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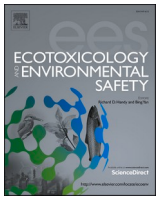

Maternal exposure to $PM_{2.5}$ induces cognitive impairment in offspring via cerebellar neuroinflammation and oxidative stress

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ABSTRACT

Available evidence suggest that exposure to $PM_{2.5}$ during pregnancy is associated with reduced cognitive function in offspring. This study aimed to investigate the effects of maternal exposure to $PM_{2.5}$ on offspring cognitive function and to elucidate the underlying mechanisms. In this work, pregnant C57BL/6 female mice were exposed to concentrated ambient $PM_{2.5}$ or filtered air from day 0.5 (=vaginal plug) to day 15.5 in the Shanghai Meteorological and Environmental Animal Exposure System, and offspring cerebellar tissues were collected on embryonic day 15.5, as well as postnatal days 0, 10 and 42. The mean $PM_{2.5}$ concentrations exposed to the pregnant mice were 73.06 ± 4.90 μg/m^3 and 11.15 ± 2.71 μg/m^3 in the concentrated ambient $PM_{2.5}$ and filtered air chambers, respectively. Maternal concentrated $PM_{2.5}$ exposure was negatively correlated with offspring spatial memory significantly as assessed by the Morris water maze. Compared with the filtered air group, $PM_{2.5}$-exposed offspring mice had reduced cerebellar microglia. Both RNA and protein levels of IL-8 and TNF-α were elevated in the concentrated ambient $PM_{2.5}$ group. $PM_{2.5}$ exposure increased the level of 8-OHG in miRNA of microglia and Purkinje cells in 6-week-old offspring. The level of prostaglandin F2α (8-iso-PGF2Aα) in the cerebellum was increased at different growing stages of offspring after gestational exposure of $PM_{2.5}$. These results suggested that maternal air pollution exposure might cause inflammatory damage and oxidative stress to the cerebellum, contributing to reduced cognitive performance in mice offspring.

1. Introduction

Epidemiological evidence have indicated that exposure to the $PM_{2.5}$, the fine particulate matter with an aerodynamic diameter of less than 2.5 μm, can negatively affect cognitive functions in humans (Babadjouni et al., 2017; Chuang et al., 2020; Liu et al., 2019). Furthermore, both the particle size and the chemical composition, such as metals, polycyclic aromatic hydrocarbons (PAHs), nitrates and sulfates, may have a significant role in PM-induced toxicity. One recently recognized mechanism is that $PM_{2.5}$ can directly damage the central nervous system, while pollutants cause respiratory and immune systems to produce harmful factors that reach the brain through peripheral circulation that lead to systemic responses, such as neuroinflammation and oxidative stress in the brain, which damages neurological cell structure and function (Costa et al., 2019; Park et al., 2021; Wang et al., 2020). Metal constituents in $PM_{2.5}$ particles have been reported to cause various

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damages to organisms, and metal components have been found in numerous tissues, including brain tissues (Choi et al., 2018). In addition to the deposition in the respiratory system, $PM_{2.5}$ could also cross the human air-blood barrier and enter the circulatory system (Lochhead et al., 2010), with smaller particles even crossing blood-brain and placental barriers (Bové et al., 2019). Furthermore, previous studies have also shown that $PM_{2.5}$ exposure during gestation causes spatial memory dysfunction and neurodevelopmental impairment in offspring (Zheng et al., 2018), but the exact mechanisms remain unclear, and few studies have examined potential mechanisms affecting cognition and neurobehavior in the offspring at different stages of growth and development.

Some studies demonstrated that exposure to $PM_{2.5}$ activates microglia and produces neuroinflammation, which impairs neuronal and synaptic functions (Kraft and Harry, 2011). $PM_{2.5}$-stimulated microglia can release pro-inflammatory cytokines, such as IL-1β、IL-8 and TNF-α (Lai et al., 2016; Li et al., 2019). Microglia activation also plays a critical role in the pruning and development of the Purkinje cell (Beckinghausen and Sillitoe, 2019), which is one of the key functional cells in the formation of cerebellar regulatory patterns that are closely related to motor development. It has been shown that pups born in the intrauterinely infected group had fewer Purkinje cell layers with significantly reduced cell size and cell density than those from healthy controls, and continued to have altered Purkinje cell morphology and impaired cerebellar function until 1 month of age (Zhang et al., 2019).

