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Metabolomics analysis explores the rescue to neurobehavioral disorder induced by maternal $PM_{2.5}$ exposure in mice

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ABSTRACT

Reproductive epidemiological studies have suggested associations between perinatal exposure to fine particulate matter ($PM_{2.5}$) and adverse birth outcomes. To explore the effects of early prenatal exposure to $PM_{2.5}$ on subsequent generations, pregnant mice were exposed to $PM_{2.5}$ or filtered clean air in whole body dynamic exposure chambers for 14 consecutive days from gestation day (GD) 1.5 to GD15.5. Neurobehavioral tests showed that spontaneous locomotion and exploratory behaviors in the offspring were significantly enhanced in the open field test. Meanwhile, metabolomics analysis suggested activation of dopamine pathway while inhibition of glycine pathway in murine brains. Administration of the DRD4 antagonist, clozapine; or supplementation of glycine receptor agonist, taurine, to mice offspring attenuated the locomotor hyperactivities to levels indistinguishable from controls. These data provide strong evidence that maternal exposure to air pollution might increase the risk for neural disorders in the offspring during critical periods of brain development.

1. Introduction

Fine particulate matter ($PM_{2.5}$) is class of particulates with aerodynamic diameter less than 2.5 μm, which readily deposits in the respiratory tract and alveoli, and is absorbed into the body through air exchange in the lungs. With the rising levels of industrial and traffic emissions, severe air pollution events have been documented in many Chinese cities. The highest levels of daily $PM_{2.5}$ has reached 369 $\mu g/m^3$ in Nanjing and 602 $\mu g/m^3$ in Shanghai in 2015 (Xu et al., 2015), raising concerns on the safety of susceptible population, such as children, the old and pregnant women.

Prenatal exposure to air pollutants may disrupt the growth and development of the fetus. A previous study suggested that maternal exposure to $PM_{2.5}$ adversely affects motor and cognitive developments in infants (Lertxundi et al., 2015). Furthermore, association between in utero $PM_{2.5}$ exposure and disruption of gene expressions in the brain-derived neurotrophic factor signaling pathway has been identified in a birth cohort study (Saenen et al., 2015). One recent study reported that prenatal exposure to polyaromatic hydrocarbons (PAHs) bound to $PM_{2.5}$ has a greater impact on the increased risk of neurodevelopmental outcomes, such as attention deficit hyperactivity disorder, in children (Perera et al., 2018). Results from animal studies also support the notion that maternal exposure to $PM_{2.5}$ markedly affects the central neural systems in offspring. For example, autism-like behavioral deficits in adult mice has been reported to be associated with perinatal exposure to concentrated ambient $PM_{2.5}$ throughout gestation followed by exposures to both dams and litters from postnatal day (PND) 2–10 (Church et al., 2017). Components of $PM_{2.5}$, such as carbon black, have been shown to increase the risk of neurodegeneration in offspring following maternal exposure (Onoda et al., 2017). However, underlying molecular mechanisms involved in understanding, intervention and prevention against such kinds of health risks remain unclear.

Small molecules in central neural system play key roles in the brain function. For example, norepinephrine (NE) and 5-hydroxy-indole acetic acid (5-HIAA) levels have been increased in concentrated air particulate (CAP) exposed rat brains (Balasubramanian et al., 2013). Metabolomics is the comprehensive study of the metabolome in cells, tissues and body fluids, which provides a close representation of

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individual's overall biological status associated with genome, diet, environment and gut microbiome (Beger et al., 2016). Several studies have successfully used metabolomics approaches to explore the toxicity of $PM_{2.5}$ exposure induced pulmonary or systemic disorders (Liu et al., 2017; Zhang et al., 2017). However, the application of metabolomics analysis to reveal $PM_{2.5}$ exposure induced neurotoxicity has not yet been reported.

In the present study, the early maternal $PM_{2.5}$-exposed mice were tested with multiple neurobehavioral tests at different stages of development. Coupled with metabolomics analysis, it has been found that suppression of glycine pathway and activation of dopamine pathway were intensively involved in hyperactivity of maternal $PM_{2.5}$-exposed mice. In addition, administration with dopamine receptor D4 antagonist or agonist of glycine receptor demonstrated potential rescue.

2. Materials and methods

2.1. Urban particulate matter ($PM_{2.5}$)

Urban Particulate Matter (Standard Reference Material (SRM) 1648a) was purchased from the National Institute of Standards and Technology (NIST), USA. This Standard Reference Material (SRM) is atmospheric particulate matter collected in an urban area. All constituents provided in SRM 1648a were naturally present in the material before processing. The mass concentration of major components in SRM 1648a were detected by a research group from NIST (Schantz et al., 2012), including 25 metals, 21 PAHs and 7 polychlorinated biphenyls (PCBs).

2.2. Animal treatment

Animals were treated humanely and all experimental protocols were approved by the Committee on Animal Use and Care of Southeast University, China. All the methods in the present study were performed according to approved guidelines.

