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Combined toxicity of amorphous silica nanoparticles and methylmercury to human lung epithelial cells

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ABSTRACT

Exposure to the ambient particulate matters (PM) has been associated with the morbidity and mortality of cardiopulmonary diseases. Compared with coarse particles, ultrafine particles (UFP) absorb or condense higher concentration of toxic air pollutants and are easily inhaled into the lung. However, the combined effects of UFP and air pollutants on human health are still poorly understood. In this study, a co-exposure in vitro model of amorphous silica nanoparticles (nano-SiO_2) and methyl mercury (MeHg) was established to investigate their combined effects and the potential joint action type. Lung adenocarcinoma cells (A549) were exposed to either nano-SiO_2 or MeHg alone, or a combination of both. Factorial design was applied to analyze their potential joint action type. Higher interfacial energy was observed in the mixed solution of nano-SiO_2 and MeHg. The intracellular content of both silicon and mercury in combination group were much higher than those in single exposure groups. In addition, the co-exposure of nano-SiO_2 and MeHg enhanced the reactive oxygen species (ROS) generation, lipid peroxidation and reduced the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-px). The excessive oxidative stress led to oxidative DNA damage as well as cellular apoptosis. Factorial design analysis demonstrated that additive and synergistic interactions were responsible for the combined toxicity of nano-SiO_2 and MeHg.

1. Introduction

Air pollution is a major environmental risk to human health. WHO estimates that air pollution leads to about 1.3 million deaths annually worldwide (WHO. Air quality and health). Epidemiological evidences confirmed that the strong links between air pollution and increased morbidity and mortality of cardiopulmonary diseases were related to the ambient particulate matter (PM) (Miller et al., 2012; Raaschou-Nielsen et al., 2013; Shah et al., 2013). The ambient PM is composed of air particles, which can be further divided into coarse (PM_{10}), fine particles ($PM_{2.5}$) and ultrafine particles (UFP, $PM_{0.1}$) (van Berlo et al., 2012). Ultrafine particles refer to particles with an aerodynamic diameter less than 100 nm. Since particles with smaller size lead to worse health effects (Breitner et al., 2011; Franck et al., 2011), the adverse effects of PM are specifically linked to the UFP. Previous studies have shown that the UFP could translocate to the circulation system (Brook, 2008; Brook et al., 2010), inducing harmful cardiovascular and pulmonary health effects (Raaschou-Nielsen et al., 2013; Valavanidis et al., 2013). Pollutants in the atmosphere are not isolated. The ambient PM is commonly found as mixtures of air pollutants such as volatile organic carbons (VOCs) and transition metals (Brook et al., 2010). Although the UFP makes up only 10% of the total mass of $PM_{2.5}$, it dominates the PM mixtures in number (Knol et al., 2009). The surface area of 20 nm UFP is 100 times higher per unit volume compared with the fine particles of 2 μm (Nel et al., 2006). The large surface area and particle numbers make the UFP have higher concentrations of adsorbed or condensed toxic air pollutants (Sioutas et al., 2005). However, the interactive effects and health impacts of exposure to combinations of UFP and air pollutants are still not well understood.

Mercury (Hg) is a global environmental pollutant that travels long distances through the atmosphere (Mahaffey et al., 2009; Steffen et al., 2012). The power plants, mining and metallurgical industry also release mercury to the ambient atmosphere,

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increasing the occupational exposure risk (Loredo et al., 2007; Gonzalez-Carrasco et al., 2011; Wang et al., 2011). Mercury in the air is easily taken up through the lungs, and about 74% of inhaled mercury is retained in human body (Syversen and Kaur, 2012). The mercury can be distributed throughout the human body via the blood circulation, which could cause cardiovascular and pulmonary health disease (Tong et al., 2010; Valera et al., 2012). Based on the *Global mercury assessment 2013* reported by United Nations Environment Program (UNEP) (Assessment, 2013), the global mercury emissions to air from anthropogenic sources is estimated as 1960 t in 2010, but the emissions are changing in the range of 1010–4070 t. Atmospheric mercury exists primarily as gaseous elemental mercury (GEM-Hg°), reactive gaseous mercury (RGM-Hg^{2+}) and particulate mercury $Hg_{(p)}$ (Fang et al., 2011). Most previous studies focused on the total mercury (THg) in outdoor urban dust and PM (Fernández-Martínez et al., 2006; Kocman et al., 2011; Steffen et al., 2012). There is only limited information about the methyl mercury (MeHg) in PM, although it was a detectable component in PM (Huang et al., 2012). Moreover, the MeHg was classified in group 2B carcinogen according to International Agency for Research on Cancer (IARC). Such organic mercury is more toxic than inorganic mercury, the bio-accumulating property makes it cause acute and chronic intoxication at low levels of exposure.

