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$PM_{2.5}$, SO_2 and NO_2 co-exposure impairs neurobehavior and induces mitochondrial injuries in the mouse brain

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HIGHLIGHTS

- $PM_{2.5}$, SO_2, and NO_2 co-exposure impairs spatial learning and memory.
- Apoptosis participates in co-exposure induced abnormal neurobehavior.
- $PM_{2.5}$, SO_2, and NO_2 co-exposure causes the mitochondrial dysfunction.
- Mitochondrial fission/fusion genes are important regulators.

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ABSTRACT

Air pollution is a serious environmental health problem that has been previously associated with neuropathological disorders. However, current experimental evidence mainly focuses on the adverse effects of a single air pollutant, ignoring the biological responses to the co-existence of these pollutants. In the present study, we co-exposed C57BL/6 J mice to $PM_{2.5}$, SO_2 and NO_2 and explored their neurobehavior, histopathologic abnormalities, apoptosis-related protein expression and mitochondrial dysfunction. The results indicate that co-exposure to $PM_{2.5}$, SO_2 and NO_2 impaired spatial learning and memory and caused abnormal expression of apoptosis-related genes (p53, bax and bcl-2). Additionally, these alterations were related to morphological changes in mitochondria, a reduction of ATP, the elevation of mitochondrial fission proteins and the downregulation of fusion proteins. These findings provide a basis for the understanding of mitochondrial abnormality-related neuropathological dysfunction in response to co-exposure to ambient air pollutants, which suggests an adaptive response to the frangibility of the central nerve system.

1. Introduction

Ambient air pollution is a complex mixture of pollutants that includes both particulate and gaseous components, and global concern over its public health impact has been increasing. Among these gradients, fine particulate matter ($PM_{2.5}$), which contains multiple chemical components that can vary dynamically with time and space, has been attracting more and more attention. $PM_{2.5}$ can be generated directly by combustion or mechanical processes and indirectly by the condensation of aerosol precursor gases (Lee et al., 2015). Water-soluble ions generally contribute to a large fraction of aerosols, and a number of field studies have been carried out to characterize water-soluble ions in fine aerosols (i.e., $PM_{2.5}$) in the past decade (Ocskay et al., 2006; Gao et al., 2012). However, sulfur dioxide (SO_2) and nitrogen oxides (NO_X) are the precursors of the formation of sulfate and nitrate PM, including acid and non-acid aerosols (secondary water-soluble aerosols) in the atmosphere (EPA, 2000), and secondary water-soluble ions (i.e., SO_4^{2-}, NO^{3-} and NH^{4+}) constituted a major portion of $PM_{2.5}$ (i.e., 40%–57%) in eastern China (Gao et al., 2012). Therefore, $PM_{2.5}$ and the gaseous pollutants SO_2 and NO_2 coexist in the atmosphere and play important roles in the process of secondary aerosol pollution.

Increasing numbers of epidemiological studies have indicated that air pollutants not only cause increased morbidity of respiratory diseases but are also associated with neuropathological

Abbreviations: $PM_{2.5}$, fine particulate matter; SO_2, sulfur dioxide; NO_X, nitrogen oxides; HE, hematoxylin-eosin; PBS, phosphate buffer saline; TEM, transmission electron microscopy; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; NC, nitrocellulose; BSA, bovine serum albumin; Mfn, mitofusin; OPA1, optic atrophy 1; Fis1, mitochondrial fission protein 1; Drp1, dynamin-related protein 1.

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dysfunction (Block and Calderón-Garcidueñas, 2009; Guxens and Sunyer, 2012; Ranft et al., 2009; Calderón-Garcidueñas et al., 2015). Specifically, prolonged exposure to particulate matter has the potential to change the brain's inflammatory phenotype and facilitate the development of early Alzheimer's disease-like pathology (Field et al., 2010; Smith et al., 2012). SO_2 exposure disturbed neurobehavior and repressed memory-related kinase activation and glutamate receptor gene expression via neuroinflammation in rats and tended to cause neurodegeneration (Hong et al., 2002; Szyszkowicz et al., 2009; Smith et al., 2012; Yao et al., 2015). Additionally, an increased risk of nervous diseases, including neurodegenerative disorders, vascular dementia, and autism spectrum disorder, was induced by another important air pollutant, NO_2 (Zhu et al., 2012; Li and Xin, 2013; Jung et al., 2013; Kim et al., 2014). These findings highlight the contribution of $PM_{2.5}$, SO_2 and NO_2 to air pollution-related neurological dysfunction.

Neuronal apoptosis commonly occurs in neurological diseases, and the process is hallmarked by p53, the Bcl-2 family, c-myc and other factors. Mitochondria exist as a dynamic network and play crucial roles in numerous biochemical functions, including energy production, apoptosis induction, calcium signaling and cell growth (Hoppins, 2014). The activation of the mitochondrial pathway is an important indicator of the occurrence of apoptosis (Sassone et al., 2013; Peng et al., 2015). In vivo, mitochondria form elongated tubules that constantly undergo fission and fusion to maintain an interconnecting network (Hoppins, 2014), which are essential for maintaining normal cell functions (Westrate et al., 2014). A tightly controlled balance between fusion and fission is of utmost significance in the high energy-demanding biological process, which has a strong association with brain development and neurodegenerative diseases (Rousset et al., 2012). Mitochondrial dysfunction has been observed following individual exposure to SO_2, NO_2, black carbon and $PM_{2.5}$ (Qin et al., 2012; Meyer et al., 2013; Colicino et al., 2014; Yan et al., 2015). However, little is known about whether co-exposure to air pollutants could trigger mitochondrial dysfunction and neurobehavior impairment.

