Toxicology 380 (2017) 94–103

Contents lists available at ScienceDirect

Toxicology

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

Role of oxidative stress and DNA hydroxymethylation in the neurotoxicity of fine particulate matter

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ARTICLE INFO

Article history:
Received 10 December 2016
Received in revised form 22 January 2017
Accepted 26 January 2017
Available online 30 January 2017

Keywords:
Fine particulate matter
Neurocytotoxicity
DNA hydroxymethylation
Oxidative stress
Neurite outgrowth
Synapse formation

ABSTRACT

Epidemiological studies have implicated fine particulate matter ($PM_{2.5}$) as a risk factor for neurodegenerative diseases and neurodevelopmental disorders. However, the underlying molecular mechanisms and the influences of different components remain largely elusive. Here, we extended our previous work to investigate the role of oxidative stress and DNA hydroxymethylation in neuronal pathology of $PM_{2.5}$. We found $PM_{2.5}$ and its extracts (water-soluble extracts, organic extracts and carbon core component) differentially caused cell cycle arrest, cell apoptosis and the cell proliferation inhibition in neuronal cells. These effects were mechanistically related to each other and oxidative stress, suggesting $PM_{2.5}$ and toxic compounds adsorbed on the particles may cause different types of brain damages. In addition, $PM_{2.5}$ and its organic extracts increased global DNA hydroxymethylation and gene-specific DNA hydroxymethylation of neuronal genes, and subsequently interfered with their mRNA expression. The impairments in neuronal progression characterized with decreased length of neurite and reduced mRNA expression of neuronal markers and synaptic markers. The blocking effects of antioxidants demonstrated the involvement of oxidative stress-mediated hydroxymethylation abnormalities in $PM_{2.5}$-induced defects in neurite outgrowth and synapse formation. Our results first revealed the role of oxidative stress-mediated abnormal DNA hydroxymethylation in neuronal impairments of $PM_{2.5}$, and thoroughly evaluated the neurocytotoxicity of different components.

1. Introduction

Epidemiological studies have shown the association of ambient particulate air pollution with diseases of the central nervous system (CNS), including stroke, Alzheimer's disease, Parkinson's disease, cognitive impairments and neurodevelopmental disorders (Calderón-Garcidueñas et al., 2015; Flores-Pajot et al., 2016). It is reported that fine particulate matter ($PM_{2.5}$) exposure was correlated to increased expression of markers of neurodegenerative disease pathologies (Calderón-Garcidueñas et al., 2015, 2016). Perinatal exposure to $PM_{2.5}$ and early life exposure to air pollution may increase the risk of children autism (Becerra et al., 2013; Wong et al., 2015). Although, it has been suggested that various components of particulate matter, such as ultrafine particles and chemical components can translocate to the CNS where they can activate adverse biological responses (Block and Calderón-Garcidueñas, 2009; Heusinkveld et al., 2016; Solaimani et al., 2016), the contribution of different components and the underlying molecular mechanisms for CNS pathology of $PM_{2.5}$ remain largely elusive.

Unlike most cell types, neurons are believed to have permanently blocked their capacity to proliferate once they are differentiated in adult nervous system (Frade and Ovejero-Benito, 2015). Nevertheless, recent evidence indicates the existence of a cell cycle reactivation in specific populations of neurons, and

Abbreviations: $PM_{2.5}$, fine particulate matter; CNS, central nervous system; ROS, reactive oxygen species; 5-hmC, 5-hydroxymethylcytosine; 5-mC, 5-methylcytosine; Tet, ten-eleven translocation; Pw, water-soluble extracts; Po, the organic extracts; Pc, the carbon core component; PAHs, polycyclic aromatic hydrocarbons; MEM/F12, Modified Eagle's medium/F12; FBS, fetal bovine serum; MAP-2, microtubule-associated protein 2; TUJ1, β3-tubuin; Pwo, the mixture of Pw and Po; NAC, *N*-acetylcysteine; GSH, glutathione; LDH, lactate dehydrogenase; PI, propidium iodide; 8-OHdG, 8-hydroxy deoxyguanosine.