It is important to evaluate pulmonary toxicity of UFP and air pollutants, since respiratory tract is directly exposed to the atmosphere. The human lung adenocarcinoma cell line is routinely used to assess the PM as well as the mixture toxicity (Stringer et al., 1996; Sánchez-Pérez et al., 2009). It is estimated that an adult human could inhale approximately 200 billion particles per day (Donaldson et al., 2005). The UFP fraction are extremely enriched in many chemical compounds, including organic carbon species and trace metals (Sioutas et al., 2005). Facilitated transport of these adsorbed pollutants may happen when UFP is inhaled deep into the lung and transported into the blood and deposit in target organs. Some of the soluble constituents may leach from the UFP, elevating pollutants concentrations in human body. Each of these pollutants may have potentially additive, synergistic or antagonistic interaction with UFP to cause adverse health effects. However, research on the interactive effects of combinations of the UFP and air pollutants were still limited. The potential interaction between UFP and air pollutants needs to be considered.

2. Materials and methods

2.1. Silica nanoparticles and chemicals

Amorphous silica nanoparticles (nano-SiO_2) were prepared by the Stöber method (Sun et al., 2011); the nano-SiO_2 was sterilized in an autoclave and suspended in distilled water. Stock solution of Methyl mercury chloride (MeHgCl, 10 mM from Merck, Germany) was prepared in anhydrous ethanol and normal saline with a volume ratio of 1:4 and stored at 4 °C.

2.2. Characterization of nano-SiO_2

The particle size and distribution of silica nanoparticles were measured by transmission electron microscope (TEM) (JEOL, Japan) and ImageJ software. The hydrodynamic sizes and zeta potential of autoclaved nano-SiO_2 in distilled water or in serum-free RPMI-1640 medium were measured by dynamic light scattering (DLS) using zeta electric potential granulometer (Malvern, Britain). Nano-SiO_2 was ultrasonicated for 5 min, mixed with MeHg and measured immediately.

2.3. Surface tension measurement

The interfacial effect caused by the nano-SiO_2, MeHg and their mixture was determined based on the pendant drop method using an optical contact-angle measuring device (Kruss DSA30). Typically, a drop of sample solution (like nano-SiO_2, MeHg or their mixture solutions) was jetted and suspended for 10 min. Images were recorded by a camera and then analyzed using the DROP image advance software which calculated the surface tension using the Young–Laplace equation. All experiments were performed at room temperature.

2.4. Cell culture and CO-exposure of nano-SiO_2 and/or MeHg

The human lung adenocarcinoma cell line (A549) was purchased from Cell Resource Center, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. The cells were cultured in RPMI-1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin and maintained at 37 °C in an incubator containing 5% CO_2. The cells were exposed to nano-SiO_2 alone, MeHg alone, or a combination of nano-SiO_2 and MeHg. Suspension of silica nanoparticles were dispersed by sonicator (160 W, 20 kHz, 5 min) before added into culture medium to minimize their aggregation. The treatment groups of factorial design experiments were described in Table 1.

2.5. Cell viability

The effect of nano-SiO_2, MeHg and their mixture on cell viability were assessed using the MTT assay. Cells were washed twice with PBS and then treated with nano-SiO_2 and/or MeHg respectively. After 24 h incubation, 10 μl MTT was added to each well at 5 mg/ml and further incubated for another 4 h, followed by an addition of 150 μl of dimethylsulfoxide (DMSO). Optical density was then acquired with a microplate reader (Themo Multiscan MK3, USA) at 492 nm.

2.6. Analysis of SI and Hg uptake

The A549 cells were seeded in a 10-cm Petri dish and were cultured as described above. Cells were treated with nano-SiO_2 and/or MeHg respectively; washed with PBS three times and trypsinized. After centrifugation, the cell pellets were digested in 3 mL of nitric acid (HNO_3) and 1 mL of hydrogen peroxide (H_2O_2) with ultrasound. These clear acidic solutions were diluted for further testing. The intracellular mass of nano-SiO_2 and MeHg were measured by detecting the silicon and mercury content with ICP-OES (Varian 710-ES) and Atomic fluorescence spectrometer (AFS-920).

2.7. Intracellular reactive oxygen species (ROS) measurement

Intracellular ROS were measured by flow cytometry with 2.7-dichlorofluorescein diacetate (DCFH-DA) (Beyotime, China) as a probe. After 24 h exposure, cells were washed twice with PBS and co-incubated with serum-free RPMI-1640 medium containing

Table 1
Factorial design experiments were applied based on the description.

SiO_2	MeHg	
	−	+
−	0	5 μM
+	25 μg/mL	25 μg/mL+5 μM