Sterile inflammation has been found to lead to increased IL-8 expression, lipid peroxidation products and oxidative stress to DNA that can damage the guanine nucleotide base to form 8-oxoguanine (o8G) base, which is considered one of the most lethal base disruptions associated with cell cycle arrest. RNA is more prone to o8G modifications than DNA (Simms and Zaher, 2016). Recently, Seok H. et al. reported the existence of position-specific o8G oxidation of miRNA, a post-transcriptional regulator targeting mRNA, the causality between such epitranscriptional modification and reactive oxygen species, as well as its pathophysiological relevance to cardiac hypertrophy via the alteration of miRNA targeting (Seok et al., 2020). However, to the best of our knowledge, o8G modification of miRNA has barely been investigated in other tissues thus far, especially in the brain. In addition, numerous studies have shown that miRNA dysregulation is associated with exposure to $PM_{2.5}$, carbon black nanoparticles and diesel exhaust particles (Bleck et al., 2013; Farraj et al., 2011; Ku et al., 2017a; Sanchez et al., 2020), also mediates the inactivation of microglia (Brás et al., 2020) and the degeneration of Purkinje cell (Schaefer et al., 2007), causing neuroinflammation in the brain (Brás et al., 2020; Mandolesi et al., 2017). Therefore, we hypothesized that oxidative stress might lead to site-specific oxidation of miRNAs in functional cerebellar cells, thereby perpetuating fetal-derived brain damage due to intrauterine aseptic inflammatory responses into childhood or even adulthood. We aim to explore the effects of parental $PM_{2.5}$ exposure on the cognitive function of offspring mice, along with the pathophysiological mechanisms of neuroinflammation and oxidative stress. We hope that the results will provide more references for the studies related to environmental exposure and neurodevelopment health.

2. Methods

2.1. Animal exposure to $PM_{2.5}$

Barrier-raised SPF 6-week-old C57BL/6 mice were obtained from Jiangsu Gempharmatech Co., Ltd (Certification No. 202016448). Thirty female mice were mated with males to establish pregnancies, which were confirmed by vaginal plugs. Then the pregnant mice were randomly divided into two groups, exposure to filtered air (FA) or exposure to concentrated ambient $PM_{2.5}$ (CAP), respectively. The exposures of FA and CAP were performed in the Shanghai Meteorological and Environmental Animal Exposure System (Shanghai-METAS), which was described in details previously (Du et al., 2018; Yang et al., 2019). Real-time concentrations of $PM_{2.5}$ were measured via pDR-1500 (Thermo Scientific, Waltham, MA, USA), and samples of $PM_{2.5}$ were simultaneously collected on Teflon filters for accurate exposure concentration determination. The exposure was conducted 8 h per day, 7 days per week for consecutive 15 days. Pregnant mice were transferred to an animal housing facility to produce offspring after the exposure. The fetal mice on embryonic day 15.5, and newborn mice on postnatal days 0, 10 and 42 (N = 10 pups, randomly selected from 3 to 4 breeders, in each group) were euthanized and the cerebellar tissues were collected thereafter. The Animal Experimental Ethics Committee in the Department of Laboratory Animal Science at Tongji University approved this study with the approval number TJBG09721 (Fig. 1).

2.2. Open field test

Mice were placed in an open box of 40 × 40 × 50 cm^3 and were allowed to freely wander for at least 6 min before the test. The open field box was cleaned with 75 % alcohol solution before each mouse was introduced. The field was divided into 4 rows × 4 columns yielding 16 zones. The total distance travelled, average speed, distance travelled in the center area (20 cm × 20 cm), average speed in the central area, and the number of entries into central zones were analyzed.

