Journal of Hazardous Materials 369 (2019) 180–190

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Ambient PM2.5 caused depressive-like responses through Nrf2/NLRP3 signaling pathway modulating inflammation

Chen Chu[a], Haiya Zhang[b], Shijie Cui[c], Bin Han[a], Lixiao Zhou[a], Ning Zhang[a], Xuan Su[a], Yujie Niu[b,c], Wen Chen[d], Rui Chen[e], Rong Zhang[a,c,*], Yuxin Zheng[f,**]

[a] Department of Toxicology, Hebei Medical University, Shijiazhuang, 050017, PR China
[b] Deportment Occupational Health and Environmental Health, Hebei Medical University, Shijiazhuang, 050017, PR China
[c] Hebei Province Key Laboratory of Environment and Human Health, Shijiazhuang, 050017, PR China
[d] Department of Toxicology, School of Public Health, Sun Yat-sen University, Guangzhou, 510080, PR China
[e] Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Dingjiaqiao 87, Nanjing, 210009, PR China
[f] Department of Toxicology, Public Health College, Qingdao University, 266000, Qingdao, PR China

ARTICLE INFO

Keywords:
PM2.5
Depression
Inflammation
NLRP3
Nrf2

ABSTRACT

PM2.5 pollution has been associated with numerous adverse effects including cardiovascular, respiratory and metabolic diseases as well as emotional disorders. However, the potential mechanism has not known clearly. Twenty-four rats were divided into 3 groups and exposed to various airs: filtered air (FA), unfiltered air (UA) and concentrated PM2.5 air (CA), respectively. Thirty wild type (WT) and 30 Nrf2 knockout (KO) mice were divided into 2 groups and exposed to FA and UA, respectively. The changes of neurobehavioral function, neurotransmitter secretion, toxic elements deposition, oxidative stress and the inflammation in prefrontal cortex were investigated during 9–12 weeks with/without PM2.5 exposure. Results showed that CA rats and KO-UA mice emerged obviously depressive-like responses. Li, Be, Al, Cr, Co, Ni, Se, Cd, Ba, Ti and Pb could deposit in the prefrontal cortex of rats after PM2.5 exposure. The neurotransmitters were significantly disorder in prefrontal cortex of CA rats. The NLRP3 signaling pathway was more activated in $Nrf2^{-/-}$ than WT mice after PM2.5 exposure for 9 weeks. Nrf2/ NLRP3 signaling pathway modulating the inflammation might play an important role in the depression induced by ambient PM2.5.

1. Introduction

Ambient particulate matter 2.5 (PM2.5) poses a significant risk to public health worldwide and the PM2.5 pollution has been associated with numerous adverse health effects [1]. World Health Organization (WHO) reports that in 2012 around 7 million people died - one in eight of total global deaths - as result of air pollution exposure. According to the WHO global burden of disease (GBD) study, ambient PM2.5 was responsible for 3.2 million premature deaths worldwide annually, and 1.2 million premature deaths in China alone [2]. These data suggest that PM2.5 is now one of the world's largest environmental health risks. Therefore, the WHO ambient air quality guidelines suggest an annual mean PM2.5 concentration limit of 10 μg/m^3 and 25 μg/m^3 for the 24-hourly mean to prevent the adverse effects of PM2.5 on public health [3].

Recent study had reported adverse associations between air pollution exposures and the nervous system [4]. Increases in PM2.5 exposure was associated with depression, emotional symptoms and suicide attempts or elevated anxiety [5,6]. Consistent with these findings, time-series studies examining acute associations had reported increased depression-related hospital admissions with increasing pollution levels [7–9]. A significant association between PM2.5 and hospital admission for mental and behavioral disorders was found in Shijiazhuang, as one of the most polluted cities in China [10]. There was association between chronic exposures to PM2.5 and onset of depression among cohorts in the US [11,12].

