Ecotoxicology and Environmental Safety 163 (2018) 47–55

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Gene profiles to characterize the combined toxicity induced by low level co-exposure of silica nanoparticles and benzo[*a*]pyrene using whole genome microarrays in zebrafish embryos

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ARTICLE INFO

Keywords:
Silica nanoparticles
Benzo[*a*]pyrene
Genome microarrays
Combined toxicity
Zebrafish embryos

ABSTRACT

Several studies have suggested that air pollutants combine exposure have greater adverse effects. However, limited studies were available on the combined toxicity of silica nanoparticles (SiNPs) and benzo[*a*]pyrene (B[*a*]P). The study was to evaluate the toxic effect and mechanisms of low-dose exposure of SiNPs, B[*a*]P and co-exposure in zebrafish embryos. In this study, zebrafish embryos received intravenous microinjection of SiNPs and B[*a*]P, and then was used to select differentially expressed genes by microarray analysis. Multiple bioinformatics analyses and STC analysis were done to identify key genes, pathways and biological processes and the expression trend of genes in each group. 1) 3065 differentially expressed genes were identified in zebrafish embryos. 2) These differentially expressed genes were involved in multiple biological processes and cellular processes such as immunity, response to stimuli, cell proliferation, adhesion, signaling transduction, and embryonic development. 3) Dynamic Gene Network analysis was used to identify a subgroup of 26 core genes that involved in multiple biological processes and cellular processes. 4) Pathway analysis and Signal-net analysis indicated that the MAPK signaling pathway, calcium signaling pathway, p53 signaling pathway, PI3k/Akt signaling pathway, and several pathways associated with immune response were the most prominent significant pathways induced by co-exposure of SiNPs and B[*a*]P in zebrafish embryos. Our study demonstrated that the molecular actions of co-treated with SiNPs and B[*a*]P on the immune system, inflammatory process and cardiovascular development had more severe toxicity than single exposure.

1. Introduction

Air pollution has been closely linked with the increasing of cardiovascular disease. WHO reported that noncommunicable diseases (NCDs) kill about 41 million people each year, equivalent to 71% of all deaths globally, and cardiovascular diseases account for most NCD deaths, while study on the combined effects of the particulate matter (PM) components on cardiovascular system was still limited. The ambient particulate matter is usually a complex mixture of particles constituted by polycyclic aromatic hydrocarbon (PAH), volatile organic carbon (VOC), other elemental and organic and inorganic components, etc. (Chen et al., 2016). A great deal of studies has shown that the level of toxic effect of PM is related to the particle size. The Ultrafine particles (UFPs), aerodynamics diameter is less than 100 nm, which pose the greatest danger because of their high content of organic chemicals and prooxidative potential (Araujo et al., 2008). However, up to now, studies on co-exposure of UFPs and air pollutants induced the cardiovascular toxicity are very limited.

In recent years, nanotechnology environmental health and safety (nanoEHS) is getting more and more attention (Lin et al., 2017). Silica nanoparticles (SiNPs) as a representative component of earth shell and mineral dust, and are widely used to study atmosphere (Zhao et al., 2011). SiNPs are also as drug delivery carriers applied in biomedical fields through intravenous injection (Chakravarty et al., 2015). SiNPs have been found to pass through skin, blood-brain barrier, blood-placental barrier, and induce oxidative stress, DNA damage, and apoptosis (Nabeshi et al., 2011; Yamashita et al., 2011; Yoshida et al., 2011). Our previous studies demonstrated that the exposure of SiNPs induced

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https://doi.org/10.1016/j.ecoenv.2018.07.059
Received 27 February 2018; Received in revised form 12 July 2018; Accepted 15 July 2018

embryotoxicity, disturbed the heart formation and development by inhibiting the angiogenesis (Duan et al., 2016a, 2013; Guo et al., 2016). Thus, SiNPs have adverse effects on living organism.

The polycyclic aromatic hydrocarbons (PAHs) are produced from incomplete combustion of organic materials or fossil fuels. Among them, Benzo[*a*]pyrene (B[*a*]P) is highly toxic and the most commonly kinds of PAHs in environment (Knecht et al., 2017). It is reported that exposure to B[*a*]P could induce the significant changes in expression level of genes for regulating cardiovascular developmental defects (Huang et al., 2012). B[*a*]P also has been shown to promote the generation of DNA adducts or trigger DNA oxidative alterations (Baird et al., 2005). However, few studies had assessed the combined effects of SiNPs and B[*a*]P in living organism.

The zebrafish with high homology with human genes, its transparency and external developmental characteristics make it widely used in cardiovascular development and cardiovascular disease studies (Asnani and Peterson, 2014; Vargas and Vasquez, 2016). Genome-wide transcriptional analysis is widely used in many studies for assessing biomaterial nanotoxicity and analyzing the mechanism of disease (Stoughton, 2005). There is evidence to show that the zebrafish has been used to recapitulate a number of cardiovascular disease processes ranging from congenital heart defects to arrhythmia to cardiomyopathy (Fako and Furgeson, 2009).