2.3. Morris water maze test

Morris water maze test was performed in a round white pool of 120 cm in diameter and 50 cm in depth. The pool was filled with water of 30 cm in depth, which was maintained at 22 ± 1 °C. The platform was 6 cm in diameter and 1 cm beneath the surface of the water. And the test was conducted for six consecutive days. On each day of the testing, the platform was placed in the same quadrant. In the training phase (the first five days), the mice were given four successive 60-second trials per day, starting from a different quadrant each time. The same quadrant start pattern was applied during the entire test. For statistical analysis, the four daily trials were averaged to determine latency to find the platform (up to 60 s). Once a mouse reached the platform, it was allowed to sit for 30 s. If it failed to reach the platform within 60 s, it would be gently guided towards the platform. When mice found and jumped off from the platform, a new trial began. Mice that did not find the platform were given a latency score of 60 s. The probe test was conducted on the day 6 as the second phase of the experiment. The platform was removed during this phase, and the mice started from the opposite quadrant. Recorded distances and swimming times were taken in each quadrant, including the goal quadrant which previously held the platform.

2.4. miRNA isolation

The methods to isolate, purify and culture primary murine microglia (Bohlen et al., 2019) and Purkinje cells (Tjaden et al., 2018) from mouse cerebella were reported previously. Then the miRNA was extracted from primary isolated cells using a mirVana™ miRNA isolation kit (Ambion, AM1560) following the manufacturer's instructions. The level of oxidative nucleic acid modification marker 8-OHG was measured using OxiSelect™ Oxidative RNA Damage ELISA kit (XY-8-epi-PGF2α-MU, Shanghai XinYu Biotech Co., Ltd).

2.5. ELISA

The levels of inflammatory cytokines IL-8 (XY-IL-8-MU, Shanghai XinYu Biotech Co., Ltd) and TNF-a (XY-E11944, Shanghai XinYu Biotech Co., Ltd), oxidative stress biomarker 8-iso-prostaglandin F2α (STA-325, OxiSelect), and oxidative nucleic acid modification marker 8-OHG were measured using an ELISA kit according to the manufacturer's instructions. Briefly, we added standard and experimental reagents to the samples, incubated them for 30 min at 37 °C, washed them five times,

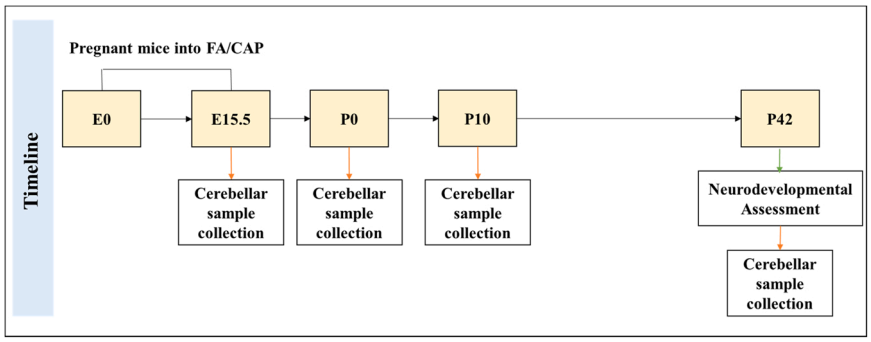

Fig. 1. Schematic timeline of the experimental design. E represents the embryonic day and P represents the postnatal day.

then added enzyme and chromogen solutions. Within 15 min of adding the stop solution, the absorbance was measured. The experiment was triplicated for each sample.

2.6. Immunohistochemistry

Cerebellar samples were collected and placed in 4 % paraformaldehyde for 24 h. Cerebellar tissues were made into 10-μm sections, dehydrated, paraffin-embedded, and sectioned. Paraffin sections were routinely dewaxed and rehydrated, blocked for endogenous peroxidase activity, antigen repair, and closed. Samples were incubated overnight at 4 °C with a primary antibody (rabbit anti-Iba1, ab178846, Abcam) and then with a broad-spectrum secondary antibody (D-3004, Shanghai Changdao Biotechnology Co., Ltd,). DAB substrate was then added, followed by counterstaining with hematoxylin. Five fields of view were randomly taken under an inverted fluorescence microscope at 200 ×, and protein expression of each indicator in the same field of view (i.e., the percentage of the brownish-yellow region to the total area of the view) was calculated using Image J analysis software.