However, the current insufficient epidemiological evidences are hard to explain the mechanism which would hold back the treatment and prevention of psychological diseases and mental disorders induced by PM2.5 exposure. PM2.5 has complex constituents including heavy

* Corresponding author at: Dept. Toxicology, Hebei Medical University, 361 Zhongshan east Rd., Shijiazhuang, Hebei 050017, PR China; Dept. Toxicology, Public health College, Qingdao University, 266000, Qingdao, PR China.
** Corresponding author at: Dept. Toxicology, Public health College, Qingdao University, 266000, Qingdao, PR China.
E-mail addresses: rongzhang@hebmu.edu.cn (R. Zhang), yxzheng@qdu.edu.cn (Y. Zheng).

https://doi.org/10.1016/j.jhazmat.2019.02.026
Received 4 August 2018; Received in revised form 6 February 2019; Accepted 8 February 2019
Available online 10 February 2019
0304-3894/ © 2019 Elsevier B.V. All rights reserved.

metals and PAHs [13], which towards to result in depressive responses and increased hippocampal pro-inflammatory cytokines [14]. The nucleotide-binding domain and leucinerich repeat protein 3 (NLRP3) inflammasome is reported as a central mediator by which psychological and physical stressors could contribute to the development of depression [15].

In the literature, NLRP3 had been associated with oxidative stress [16]. After phagocytosing exogenous particles, macrophages could generate a large amount of reactive oxygen species (ROS) [17], which in turn was known to play an important role in depression [18,19]. Excessive ROS could bind to the inflammasome assembly and finally activated NLRP3 inflammasome [17]. PM2.5 extracts could induce inflammatory responses through NLRP3 inflammasome pathway activating then mediate ROS generation and trigger the following oxidative stress [20].

Nuclear factor E2-related factor-2 (Nrf2) is an essential transcription factor that mediates cellular antioxidant responses. PM2.5 exposure significantly increased the expressions of Nrf2 and Nrf2-regulated antioxidant genes then performed anti-oxidative stress effect [21]. Nrf2-mediated defenses could be activated by PM2.5-induced ROS and many functions as signaling molecules [22]. Though there were evidences of the oxidative stress and inflammation response to depression induced by PM2.5 [23,24], the interaction mechanism of oxidative stress and inflammation regulated by Nrf2 has not been known clearly. Nrf2 signal pathway took responsibility to PM2.5-caused hypothalamus inflammation [25] and its critical proinflammatory effect was mediated by NLRP3inflammasome activation. The NLRP3 inflammasome contains a C-terminal LRR, a central NACHT domain, and an N-terminal pyrin domain (PYD). NLRP3 binds to the adaptor protein, apoptosis speck-like protein, containing a CARD domain (ASC) which in turn recruits and activates Caspase-1. Biochemical analysis revealed that Nrf2 appeared in the ASC-enriched cytosolic compartment after NLRP3 inflammasome activation [26]. Therefore, the Nrf2/ NLRP3 signal regulatory oxidative stress and inflammation interaction mechanism was hypothesized to explain the depressive-like responses induced by sub-chronic PM2.5 exposure.

Here, sub-chronic PM2.5 exposure animal models were established to explore the depression and it's perhaps mechanism. The evaluation of trace or toxic elements deposition in brain partly explained the direct reason of depressive-like responses in rats. Nrf2 knockout mice were used to study the regulating role of Nrf2/ NLRP3 signaling pathway modulating inflammation on the depression induced by ambient PM2.5. The present study suggested toxic elements deposited in prefrontal cortex after PM2.5 exposure. And PM2.5 exposure triggered Nrf2/ NLRP3 signaling pathway mediating inflammation, then resulting in depressive-like responses.

