JOURNAL OF ENVIRONMENTAL SCIENCES XX (2017) XXX–XXX

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JES JOURNAL OF ENVIRONMENTAL SCIENCES www.jesc.ac.cn

Abnormal energy metabolism and tau phosphorylation in the brains of middle-aged mice in response to atmospheric $PM_{2.5}$ exposure

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ARTICLE INFO

Article history:
Received 30 March 2017
Revised 28 June 2017
Accepted 29 June 2017
Available online xxxx

Keywords:
Fine particulate matter ($PM_{2.5}$)
Mitochondrial structure and function
Energy metabolism
Reactive oxygen species (ROS)
Tau phosphorylation

ABSTRACT

In light of the accelerated aging of the global population and the deterioration of the atmosphere pollution, we sought to clarify the potential mechanisms by which fine particulate matter ($PM_{2.5}$) can cause cognitive impairment and neurodegeneration through the alteration of mitochondrial structure and function. The results indicate that $PM_{2.5}$ inhalation reduces ATP production by disrupting the aerobic tricarboxylic acid (TCA) cycle and oxidative phosphorylation, thereby causing the hypophosphorylation of tau in the cortices of middle-aged mice. Furthermore, excessive ROS generation was involved in the impairment. Interestingly, these alterations were partially reversed after exposure to $PM_{2.5}$ ended. These findings clarify the mechanism involved in mitochondrial abnormality-related neuropathological dysfunction in response to atmospheric $PM_{2.5}$ inhalation and provide an optimistic sight for alleviating the adverse health outcomes in polluted areas.

Introduction

Population aging is a pervasive and unprecedented phenomenon that cannot be overlooked. People aged 60 years and older are estimated to become approximately 22% of the global population and to exceed the number of young people for the first time in history by 2050 (World Population Ageing: 1950–2050. Population Division, DESA, United Nations, 2006). As a result, the health problems of aging individuals have become an important social issue. Among these, the number of individuals with illnesses associated with cognitive impairment such as dementia, including Alzheimer's disease (AD) and Parkinson's disease (PD), has reached 24 million and is predicted to quadruple by the year 2050 (Reitz and Mayeux, 2014). An epidemiological investigation showed that approximately 40% of people aged 85 years and over suffer from AD and 10% suffer from PD (de Lau and Breteler, 2006; Ferri *et al.*, 2005). While the majority of AD and PD cases are sporadic, inheritable genetic mutations play a small role in their etiologies (Dosunmu *et al.*, 2007; Heusinkveld *et al.*, 2016). Environmental factors such as pesticides, metals, and atmospheric pollutants have been postulated to contribute to the pathogenesis of AD and PD (Yan *et al.*, 2016a, 2016b; McAllum and Finkelstein, 2016; Block and Calderón-Garcidueñas, 2009). Exposure to atmospheric fine particles ($PM_{2.5}$) has been linked with numerous diseases, both local (in the lung) and systemic (in extra-pulmonary sites, such as cardiovascular and neuronal tissues) (Liu *et al.*, 2017; Kioumourtzoglou *et al.*, 2016). A lifespan study found that atmospheric pollution is associated with the quantifiable impairment of brain development in young people and with cognitive decline in the elderly (Clifford *et al.*, 2016). A meta-analysis based on 108 papers

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http://dx.doi.org/10.1016/j.jes.2017.06.037
1001-0742/

Please cite this article as: Gao, R., et al., Abnormal energy metabolism and tau phosphorylation in the brains of middle-aged mice in response to atmospheric $PM_{2.5}$ exposure, J. Environ. Sci. (2017), http://dx.doi.org/10.1016/j.jes.2017.06.037

revealed a higher risk of death for older populations (0.64%) than for younger populations (0.34%) per 10 μg/m^3 increase in particulate matter (PM) (Bell *et al.*, 2013). Although many epidemiological studies have described the adverse effects of $PM_{2.5}$ on cognitive function in middle-aged people (Ailshire and Crimmins, 2014; Schikowski *et al.*, 2015), there have been limited laboratory investigations into the mechanism of injury. Casanova reported that long-term $PM_{2.5}$ exposure might accelerate the loss of both gray matter and white matter in the brains of older women (Casanova *et al.*, 2016). However, whether the molecular targets of neurological degenerative damage are influenced by $PM_{2.5}$ and whether these effects can be reversed remain unclear.