2.7. Quantitative real-time PCR

The RNA was extracted with TRIzol reagent (Invitrogen), reverse-transcribed to cDNA with a Thermo Scientific RevertAid first-strand cDNA synthesis kit as directed by the manufacturer. Quantitative real-time PCR (RT-qPCR) was performed using SYBR Green PCR Master Mix (Invitrogen) and detected by an ABI PRISM 7300 Sequence Detection System (Applied Biosystems). By using the comparative threshold (Ct) method, target mRNA levels were normalized to housekeeping GADPH levels. This study used sequence-specific primers summarized in Table 1. Each sample was run in triplicate with the relative quantity of each transcript normalized by the housekeeping gene and expressed as a fold change over the control.

2.8. Statistical analyses

The data was analyzed using IBM SPSS statistic 23.0, and we expressed the data as means ± standard deviation. To compare the differences between two groups, the student's t-test was used for variables with normal distribution, otherwise, a Wilcoxon signed-rank test was used. Statistical significance was determined by a P-value lower than 0.05.

Table 1
PCR primers.

Gene	Direction	Primer sequence (in 5′−3′ direction)
Cxcr2	Forward	TTTGAGGGTCGTACTGCGTATC
	Reverse	TGGGCCTTAAAGAGGGTGC
TNF	Forward	GGCGGTGCCTATGTCTCAG
	Reverse	CCTCCACTTGGTGGTTTGTG
GADPH	Forward	CTGCCCAGAACATCATCC
	Reverse	CTCAGATGCCTGCTTCAC

3. Results

3.1. Maternal air pollution exposure impact the cognitive performance in mice offspring

3.1.1. $PM_{2.5}$ exposure impacted the exploratory behavior changes measured by open field test

An open field test was conducted to assess primarily exploratory behavior in mouse offspring at 6 weeks old. Anxiety-like behavior was associated with time spent in the center of the field. The total travelled distance and average speed was not significantly different between the two groups (Fig. 2A and B). The distance and the speed travelled or time spent in the central area of the field were analyzed, which showed no significant difference between the two groups either (Fig. 2C, D, and E).

3.1.2. $PM_{2.5}$ exposure reduced the learning and spatial memory tested by the Morris water maze

Morris water maze test was performed on mouse offspring at 6 weeks old to evaluate their spatial learning and memory ability. Our results showed that the platform latency on day 5 was shorter than that on the first day of training, but there was no significant difference between the two groups in escape latency (Data for day 5: FA: 37.88 ± 23.36 s; CAP: 41.87 ± 22.98 s; $p > 0.05$) in the Morris water maze test (Fig. 3A). However, there were significant differences in the target quadrant crossing times (Data for day 6: FA: 4.90 ± 1.66; CAP: 2.60 ± 1.35), target quadrant stay time (FA: 13.37 ± 6.87 s; CAP: 7.63 ± 4.71 s) and the distance travelled in the target quadrant (FA: 2013.40 ± 718.68 mm; CAP: 1222.84 ± 701.82 mm) between the two groups during the probe trial (Fig. 3B-D, $p < 0.05$).

3.2. $PM_{2.5}$ exposure changed the cerebellar microglia presence measured by Immunohistochemistry

Microglia activation is thought to be a marker of neuroinflammation and has been shown to contribute to the pathogenesis of several brain disorders, including neurodegenerative diseases (Zheng et al., 2018), and intrauterine infection has been shown to cause an increase in microglia in the brains of offspring mice (Juckel et al., 2011). We used Iba1 as the marker to label microglia (the brownish-yellow cells in Fig. 4A) in the paraffin-embedded cerebellar sections. The microglia percentage in the cerebellum was the largest on the embryonic day 15.5 in mice of the CAP group, and gradually decreased with time on the postnatal days 0, 10, 42 (Fig. 4B). In comparison, the presence of cerebellar microglia in the FA group was significantly lower than that in the CAP group at all stages, with an overall general trend of decreasing as the mice grew. The observation of microglia proliferation supported our hypothesis of induced inflammation upon $PM_{2.5}$ exposure.