In this study, we exposed C57BL/6 J mice to $PM_{2.5}$, SO_2 and NO_2 and determined spatial learning and memory, histopathologic abnormality, apoptosis-related protein expression and mitochondrial dysfunction (mainly including mitochondrial ultrastructure, ATP content and mitochondrial fission/fusion) in the brain. The study attempted to provide experimental evidence for the increased risk of neurological disorders in response to co-exposure to ambient air pollutants.

2. Materials and methods

2.1. $PM_{2.5}$ sampling

$PM_{2.5}$ were acquired with middle volume air samplers (TH-150CIII and TH-150C, Wuhan, China) from Shanxi University (112°57′E longitude, 37°73′N latitude), Taiyuan City, Shanxi Province of China. The middle volume air samplers were set on the top of the living area of a building far from obstacles to obtain sufficient free-moving air. The flow rate of the sampler was 100 L min^{-1}, and the sampling time was 22 h each day during the period from November 2013 to February 2014. The PM samples were collected onto a quartz filter membrane (Φ90 mm, Munktell, Sweden), and a $PM_{2.5}$ suspension was prepared by soaking filters with $PM_{2.5}$ in Milin-Q deionized water, followed by immersion and ultrasonic vibration more than three times. Then, the $PM_{2.5}$ suspension was processed with a vacuum freeze-drying technique. The dried samples were diluted with sterilized 0.9% physiological saline and swirled for 10 min before using them for animal experiments, and an aqueous suspension was pooled and frozen at −20 °C.

2.2. Animal treatment

Healthy male C57BL/6 mice (8 weeks old) were obtained from Beijing HFK Bioscience Co., LTD (Beijing, China). The mice were housed under standard conditions (24 ± 2 °C, 50 ± 5% humidity, and 12:12 h light:dark cycle). After one week of adaptation, the mice were randomly divided into three groups. For the control group, mice were exposed to filtered air (6 h/d, 28 d) and received an oropharyngeal aspiration of saline every other day. For the lower concentration group, mice were treated with 0.5 mg m^{-3} SO_2 and 0.2 mg m^{-3} NO_2 via dynamic inhalation simultaneously (6 h/d, 28 d) followed by oropharyngeal aspiration of $PM_{2.5}$ 1 mg kg^{-1} every other day. For the higher concentration group, mice were exposed to 3.5 mg m^{-3} SO_2, 2 mg m^{-3} NO_2 and 3 mg kg^{-1} $PM_{2.5}$ using the same protocol. When not being treated, the animals had free access to water and standard feed. Mice were sacrificed 18 h after the final exposure. The brains were excised, quick frozen in liquid nitrogen, and stored at −80 °C. All of the animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the Ministry of Health of the People's Republic of China. The protocol was approved by the Shanxi University's Institutional Animal Care and Use Committee (Approved Animal Use Protocol Number: HZ20140503).

For dynamic inhalation, the animals were placed in a dynamic inhalation chambers without anaesthetization. SO_2 and NO_2 gases were diluted with fresh air at the intake port of the chambers to yield the desired concentrations, and the diluted gases were evenly distributed across the whole chamber by two perforated gas radiant plates, with one located in the intake port and the other connected to a gas outlet matched with an aspirator pump. The concentrations of SO_2 and NO_2 within the chambers were measured using pararosani-line hydrochloride spectrophotometry at 577 nm (Goyal, 2001) and the Saltzman colorimetric method at 540 nm (Kumie et al., 2009), respectively. For $PM_{2.5}$ administration, we used the oropharyngeal aspiration technique (Rao et al., 2003). Briefly, the mice were anesthetized with isoflurane (Yi Pin Pharmaceutical Co., Ltd., He Bei, China) and placed on a slant board, and the tongue was gently held at full extension while a 20-μL suspension of particles was pipetted onto the base of the tongue. For the control group, ambient air was cleaned with an air filter consisting of multilayer synthetic fibers to remove particles.

2.3. Behavioral tests

Morris water maze studies with mice have principally been performed to measure spatial-based learning and memory. A circular tank (diameter 100 and 75 cm in high) was filled with water rendered opaque by the addition of white, non-toxic paint. A circular platform with a diameter of 15 cm was placed 1 cm below the water surface in the center of a specific quadrant of the tank. The animals were given training sessions using visible and invisible platforms for a period of 8 consecutive days (8 sessions in total). In the non-spatial training, the mice received 3 days of training to find the same platform elevated above the water surface. Then, invisible platform training was performed continuously for 5 identical daily sessions with 4 trials per day. For each trial, the mouse was released from the wall of the tank and allowed to search, find, and stand on the platform for 10 s within the 60-s trial period. If an animal failed to reach the platform, it was gently guided to the location and was allowed to stay on it for 10 s. For each training session, the sequence designed for the animals' training was determined in a random manner that varied each day so that it was different between the separate sessions for each animal and was different for individual animals. Using an Etho Vision video tracking device (Noldus, Wageningen, Netherlands), we recorded the latency in reaching the