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http://dx.doi.org/10.1016/j.tox.2017.01.017
0300-483X/

abnormal coordination of cell cycle would induce apoptotic cell death (Frade and Ovejero-Benito, 2015; Maity-Kumar et al., 2015). Cell cycle control has been known as an important etiological event in neurodevelopment process and neurodegenerative diseases (Atwood and Bowen, 2015). Neuronal tetraploidization resulting from aberrant cell cycle events has also been described as an early hallmark of neurodegeneration (Wang et al., 2009). Cell apoptosis is a critical response of neuron to infection, starvation, DNA damage and environmental stress. Collectively, these findings provide evidence that cell cycle, cell apoptosis and neuropathology are mechanistically related. In addition, reactive oxygen species (ROS) and oxidative DNA damage significantly have been related to the abnormal adjustment of cell cycle and apoptosis, as well as increase degeneration of neurons (Jin et al., 2014; Maity-Kumar et al., 2015). A better understanding the effects of $PM_{2.5}$ and its different components on neuronal cell cycle and apoptosis as well as their relationship with oxidative stress will enable to elucidate the mechanisms for adverse CNS impacts of $PM_{2.5}$.

Recently, it has been reported that 5-hydroxymethylcytosine (5-hmC), as a newly discovered modified form of cytosine, might serve unique biological roles in neurodevelopment, aging, neurodegenerative diseases and human cancer (Liang et al., 2016; Wang et al., 2012). 5-hmC can be converted from 5-methylcytosine (5-mC) by enzymatic oxidation reaction of ten-eleven translocation (Tet) proteins, and this modification is environmental sensitive, stable and highly enriched in the brain (Madrid et al., 2016; Wang et al., 2012). Researchers have showed that 5-hmC may be from 5-mC in normal DNA in response to oxidative stress, as oxidative stress can alter the activity of the Tet protein family members (Chia et al., 2011; Delatte et al., 2015). Several evidences have shown the global 5-hmC alterations of blood cells or airway epithelial cells in responses to inhalable particulate matter, arsenic, traffic-related air pollution and chemical allergens (Chapman et al., 2016; Niedzwiecki et al., 2015; Sanchez-Guerra et al., 2015; Somineni et al., 2016). Evidence from our laboratory indicated that $PM_{2.5}$ induced significant oxidative stress and disturbed DNA methylation (5-mC) patterns in neuronal cells (Wei et al., 2016). However, no study to date has evaluated the role of 5-hmC regulation in $PM_{2.5}$-associated neuronal pathology and their association with $PM_{2.5}$-induced oxidative stress.

Here, we extended our previous work to investigate whether $PM_{2.5}$ and its different extracts could exert their neurotoxicity by oxidative stress-mediated neurocytotoxicity and DNA hydroxymethylation regulation. The investigation of neurocytotoxicity mainly focused on cell cycle regulation and cell apoptosis. The analyses of the effects induced by different types of $PM_{2.5}$ extracts aimed to evaluate the neurotoxicity of different chemical compositions.

2. Material and methods

2.1. $PM_{2.5}$ collection

$PM_{2.5}$ sampling site was located in the vicinity of Chongqing South Road. Chongqing South Road is an elevated expressway with high traffic density in the city of Shanghai, China. It mainly allows non-commercial vehicles like cars (90%) to pass through, and there are about 10% commercial vehicles like bus running on the road. There are no industry pollution sources all around the road. $PM_{2.5}$ particles largely come from exhaust emissions of non-commercial vehicles with petroleum fuel. $PM_{2.5}$ samples were collected on quartz microfiber filters (1851-865, Whatman, 203mm × 254 mm) from 26th Nov. to 21st Dec. 2014 with large-volume $PM_{2.5}$ samplers (Intelligent 2031, Qingdao Laoying Inc., China). The collected filters were stored at −20 °C until extraction.

