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Research paper

Potential hazards of superfine particles to human bronchial epithelial cells through inducing oxidative stress

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ABSTRACT

The potential hazard of superfine particles in outdoor air pollution has been paid intense attention because superfine particles can go through the respiratory system of human and target the alveoli. Moreover, nanosized particles of them can further pass through the alveolar-capillary barrier and accumulate in the lung interstitium, leading to alveolar damage and potentially the occurrence of multiple pulmonary diseases. However, the mechanism underlying the adverse effects of superfine particles remains to be elucidated. In the present study, superfine particles were demonstrated to be able to generate abiotic reactive oxidative species (ROS) and have a potential to destroy the glutathione (GSH), which in turn can cause cell injury through oxidative stress mechanism. Cell viability assessment by MTT assay showed a significant decline in the viability of human bronchial epithelial (BEAS-2B) cells upon exposure to superfine particles, which was further confirmed by cell live/dead (calcein AM/propidium iodide) assay. A series of hierarchical oxidative stress responses from the lowest to the highest protection against ROS, including heme oxygenase-1 expression, mitochondrial membrane depolarization and superoxide generation, were activated after exposure to these superfine particles. Our combined results demonstrated that superfine particles posed considerable toxicity to human bronchial epithelial cells through generating oxidative stress.

1. Introduction

It has been known for a long time that outdoor air pollution poses adverse effects on human health (Bell, M. L.; Davis, D. L., Reassessment of the lethal London fog of, 1952; Dockery et al., 1993; Griffith and Levin, 1989). Epidemiological studies have identified a positive correlation between exposure to airborne particulate matter (PM) and pulmonary morbidity and mortality (Zanobetti et al., 2000; Pope, 2004; Braga et al., 2000). The strongest correlation between mortality and PM was found for superfine particles (PM2.5) primarily derived from combustion process and industrial source (Dockery et al., 1993; Pope et al., 2002; Laden et al., 2000). Superfine particles are inhalable and can reach the deep sites of a lung, namely alveolar regions, of which nanosized particles (<100 nm) can pass through the alveolar-capillary barrier and accumulate in the lung interstitium (Oberdorster et al., 2005). A large proportion of nanosized particles has been identified in the whole population of the superfine particles. In some cities of China, the percentage of nanosized particles of the total superfine particles even reaches 87% (Ling et al., 2008). Despite these advances, the toxicity behaviors of superfine particles have not been thoroughly investigated, and the mechanisms underlying their toxicity remained largely elusive.

Previous studies have revealed superfine particles can cause diverse diseases, including lung cancer, pulmonary and cardiovascular disease, diabetes, and neurodegenerative disorders (Dockery et al., 1993; Pope, 2004; Brook et al., 2004; Zanobetti et al., 2003). Thus far, oxidative stress has been demonstrated to be a primary mechanism responsible for superfine particle-mediated toxicities (Liu et al., 2009). Oxidative stress refers to a critical imbalance between oxidation and antioxidation, and the hierarchical oxidative stress responses will be sequentially triggered including an antioxidant defense response, the initiation pro-inflammatory, and mitochondrial-mediated cytotoxicity (Nel et al., 2006). Superfine particles are capable of triggering reactive oxidative species (ROS)

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which can directly interact with antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), impairing the enzymatic activity and overwhelming the antioxidant defense system (Pamplona and Costantini, 2011). Previous studies on superfine particle-mediated toxicity paid more attention to the intracellular ROS production. However, since a large population of nanosized particles exist in superfine particles, reactive physicochemical properties, such as crystallinity defects, heterojunctions and energy band levels etc., can facilitate abiotic ROS production on particle surface, which can directly damage biomolecules or cell organelles, leading to intracellular ROS production and activation of oxidative stress (Nel et al., 2012). Furthermore, the production of intracellular ROS can further activate other signaling pathways and generate additional intracellular ROS.

In the present study, the capability of abiotic ROS generation and glutathione (GSH) depletion by superfine particles was investigated using normal human bronchial epithelial (BEAS-2B) cells. Of note, these cells were used as a representative of a lung target for airborne particulate matter in order to access the lung toxicities. Hierarchical oxidative stress responses were systemically investigated to validate the production of oxidative stress induced by superfine particles.

2. Materials and methods

2.1. Superfine particle samples

Superfine particles (PM2.5) were collected on nitrocellulose filters using Anderson G1200 samplers with a flow rate of 1 m^3/min. The sampler was set on the roof (approximately 20 m high to the ground) of a building in Chaoyang, Changchun, China. This site could be regarded as a representative region that had the mixture of residential, traffic, construction and industrial sources. Superfine particles were extracted from sampled filter strips by immersing filters in deionized water and sonicating them for 30 min. The extracted samples were then lyophilized and stored at 4 °C. All chemicals were reagent grade and used without further purification or modification unless otherwise indicated. Reagent grade water used in all experimental procedures was obtained from a Milli-Q water purification system (Millipore, Bedford, MA).