Tau is a microtubule-associated protein (MAP) primarily responsible for the assembly, maintenance and stability of microtubules. The abnormal hyperphosphorylation of tau, a hallmark of neurodegenerative illnesses (particularly AD), can decrease the stability of microtubules and disrupt cytoskeletal integrity and axonal transport (Lovestone and Reynolds, 1997; Cowan *et al.*, 2010). Recent studies have revealed an increased trend of tauopathy not only in older patients with AD (Kehoe *et al.*, 2016; Hamasaki *et al.*, 2016) but also in cognitively normal middle-aged people (potentially primary age-related tauopathy) (Crary *et al.*, 2014; Lockhart *et al.*, 2016). Emerging evidence has revealed that abnormal glucose metabolism and decreased adenosine triphosphate (ATP) production are correlated with tau hyperphosphorylation in AD patients (Szablewski, 2017; Hoyer and Lannert, 2007). Due to the high energy demands of the brain, central nervous system (CNS) functions are strongly dependent on sufficient mitochondrial production of ATP. The aging mitochondrial theory suggests that ATP deficiencies play a critical role in the accelerated aging disorders of the elderly (Scheibye-Knudsen, 2016). Thus, the aim of our present study was to clarify the possible mechanisms by which $PM_{2.5}$ causes tau phosphorylation-related cognitive impairment and neurodegeneration by affecting mitochondrial structure and function.

1. Materials and methods

1.1. $PM_{2.5}$ sampling and preparation

$PM_{2.5}$ was collected between November 2014 and February 2015 at Shanxi University (112°21–34′E longitude, 37°47–48′N latitude), Taiyuan City, Shanxi Province, China. A medium-volume air sampler (TH-150CIII, Wuhan, China) with a flow rate of 100 L/min was placed on the top of a building far from obstacles to obtain free-moving air. Samples were collected on quartz filter membranes (Φ90 mm, Munktell, Sweden) for 22 hr/d and then extracted in Milli-Q deionized water with ultrasonic processing more than three times. After vacuum freeze-drying, the samples were resuspended in sterilized 0.9% physiological saline. The aqueous suspension was pooled, frozen at −20°C and swirled for 10 min before being used for animal experiments.

1.2. Animals and $PM_{2.5}$ exposure

Female 10-month-old C57BL/6 mice were purchased from the Junke Biological Engineering Co., LTD (Nanjing, China). These mice were housed under standard conditions (24 ± 2°C, 50 ± 5% humidity, 12:12 hr light:dark cycle) and randomly divided into a $PM_{2.5}$-treated group and a control group. Mice in the $PM_{2.5}$ treatment group received oropharyngeal aspirations of $PM_{2.5}$ at 3 mg/kg after anesthetization with isoflurane (Yi Pin Pharmaceutical Co., Ltd., Hebei, China) every other day at different times. The four treatment groups included groups exposed for 2 weeks, exposed for 4 weeks, allowed to recover for 1 week after being exposed for 4 weeks, and allowed to recover for 2 weeks after being exposed for 4 weeks. Mice in the control group were treated with 0.9% saline (processed by the ultrasonic oscillation of the control membrane filter) using the same method. Mice were sacrificed, and their brain cortices were separated 24 hr after the last exposure. The cortices were immediately frozen in liquid nitrogen and then stored at −80°C until further use. All animal experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Shanxi University.

1.3. Transmission electron microscope (TEM) observation

The brain cortex tissues in mice were rapidly cut into pieces approximately 1 mm^3 and was fixed in stationary liquid. Then pieces were washed and *en bloc* stained with 1% uranyl acetate in 50% ethanol at room temperature in the dark, dehydrated by graded ethanol, and embedded in beam capsules. The 70–80-nm-thick ultrathin sections cut from the embedded tissue were collected onto grids, and then were stained with uranyl acetate and lead citrate. The mitochondrial structural were observed with an electron microscope (JEOL, JEM 1400, Japan).

1.4. Determination of the ATP content

The ATP levels were determined by the ATP detection kit according to the manufacturer's instructions (Beyotime, China). Approximately 30 mg cortex tissues were used with 240 μL lysis buffer for ATP releasing. After high speed centrifuged, the ATP content in supernatant was measured by the luciferin-luciferase method with a Thermo Scientific Varioskan Flash (Thermo Fisher Scientific, USA). The protein concentration was determined with Coomassie light blue according to the Bradford (1976). Finally, the ATP content normalized to protein concentrations were used for comparative analysis.

1.5. Real-time quantitative reverse transcription-polymerase chain reaction(PCR)

Approximately 30 mg of cortex tissue were used for total RNA extraction with TRIzol reagent (Invitrogen, USA). The RNA concentration was quantified with a NanoDropTM 2000C (Thermo, USA). Then the extracted RNA completes reverse transcription to form the first-strand complementary DNA (cDNA) using a reverse transcription kit (TaKaRa, China). The mRNA expression levels of tricarboxylic acid (TCA) cycle- and oxidative phosphorylation-related genes were determined by real-time polymerase chain reaction (PCR) on a qTOWER 2.2 real-time PCR system (Analytik Jena AG, Jena, Germany). The

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