24 h. Then 10 μL of CCK-8 solution was added into each well, and the cells were incubated for an additional 1 h at 37 °C. Optical density at 450 nm was detected by microplate reader (Themo, USA).

2.4. Hematoxylin and eosin (HE) staining

Hematoxylin, an alkaline dye, mainly stains chromatin in nucleus and ribosome in cytoplasm blue. And eosin, an acid dye, mainly stains components in cytoplasm pink or red. After administrated with 100 μg/mL of silica particles for 24 h, the cells were fixed with 95% alcohol and then stained by hematoxylin and eosin according to the manufacture's instruction (Maxim, China). The cellular morphological changes were observed under optical microscope (Olympus, Japan).

2.5. Lactate dehydrogenase (LDH) release assay

After the exposure of cells to 100 μg/mL of silica particles for 24 h, 50 μL of cell culture media was used to detect LDH activity by commercial LDH kit (Jiancheng, Nanjing, Chian) according to manufacture's instruction. Cells incubated with 0.25% Triton for 15 min served as positive control. The absorbance at 440 nm was measured by Beckman DU-640B UV–visible spectrophotometer.

2.6. Intracellular ROS assay

Intracellular ROS level was detected by using 2',7'-dichlorofluorescin diacetate (DCFH-DA), a non-polar compound, which could enter cells and be hydrolyzed into polar form 2',7'-dichlorodihydrofluorescein (DCFH). The intracellular DCFH is an oxidation sensitive fluorescent probe which could be oxidized by ROS to produce fluorescent 2',7'-dichlorofluorescin (DCF). The fluorescence intensity of DCF was positive correlated with the intracellular ROS quantity. After treated with 100 μg/mL of silica particles for 24 h, the cells were washed by PBS and incubated with 10 μmM DCFH-DA (Sigma, USA) at 37 °C for 20 min. Cells incubated with 0.3% H_2O_2 for 30 min served as positive control. The fluorescence intensities were measured by flow cytometer (FCM) (Becton Dickinson, USA), with an excitation wavelength of 488 nm and an emission wavelength of 525 nm.

2.7. DNA damage assay

After cells treatment with 100 μg/mL of silica particles for 24 h, single cell gel electrophoresis (SCGE), also known as comet assay (Zeni and Scarfi, 2010), was applied for detecting DNA damage induced by silica particles. Cells incubated with 40 μg/mL of cyclophosphamide (CP) for 24 h served as positive control. Cellular DNA damage could be visualized under fluorescence microscope (Nikon, Japan) after staining with a fluorescent DNA-binding dye propidium iodide (PI) (Sigma, USA). The fluorescent intensity of the comet tail reflects the number of DNA breaks. For each group more than 100 cells were randomly selected and the results were analyzed by CASP software then quantitated the number of cells with DNA damage and the DNA damage degree. According to the percent of DNA in the comet tail (tailDNA%), DNA damage level could be divided into five grades: grade 0 (no damage, tailDNA% less than 5%), grade 1 (slightly damage, tailDNA% within 5–20%), grade 2 (medium damage, tailDNA% within 20–40%), grade 3 (highly damage, tailDNA% within 40–95%), and grade 4 (severe damage, tailDNA% more than 95%).

2.8. Cell cycle assay

After treated with 100 μg/mL of silica particles for 24 h, cells were harvested in eppendorf tubes and centrifugated at 1000 r/min for 5 min. After supernatant removal, the cells were fixed in 1 mL ice-cold 70% ethanol and stored at −20 °C overnight. Next day, the cells were washed by PBS twice and resuspended in 450 μL of PI/RNAase mixture, containing 5 μg of PI (Sigma, USA) and 1 μg of RNAase (Sigma, USA), then incubated for 45 min at 37 °C in dark. At least 10,000 cells were collected and detected by FCM (Becton Dickinson, USA). Results were analyzed using MultiCycle V3.0 software to acquire the cell percentage of each phase in cell cycle.