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Co-exposure of silica nanoparticles and methylmercury induced cardiac toxicity *in vitro* and *in vivo*

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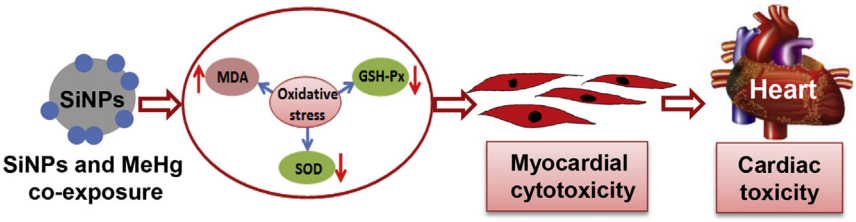
HIGHLIGHTS

- SiNPs and MeHg co-exposure caused oxidative stress, apoptosis in cardiomyocytes.
- SD rat exhibited severe injury to myocardium/heart after SiNPs and MeHg co-exposure.
- Cardiac toxicity was exerted by additive/synergistic interaction of SiNPs and MeHg.

GRAPHICAL ABSTRACT



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ABSTRACT

The released nanoparticles into environment can potentially interact with pre-existing pollution, maybe causing higher toxicity. As such, assessment of their joint toxic effects is necessary. This study was to investigate the co-exposure cardiac toxicity of silica nanoparticles (SiNPs) and methylmercury (MeHg). Factorial design was used to determine the potential joint action type. *In vitro* study, human cardiomyocytes (AC16) were exposed to SiNPs and MeHg alone or the combination. Higher toxicity was observed on cell viability, cell membrane damage in co-exposure compared with single exposure and control. The co-exposure enhanced the ROS, MDA generation and reduced the activity of SOD and GSH-Px. In addition, the co-exposure induced much higher cellular apoptotic rate in AC16. *In vivo* study, after SD rats exposed to SiNPs and MeHg and their mixture by intratracheal instillation for 30 days, pathological changes (myocardial interstitial edema) of heart were occurred in co-exposure compared with single exposure and control. Moreover obvious ultra-structural changes, including myofibril disorder, myocardial gap expansion, and mitochondrial damage were observed in co-exposure group. The activity of myocardial enzymes, including CK-MB, ANP, BNP and cTnT, were significantly elevated in co-exposure group of rat serum. Meanwhile, the cardiac injury-linked proteins expression showed an increase in SERCA2 and decreased levels of cTnT, ANP and BNP in co-exposure group. Factorial design analysis demonstrated that additive and synergistic interactions were responsible for the co-exposure cardiac toxicity *in vitro* and *vivo*. In summary, our results showed severe cardiac toxicity induced by co-exposure of SiNPs and MeHg in both cardiomycytes and heart. It will help to clarify the potential cardiovascular toxicity in regards to combined exposure pollutions.

1. Introduction

Engineered NPs are increasingly discharged into the environment with their growing production and application. SiNPs was one of the most widely used NPs which applied in food, consumer products and agriculture (Kasaai, 2015). SiNPs possessed distinct physico-chemical

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characteristics, particularly a very large surface-to-volume ratio (Oberdörster, 2010). Because of those properties, SiNPs became one of the three most produced nanomaterials in 2013 globally (Vance et al., 2015). However, the SiNPs induced toxicity on human health has aroused great attention of scientists. It's well reported that SiNPs can cause damage to cells, organs, and tissues *in vitro* (Gilardino et al., 2015; Guo et al., 2016) and *in vivo* (Du et al., 2013; Napierska et al., 2010). Because of nano-scales, SiNPs are capable of translocate into the blood stream further to cause adverse toxicity and injury to heart. As reported, after repeated intracheal instillation of SiNPs, SiNPs were detected in heart, liver and lung tissues of BALB/c mice (Yang et al., 2016). In another study, a size- and dose-dependent increase in the distribution of SiNPs was observed in the heart and serum of wistar rats after SiNPs exposure (Du et al., 2013). Moreover, there exist strong evidence that the released NPs into environment can potentially interact with pre-existing contaminants, further to cause accumulative effect and/or adverse toxic effects which are little known (Deng et al., 2017). SiNPs may adsorb and condense various contaminants to cause higher cardiac toxicity. Most previous research on SiNPs was focused on single exposure and the existing studies on co-exposure cardiac toxicity of SiNPs and pollution are limited. As such, a thorough assessment of their co-exposure cardiac toxic effects is necessary.

Mercury is one of most toxic heavy metals and an atmospheric pollutant around the world which can travel long distance in the air. Mercury can transferred *via* the blood circulation and distributed in the body, which could cause cardiopulmonary disease (Tong et al., 2010; Valera et al., 2013). Growing evidence showed that the toxicity of mercury on the heart included coronary heart disease, hypertension, cardiac arrhythmias, and myocardial infarction (Houston, 2011). There are many forms of mercury, among the list methylmercury (MeHg) has the most adverse toxic effects and was easily adsorbed (Ullrich et al., 2001). MeHg can biomagnify and bioaccumulate through the marine food chain and distribute to almost all tissues in the body (Karagas et al., 2012; Truong et al., 2015). Environmental Protection Agency (U.S. EPA) revealed that MeHg exposure can induced increased risks of heart diseases (Roman et al., 2011). Due to the adverse toxic effects on human, International Agency for Research on Cancer (IARC) classified MeHg in group 2B carcinogen (Grigor'ev Iu, 2011). Although oxidative damage is believed to be the important potential mechanism involved in cardiac toxicity (Franck et al., 2011), the mechanism of mercury induced toxicity on heart is not fully elucidated.