2.2. $PM_{2.5}$ and its extracts preparation

The preparation of $PM_{2.5}$ and its extracts are carried out as previously described (Wei et al., 2016). In briefly, the ultrasonic extraction technique was used to obtain the $PM_{2.5}$ suspensions. The pieces of $PM_{2.5}$ filters were put into sterilized ultrapure water, and were sonicated in an ultrasonic bath for 30 min at 300W below 20 °C. After repeat extraction with three times, the extracted liquids were filtrated with sterilized gauze to remove any possible fiber and then obtained $PM_{2.5}$ suspensions. All of the $PM_{2.5}$ suspensions were divided into two equal parts. Half of the suspension was freeze-dried under vacuum to obtain the $PM_{2.5}$ (the whole particle of $PM_{2.5}$). The other half of the suspensions was used for the preparation of the water-soluble extracts (Pw), the organic extracts (Po) and the carbon core component (Pc) in turn. The prepared samples were weighed and stored at −20 °C. Then we calculated the proportions of every type of extracts. In the current work, the proportions of Pw, Po, and Pc were approximately 50%, 15% and 35%, respectively.

The chemical components of $PM_{2.5}$ and its extracts were analyzed by inductively coupled plasma-mass spectrometry (ELAN DRC II, PerkinElmer, America) and inductively coupled plasma atomic emission spectrometry (SPS-8000, Leeman, USA) for 23 types of metals, and gas chromatography-mass spectrometer (Agilent7890/5975C, Agilent Technologies, USA) for 16 types of polycyclic aromatic hydrocarbons (PAHs). The chemical characteristics of $PM_{2.5}$ and its different extracts are presented in our previous reports (Wei et al., 2016). Overall, the Pw contained both metals with high solubility/bioavailability (Se, Mo, K, Rb, Zn, Ca, Sr, Cs, As and Cd) and PAHs with low number of rings (NAP, PYR, FLU and BaA). Po mainly comprised PAHs with high number of rings (DBA, PHE, BPE, IPY, BaP, BbF, BkF and CHR) with high oxidation potential. Pc was mainly composed of metals with low solubility/bioavailability (Ti, Al, Fe, Pb, Cr, Ba, Cu, Na, Ni, V and Mn).

2.3. Cell culture and treatment

SH-SY5Y human neuroblastoma cell line was purchased from Shanghai Institute for Biological Sciences, Chinese Academy of Sciences. The cells were cultured in Modified Eagle's medium/F12 (MEM/F12) supplemented with 10% fetal bovine serum (FBS), 50 IU/mL penicillin, 50 μg/mL streptomycin and 2 mM glutamine (GlutaMAX™ as donor). The SH-SY5Y cells were 100% positive for neuronal markers microtubule-associated protein 2 (MAP-2) and β3-tubulin (TUJ1) (Supplementary material 1: Fig. S1). 60%–80% confluent cells were treated with $PM_{2.5}$, Pw, Po, Pc and Pwo (the mixture of Pw and Po) dispersed in MEM/F12 medium with 10% FBS. For cell viability assay, the cells were treated for 72 h with 0, 2.5, 5, 10, 20, 40, 80, 160 and 320 μg/mL $PM_{2.5}$. The concentrations of Pw, Po, Pc and Pwo were calculated by multiplying $PM_{2.5}$ concentration by their individual proportions (Pw, 50%; Po, 15%; Pc, 35%). Thus, the cells were treated for 72 h with 0, 1.25, 2.5, 5, 10, 20, 40, 80 and 160 μg/mL Pw; 0, 0.375, 0.75, 1.5, 3, 6, 12, 24 and 48 μg/mL Po; 0, 0.875, 1.75, 3.5, 7, 14, 28, 56 and 112 μg/mL Pc. For Pwo treatment, the doses were the combination of Pw and Po with series concentrations. The oxidative stress mechanism was evaluated by using antioxidants *N*-acetylcysteine (*NAC*; 5 mM) and glutathione (GSH, 10 mM). The inhibitors were co-applied with 80 μg/mL $PM_{2.5}$ for 72 h.

2.4. CCK-8 assay

Cell viability was measured by CCK-8 assay. Briefly, SH-SY5Y cells were seeded in 96-well plate in MEM/F12 medium supplemented with 10% FBS. After treatment, the cells were incubated with CCK-8 (20 μL) for 2 h. Then, the culture plate was