Simultaneously, limited studies evaluated the combined toxicity of SiNPs and B[*a*]P by comprehensive genome-wide transcriptional analysis in zebrafish embryos. Previous study from our laboratory demonstrated that low dose level of SiNPs and B[*a*]P combination triggers inflammatory response and blood hypercoagulable in zebrafish (Duan et al., 2016b). To have a comprehensive understanding of combined effects triggered by SiNPs and B[*a*]P in vivo, we performed a genome-wide transcriptional analysis. This study provides a persuasive evidence for the transcriptional effects of combined environmental pollutants on living organism.

2. Materials and methods

2.1. Characteristics of SiNPs and B[a]P

The prepared method and characterized of SiNPs have been described as previously studied (Duan et al., 2013, 2014b). B[*a*]P (Purity is greater than 99%) was bought from Sigma Aldrich (St. Louis, MO, USA). The physical properties about SiNPs and B[*a*]P have been assessed and described detailedly in our previous works (Duan et al., 2016b).

2.2. Zebrafish husbandry

Zebrafish acquired from Hunter Biotechnology, the accreditation number is 001458. It is accredited by the International Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The zebrafish husbandry has been described detailedly in our previous studies (Duan et al., 2016b). All zebrafish embryos come from the same batch of eggs.

2.3. Exposure method

After Zebrafish embryos were anesthetized at 48 hpf, the different concentrations of SiNPs, B[*a*]P, and SiNPs + B[*a*]P were injected at the ventral end of the Duct of Cuvier (DC) under a stereomicroscope (Nikon, Japan) and the pulse time was controlled to deliver 10 nL of SiNPs, B[*a*]P, and SiNPs + B[*a*]P, respectively. The control group was replaced by ultrapure water of equal volume. Zebrafish embryos were moved to 6-well microplates and continue to train for 24 h after intravenous microinjection.

2.4. Malformation assessment

The NOAEL levels were confirmed according to the modified morphological assessments (Panzica-Kelly et al., 2010). As previously studied, 3 mg/mL of SiNPs and 0.125 mg/mL of B[*a*]P were chosen as the NOAEL dosage (Duan et al., 2016b). Zebrafish malformation was defined by estimating the morphological anatomic structures or organ systems. What's more, the cardiac toxicity phenotype such as heart rate, atria/ventricular ratio and pericardial edema were also assessed.

2.5. Microarray analysis

Total RNA was extract from 50 embryos per treatment group using TRIzol reagent (Invitrogen, Canada), which was purified with a RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The mRNA expression profiling found 59,302 gene-level probe sets by Zebrafish Gene 1.0 ST Array (Affymetrix Gene Chip®, USA). The microarray analysis was performed by Affymetrix® Expression Console Software. Three repetitions were used in microarray analysis.

2.6. Identify differentially expressed mRNAs

Random variance model (RVM) *t*-test was used to identify differential expression genes in microarray data analysis. RVMF-test was used to filter the differentially expressed genes in virtue of the advantage in small samples, it can improve the degree of freedom effectively (Clarke et al., 2008; Wright and Simon, 2003). $P < 0.05$ was the differential expression genes regulated up or down. These *p* values were adjusted by FDR (Reiner et al., 2003).

2.7. GO analysis

The primary function of differential genes was analyzed by Gene ontology (GO) analysis. Fisher's exact test and $\chi 2$ test were applied to classify the GO category, and p value was corrected by FDR (Dupuy et al., 2007), and $p < 0.05$ was seen as the standard of difference screening.

2.8. STC and STC-GO analysis

The gene expression time series and the most probable set of clusters generating the observed time series were profiled using the series test of cluster (STC). Due to different signal density change tendencies of genes under different situations, we clustered short time-series gene expression data to clear and definite some unique profiles. GO of significant STC cluster profiles were performed by the two-sided Fisher's exact test. $P < 0.05$ was seen as the significant GO categories.

2.9. Dynamic gene network analysis

A co-expression network was built using the normalized signal intensity of significant differential genes. The Pearson's correlation was used to filter the significant correlation gene pairs. The gene-gene interaction network was built according to the correlation between genes. In network, a node represents a gene, and the edges between genes describe their interactions (Prieto et al., 2008). Different nodes were marked with different degrees, respectively. The node usually has higher degree at the center of the network and stronger ability to regulate adjacent genes. In addition, k-core in graph theory was used to depict the characteristics of the network. According to the relationship between genes, they were divided into different subnetworks, and marked with different colour.

2.10. Pathway analysis

Pathway analysis is the interaction network of the significant