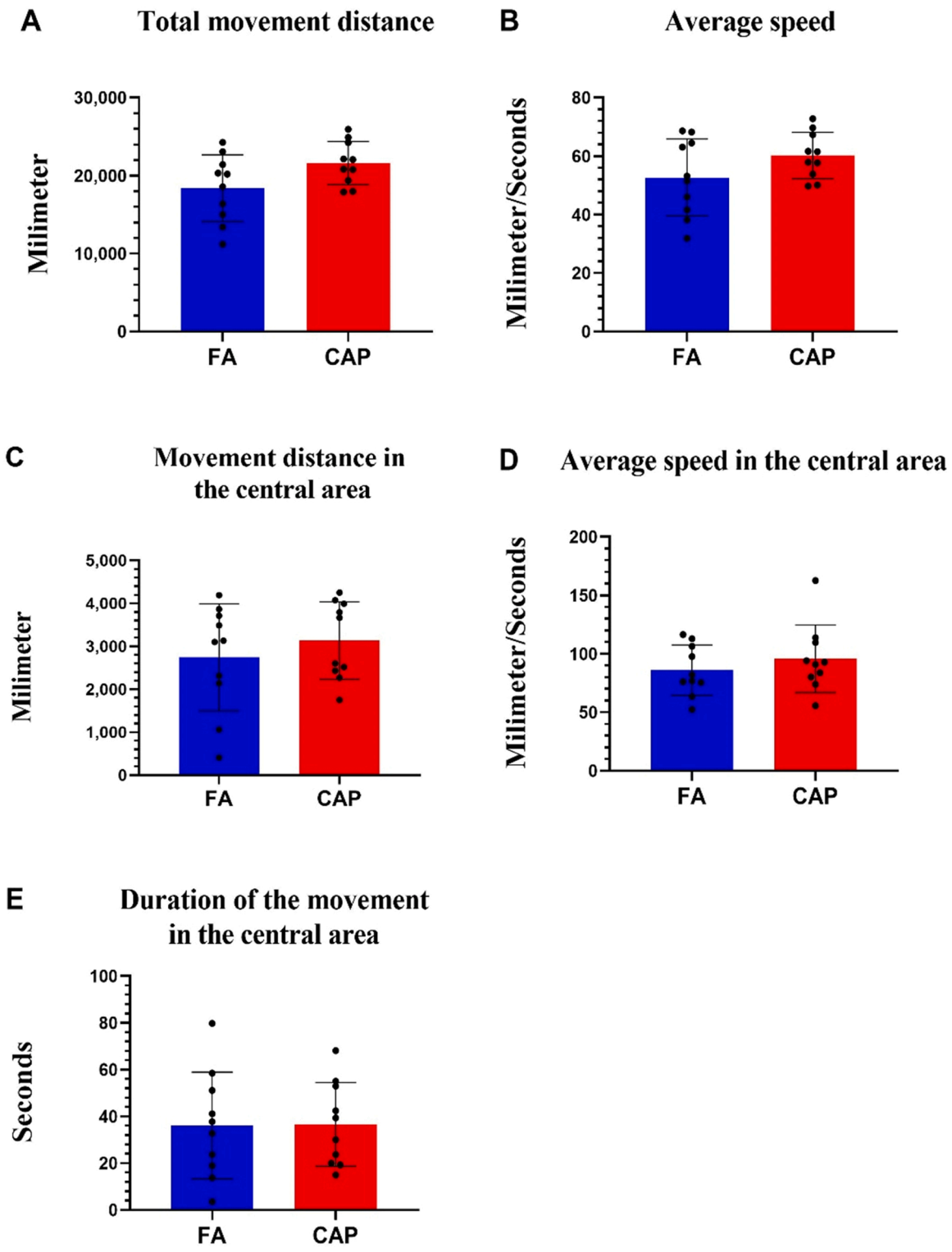

Fig. 2. Effects of $PM_{2.5}$ exposure on locomotor activity in the open-field test. (A) Total movement distance; (B) Average speed; (C) Movement distance in the central area; (D) Average speed in the central area; and (E) Duration of movement in the central area. n = 10.