2.2. Physicochemical characterization

The field emission scanning electron microscopy (FESEM) images were taken using a XL30 ESEM FEG operated at an acceleration voltage of 10.0 kV. The transmission electron microscopy (TEM) images were taken using a JEOL microscope (1200EX II) operated at 80.0 kV. Zeta potential and dynamic light scattering (DLS) data were obtained using a Malvern Nanosizer ZS. Inductively coupled plasma optical emission spectroscopy (ICP-OES) (ICP6300, Thermo Scientic, USA) was applied to determine the contents of the 10 metal elements (V, Mn, Fe, Co, Ni, Cu, Zn, Ge, Ba, and Ce) relative to superfine particles.

2.3. Abiotic ROS measurement

The stock solution of 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) was prepared by mixing 50 μg of H_2DCFDA with 17.3 μL of ethanol, followed by addition of 692 μL of 0.01 mol L^{-1} sodium hydroxide solution. The resulting solution was incubated for 30 min, and 3500 μL of a sodium phosphate buffer (25 mmol L^{-1}, pH = 7.4) was added to form 29 μmol/L DCF solution. To each well of a 96 multiwell black plate (Costar, Corning, NY) we added 80 μL DCF working solution. Subsequently, particle suspensions were added to each well, followed by 2 h incubation. DCF fluorescence emission spectra in the range of 500–600 nm were collected using a SpectraMax M3 microplate reader (Molecular Devices Corp., Sunnyvale, CA, USA) with an excitation wavelength of 490 nm.

2.4. Abiotic GSH measurements

The amount of GSH was determined by 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) methods. To each well of a 96 multiwell black plate (Costar, Corning, NY) we added 100 μL of 5 mM GSH and 10 μL of superfine particle suspension, followed by 2 h incubation. 10 μL of 2 mM DNTB in PBS solution was added to each well. After 25 min incubation, the absorbance at 412 nm proportional to GSH amounts was measured using a SpectraMax M3 microplate reader.

2.5. Cell culture

Human bronchial epithelial (BEAS-2B) cells were cultured in bronchial epithelial basal medium (BEBM) (Lonza, Walkersville, MD), supplemented with growth factors from the SingleQuot kit (Lonza) to reconstitute BEGM. Cells were passaged at 70–80% confluency every 2–4 days.

2.6. MTS assay

Cell viability was determined by a MTS assay. 1×10^4 cells in 100 μL of culture medium were plated in each well of a 96-mutiwell plate for overnight growth. Then, the culture medium was removed, and cells were treated for 24 h with 100 μL of 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100, 200 and 400 μg mL^{-1} particle suspensions. After the treatment, the medium was removed and followed by washing of the plates three times with PBS. Each well received 100 μL of culture medium containing 16.7% of MTS stock solution for an hour at 37 °C in a humidified 5% CO_2 incubator. The plate was centrifuged at 2000*g* for 10 min in Xiangyi L535R with a microplate rotor to spin down the cell debris, and 80 μL of supernatant was transferred into a new 96-well plate. The absorbance of the formazan was read at 490 nm on SpectraMax M3 microplate reader.

2.7. Live/dead cell assay

Cells were exposed to superfine particle suspensions at 100, 200, and 400 μg mL^{-1} for 24 h. After being washed with phosphate-buffered saline (PBS), cells were stained with calcein AM and propidium iodide (PI) for 30 min. Then, cells were washed with PBS three times, and cellular fluorescence was observed using Olympus BX-51 optical system microscope (Tokyo, Japan) with 10 × objective.

2.8. Intracellular ROS detection

Intracellular ROS was detected by DCF. 400 μL of 4×10^5 BEAS-2B cells were plated in each well of a 24-well plate for overnight growth. The culture medium was removed and cells were exposed to 400 μL of 12.5, 50, and 200 μg/mL superfine particle suspensions for 24 h. Cells were washed three times with PBS, and were cultured with 200 μL of H_2DCFDA (10 μM) staining solution at 37 °C for 30 min. Cells were washed again three times with PBS, and DCF fluorescence intensity was measured using a SpectraMax M3 microplate reader.

2.9. 2.8. Western blot analysis for HO-1 expression

1.6×10^5 cells in 1.6 mL culture medium were seeded into each well of the six-well plate. After overnight growth, cells were treated with 1.6 mL of 100, 200, and 400 μg mL^{-1} superfine particle suspensions for 24 h. Proteins were separated by electrophoresis in a 8–16% sodium dodecylsulfate polyacrylamide gel (SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) Immobilion P membrane (Millipore Corp., USA). Then, the membranes were blocked for 1 h at room temperature in Tris buffered saline containing 0.1% v/v Tween-20 (TBS/T) buffer and 5% nonfat dry milk, followed by 2 h incubation with anti-human HO-1 monoclonal antibody (dilution 1:500) (ENZO Life Sciences,