2. Materials and methods

2.1. Whole-body ambient inhalational protocol

Male, pathogen-free Sprague-Dawley rats at 6 weeks of age were purchased from the Animal Experimental Center of Hebei Medical University and acclimated for a week before the commencement of inhalation. The humidity was 50% and the temperature was 25–26 °C in the cages with a 12 h light/dark cycle. Twenty-four rats were divided randomly into 3 groups and exposed to filtered air (FA), unfiltered air (UA) and concentrated PM2.5 air (CA) by real time ambient particulate matter exposed chambers, respectively. The FA, UA and CA rats were exposed to clean air in the chamber with HEPA-filters, atmosphere in the chamber without HEPA-filters and concentrated PM2.5 atmosphere, respectively. The exposure time was 6 h per day, 6 days per week from May 1st, 2017 to July 24th, 2017 at a total of 12 weeks. $Nrf2^{-/-}$ (KO) mice were used to explain the effects of Nrf2 signaling pathway on the depression induced by ambient PM2.5. Thirty male wild type (WT) and 30 male $Nrf2^{-/-}$ (KO) mice at 6-week age were divided randomly into two groups, respectively. WT-FA and KO-FA mice were exposed to clean air whereas WT-UA and KO-UA mice were exposed to unfiltered air, respectively. The mice were exposed for 6 h per day, 6 days per week from May 1st, 2017 to July 3th, 2017 at a total of 9 weeks. Animals were housed in chamber with HEPA-filters after exposure. Mice and rats began a series of behavioral testing at 8th and 11th week and then executed (Fig. S1A). The present protocol was approved by the Committee of the Ethics Animal Experiments of Hebei Medical University (IACUC-Hebmu-20170116) and carried out under the institutional guidelines for ethical animal use.

2.2. The exposure equipment and parameters monitor

The concentrated PM2.5 was generated using a PM2.5 concentration enrichment system (VACES) with a concentration monitor (HRH-7886, Beijing Huironghe Technology Co., Ltd. Beijing, China, Fig. S1B), which located at Shijiazhuang, China. The surroundings of exposure equipment is near a major traffic arterial (Black line in Fig. S1C), 2.4 km north of the local National Air Pollutant Surveillance (NAPS) monitoring stations (Point A), and 1.1 km north of a pharmaceutical factory (Point B) as well as 2.1 km north of a coal-fired power plants (Point C).

The meteorological condition inside the chambers was closely monitored to keep a relatively constant temperature (20–25 °C), humidity (40–60%), ventilation frequency (18–20/h) and noise (30–35 dB). The concentrations of ambient PM were monitored using an Aerosol Detector DUSTTRAKTM II-Model 8530 (TSI Instrument, Shoreview, MN) and the spectrum of particle size was analyzed by an Aerodynamic Particle Sizer (APS) Spectrometer 3321 (TSI Instrument, Shoreview, MN). During the exposure periods, we collected the PM2.5 on the Teflon filter by High-Volume Air Samples (The Thermo Scientific, Franklin, MA [27] and then PM2.5 was extracted. Briefly, the filters were cut into small pieces and taken into a 50 mL centrifuge tube. After an ultrasonic processing in ice cold water for 30 min, then shaking for 20 min on a shaker and repeating the processes three times, the extracts were passed sequentially through a sterile 40 μm nylon filter (Corning Life Sciences, Corning, NY) and dried by lyophilization [28]. The PM 2.5 extraction powder was stored in −20 °C until used. The levels of NO_2, SO_2 and O_3 in chambers were monitored by the gas detector GasAlertMicro 5 PID (BW Canada) during the exposure to compare the differences among the different chambers.

2.3. Behavioral tests

2.3.1. Open field test (OFT)

OFT detects hyperactivity by movement and anxious behavior. A 60 cm × 60 cm × 25 cm square black box was used to rat and a 30 cm × 30 cm × 25 cm square white box was used to mice. Each rat or mouse was placed in the box and observed for five minutes. The amount of traveling time and distance in the center area of the maze were recorded [29].

2.3.2. Novelty suppressed feeding test (NSF)

An open-field arena (60 cm × 60 cm × 25 cm) with several pellets of food placed on a piece of white paper (10 × 10 cm) in the center was used to detect the exploratory behavior. The rats were fasting for 24 h before the test. Next day, the rat was individually placed in a corner of the cage. The latency that the rats approached to foods and began to eat was recorded. The observed maximum time was for 10 min [29].

2.3.3. Sucrose preference test (SPT)

SPT was used to evaluate the anhedonia of animals. Each rat was isolated in one cage with two bottles. One bottle is containing water and another is containing 3% sucrose solution. The rats could choose the two bottles freely. The intakes from each bottle were checked daily for total 3 days. The bottles were shifted daily throughout the test to avoid potential side preferences [30]. The sucrose preference of rat in