3.3. $PM_{2.5}$ exposure increased miRNA 8-OHG modification in microglia and Purkinje cells

We measured and compared 8-OHG modification between CAP and FA groups. After offspring mice reached 6 weeks of age, cerebellar tissues were taken for primary isolation of microglia and Purkinje cells, and miRNA was extracted from them separately using kits according to the manufacturer's protocol (Fig. 5). The levels of 8-OHG in microglia and Purkinje cells' miRNA in the CAP group were significantly higher than those in the FA group ($p < 0.01$). It indicated that the degree of miRNA damage was higher in the CAP group, suggesting dysregulation of miRNA targeting and function.

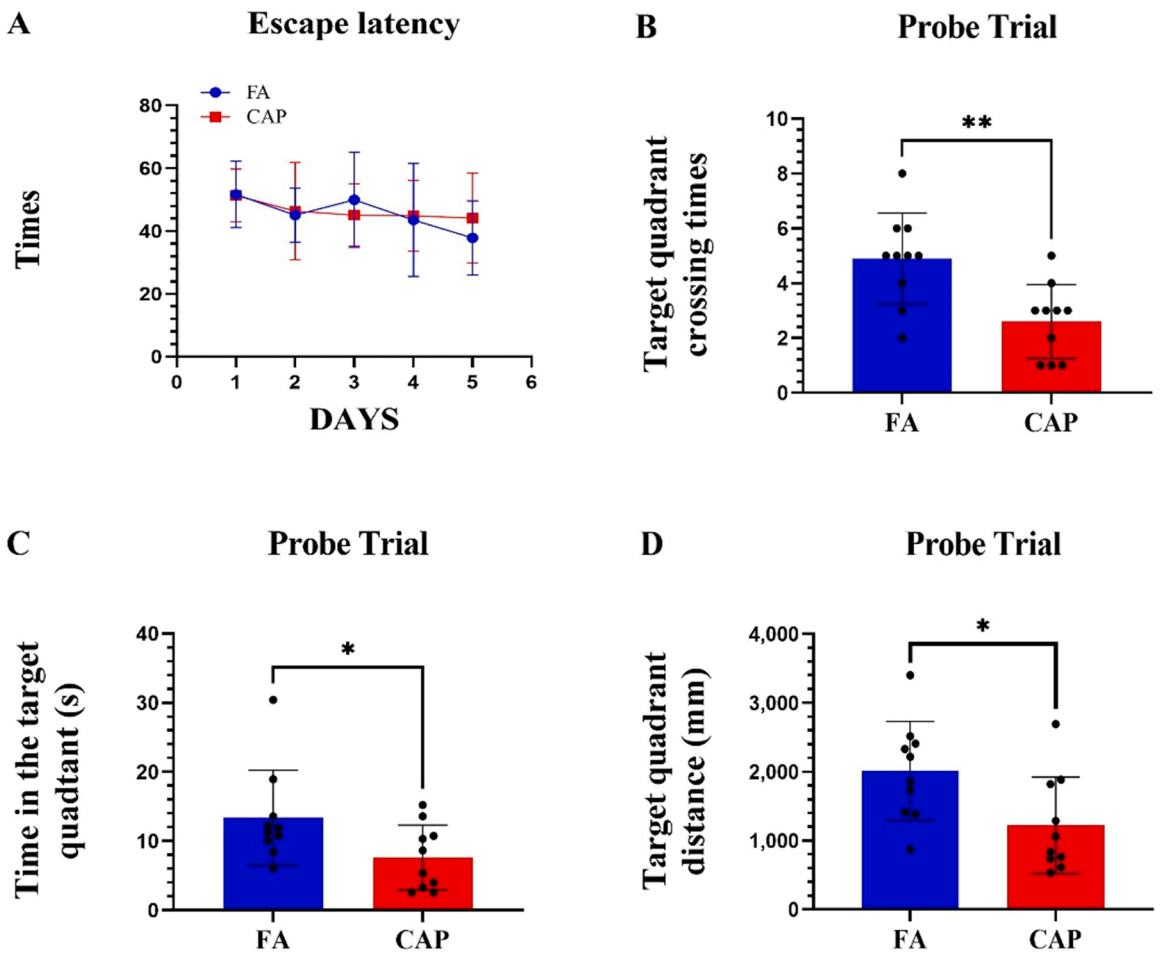

Fig. 3. Behavioral changes revealed in Morris water maze test. (A) Morris water maze test positioning flight test average latency of 5 days; (B) the Average number of passes; (C) Average target quadrant stay time; (D) Average target quadrant activity distance. * $p < 0.05$, * * $p < 0.01$, n = 10.

3.4. $PM_{2.5}$ exposure induced inflammatory cytokines in cerebellar tissue

The protein levels of TNF-α (A) and IL-8 (B) and mRNA expression of TNF-α (C) and IL-8 (D) after 15 days of $PM_{2.5}$ exposure were detected by ELISA and real-time PCR, respectively. * $p < 0.05$, * * $p < 0.01$, n = 6 offsprings from 3 to 4 breeders per group.

We wanted to see if maternal exposure to $PM_{2.5}$ would have an effect on the level of cerebellar inflammation in the offspring, to this end, we assessed inflammatory cytokines including IL-8 and TNF-α in cerebellar tissue by ELISA for protein levels (Fig. 6 A and B) and RT-PCR for mRNA expression (Fig. 6 C and D). We compared them in two groups at four stages. The CAP group showed a significant increase in the expression of the two cytokines at both mRNA and protein levels at all stages of mouse growth. Intriguingly, the effects of $PM_{2.5}$ exposure on cytokine induction gradually approached the control group during postnatal development, though statistical differences still existed ($p < 0.05$).

3.5. $PM_{2.5}$ exposure caused lipid peroxidation in cerebellar tissue