A total of 24 pregnant ICR mice (gestation day (GD) 0.5) were purchased from the College of Veterinary Medicine Yangzhou University. Mice were maintained under a 12/12 h dark/light cycle, with free access to food and water. The mice were randomly divided into two groups (with 12 pregnant mice each group), namely control and $PM_{2.5}$-treated group. Starting on GD 1.5, the mice were exposed daily to $PM_{2.5}$ (0.4 mg/m^3) or filtered clean air, respectively, in whole body dynamic exposure chambers for 6 h per day from 9 a.m. to 3 p.m., until GD 15.5 (for 14 consecutive days). The dynamic inhalation exposure chamber is outfitted with an aerosol generator (Beijing HuiRongHe Technology Co. Ltd., China). The levels of $PM_{2.5}$ in the chamber were detected by real-time air quality monitoring equipment (CEL-712 Microdust Pro, CASELLA CEL Inc, USA). The average concentration of $PM_{2.5}$ was 0.4 mg/m^3, which is the lowest stable level of suspending $PM_{2.5}$ in air produced by the aerosol generator under current condition. The temperature in the chambers was set to 22.5 °C. The pregnant mice were maintained one per polycarbonate cage on corncob bedding until delivery.

The body weights of pregnant mice were recorded every 3 days until delivery. At parturition, the number of live and dead pups was recorded. On PND3, gender of live offspring was examined by comparison of urogenital-anal distance and individual animals within each litter were identified by paw tattoo. On postnatal day 5, the litters were randomly reduced to 6 pups (3 male and 3 female) for each of the dams.

2.3. Developmental landmarks of development

Each pup was observed daily for the appearance of developmental landmarks. Body weights were measured every three days for each mouse from PND5 to PND41 then every week from PND 42 to PND 70. Starting on PND3, bilateral ear detachment, hair growth and eye opening were recorded for each pup. The criteria for bilateral ear detachment and eye opening are that both pinnae are fully detached, as well as both eyes visible, even if partially open.

2.4. Reflex development tests

Four pups (2 male and 2 female) from each litter were tested for reflex development using reflex righting, cliff avoidance and negative geotaxis tests (Cole et al., 2012; Li et al., 2015).

On PND 4, 7, and 10 pups were tested for reflex righting beginning at 9:00 a.m. Pups were placed in a supine position and the time to right onto all four paws was measured. The scores for successful righting were set as follow: 3 for righting within 10 s; 2 for more than 10 s but within 20 s, 1 for more than 20 s but within 30 s. If mice did not complete the task within 30 s, the test was terminated and recorded as 30 s.

On PND 6, cliff avoidance was measured using a square plexiglass platform, 25 cm above the surface of bench. Each pup was placed with front paws and snout over the edge. The time of pup turn 180° away from the cliff was recorded.

On PND 7, negative geotaxis was measured, with a rest period of at least 15 min following reflex righting test. Pups were placed head-downward on a 30° rough wood incline and the time to turn 180° to face upward was measured.

The scores for cliff avoidance and negative geotaxis were set as follow; 2 for response within 30 s, 1 for response within 60 s, and 0 for no response within 60 s.

2.5. Open field test (OFT)

Four offspring (2 male and 2 female) from each litter were tested for locomotor activities and behavioral responses to an unfamiliar environment using open field test (PFT) on PND 28. Mice were individually placed in an acrylic box (40 cm × 40 cm) within a soundproof box and allowed to move freely for 3 min. After the test, the mice were returned to their home cages. Parameters including total distance (cm), duration of immobility (sec), distance travelled within the central area (cm), time spent in the central area (sec), frequencies of zone transition (n) and movement speed (cm sec^{-1}) were recorded and analyzed with video tracking software (Ethovision XT, Noldus Information Technology, Netherland).

2.6. Metabolomics analysis

A total of 8 F1 mice (4 male and 4 female) from control and maternal $PM_{2.5}$ exposure groups were randomized decapitated for metabolomics analysis 24 h after the OFT. The whole brain tissues were collected, half of the brain tissue was stored in liquid nitrogen, and the other half brain tissue were prepared and analyzed as previously described (Li et al., 2017). Briefly, mouse cortex samples were homogenized with 50% methanol (1 ml) and the supernatant was obtained after centrifugation at 12,000 rpm for 15 min. About 350 µl of the supernatant was transferred into a 2 ml vial and blow-dried by nitrogen. Afterward, 80 µl of methoxyamine was added and reacted for 2 h at 37 °C. Finally, 100 µl of bis (trimethylsilyl)trifluoroacetamide (BSTFA) reagent (containing 1% TMCS) was added to the mixture and reacted for 60 min at 70 °C. The sample was cooled down to room temperature and then analyzed by GC/TOF/MS (Agilent 7890 coupled with a Pegasus 4D TOF). 1 µl of the sample was injected in split less mode at a gas flow rate of 1 ml/min. The program was set at 80 °C for 0.2 min, raised to 180 °C at a rate of 10 °C/min, then to 240 °C at a rate of 5 °C/min, and finally to 290 °C at a rate of 20 °C/min.

2.7. RNA isolation and quantitative real-time PCR assay

Six mice (3 male and 3 female) per group were decapitated 24 h