It is meaningful to evaluate combined toxic effects of SiNPs and environmental pollutants such as MeHg. The AC16 cell line is used to assess the co-exposure cardiac toxicity, since AC16 cell line is often used *in vitro* studies of heart function and cardiac toxicity (Cui et al., 2017). Meanwhile, SD rats were used to assess the cardiac injury of the co-exposure *in vivo*. Our previous study found that the combined of SiNPs and MeHg exhibited higher toxicity on vascular endothelium in zebrafish embryos (Duan et al., 2016). But the co-exposure cardiac toxicity of SiNPs and MeHg was unknown. Our research will assess the myocardial injury *in vitro* and cardiac injury *in vivo* induced by co-exposure of SiNPs and MeHg. It will help to reveal the toxicity of NPs and environmental pollutants on the heart and also provide evidences for the potential cardiovascular toxicity regards to combined exposure pollutions.

2. Materials and methods

2.1. SiNPs and MeHg preparation

We got the amorphous silica nanoparticles (SiNPs) using the Stöber technique (Sun et al., 2011). Methyl mercury chloride was purchased from Merck (Germany). SiNPs were autoclaved and suspended in deionized water in reserve. In order to minimize the aggregation, solution of SiNPs were resuspended by sonicator (Weiye, JK-DY100, China) for 5 min (160 W, 20 kHz) before adding to culture medium. SiNPs was observed by transmission electron microscope (TEM) (JEOL, JEM1400plus, Japan), next image J software were used to measure the size and distribution of the SiNPs. The hydrodynamic size was measured by dynamic light scattering (DLS). Meanwhile Zeta electric potential granulometer (Malvern, Worcestershire, UK) was applied for measuring zeta potential of SiNPs in three different solution media. SiNPs were sonicated for 5 min and then took the next measurement immediately.

2.2. Cell culture, cell viability and co-exposure design

AC16 was obtained from the Cell Resource Center of Shanghai Institutes for Biological Sciences (SIBS, Shanghai, China). AC16 were cultured in DMEM (Corning, USA) mixed with 10% fetal bovine serum (Gibco, USA), and incubated at in an incubator containing 5% CO_2 at 37 °C. Before treating, solution of SiNPs was sonicated for 5 min. The effects of SiNPs and MeHg and their combination on cell viability were detected using Cell Counting Kit (CCK-8) (KeyGEN, China) according to the protocol. 1×10^4 cells per well of AC16 cells with were seeded into 96-well plates for 24 h, next treated by single SiNPs and MeHg treatment or their mixture. We first tested the cell viability at 3.125, 6.25, 12.5, and 25 μg/mL concentration of SiNPs and 0.5, 1, 2, 4 and 8 μM concentration of MeHg. No significant cytotoxic concentrations of SiNPs and MeHg were chosen as the single exposure and the mixture of SiNPs and MeHg as the co-exposure. Thus, the groups were set as SiNPs (6.25 μg/mL), MeHg (0.5 μM), and SiNPs + MeHg (6.25 μg/mL + 0.5 μM), with pure culture medium as control group. Control group was treated with the same volume of DMEM. Then the concentrations of no significant cytotoxicity of SiNPs and MeHg were used for the measurement of the co-exposure's toxicity and the next other measurement *in vitro*.

2.3. Animal groups and treatment

All the animal studies were approved in accordance to the guidelines for care and use of laboratory animals of National Institute of Health (NIH) (China). The Animal experiments and experimental animal welfare committee of Capital Medical University (CCMU) had approved our animal research. A number of 40 six-week-old Sprague Dawley (SD) male rats (200 ± 20 g) were brought from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and all of the rats were with specific pathogen free (SPF). Forthy (40) rats were assigned to four groups matched with weight randomly. Rats of four groups were treated with normal saline (control), SiNPs, MeHg and their combination (SiNPs + MeHg) by intratracheal instillation (once every 3 days for 10 times). The control group received equivalent volume of normal saline. The doses of SiNPs were converted according to the lowest dose (2 mg/kg body weight) of SiNPs in a rat inhalation study of short term (Du et al., 2013). The dose of MeHg (9.882 μg/kg body weight) used in this animal study was converted based on the National Ambient Air Quality Standard (NAAQS) (GB 3095-2012) (China). As to minimize the agglomeration, SiNPs were sonicated for 10 min before the intratracheal instillation.

2.4. Measurement of LDH release

The treatment groups for lactate dehydrogenase (LDH) measurement was designed as SiNPs (6.25 μg/mL), MeHg (0.5 μM), and SiNPs + MeHg (6.25 μg/mL + 0.5 μM), with pure culture medium as control group. After AC16 exposed to single treated (SiNPs or MeHg) and the co-exposure (SiNPs + MeHg) for 24 h, then 100 μL supernatant of culture medium was get for LDH activity measurement. Since the release of intracellular LDH is an indicator of the integrity of the cell membrane damage and cell death. Lactate dehydrogenase (LDH) kit (Jiancheng, China) was used to measure the LDH activity in culture medium according to the manufacturer's protocol. Supernatants of cell medium were