To observe whether maternal exposure to $PM_{2.5}$ affected offspring cerebellar oxidative stress levels, as shown in Fig. 7, we compared the levels of 8-iso-PGF2α in cerebellar tissue in the CAP and FA groups. Similar to inflammatory cytokines, the elevation of 8-iso-PGF2α in the CAP group tended to attenuate over the course of offspring growth, but there was always a significant difference between the two groups.

4. Discussion

Recently, many studies have demonstrated that there is a negative correlation between exposure to air pollution and various damages to cognitive functions (Taylor et al., 2017). Studies have shown that metal constituents attached to $PM_{2.5}$ particles can cause sustainable damage to the body, and metal component deposition has been found in many tissues including the brain (Ku et al., 2017b), which can cause neurotoxic effects on animals in the growing period and lead to neuroinflammation (Karri et al., 2016). There have been many human and animal studies in which exposure to air pollution may damage brain development and perhaps contribute to neurodevelopmental disorders (Cory-Slechta et al., 2018; Lee et al., 2016; Payne-Sturges et al., 2019). In addition, epidemiological studies also indicate strong associations between exposure to air pollution in utero and/or early in life and risks of neurobehavioral developments such as Autism spectrum disorder (ASD) (Guxens et al., 2014; Kicinski and Nawrot, 2015).

Notably, different from previous studies by intratracheal instillation, the mice in the current study were exposed to CAP or FA using a whole-body exposure system, which mimics the “real-world” exposure to environmentally relevant $PM_{2.5}$ in humans (Yang et al., 2019). The offspring mouse model was constructed by exposing pregnant mice to high concentrations of $PM_{2.5}$ in the first 15 days of pregnancy. Behavioral tests were conducted in the offspring, and open-field tests indicated that $PM_{2.5}$ exposure did not significantly affect general exploratory or anxiety-like behavior in these mice. However, a result from an elevated plus maze test showed that anxiety-like response in offspring exposed to $PM_{2.5}$ during gestation was correlated with their genders (Zhao et al.,

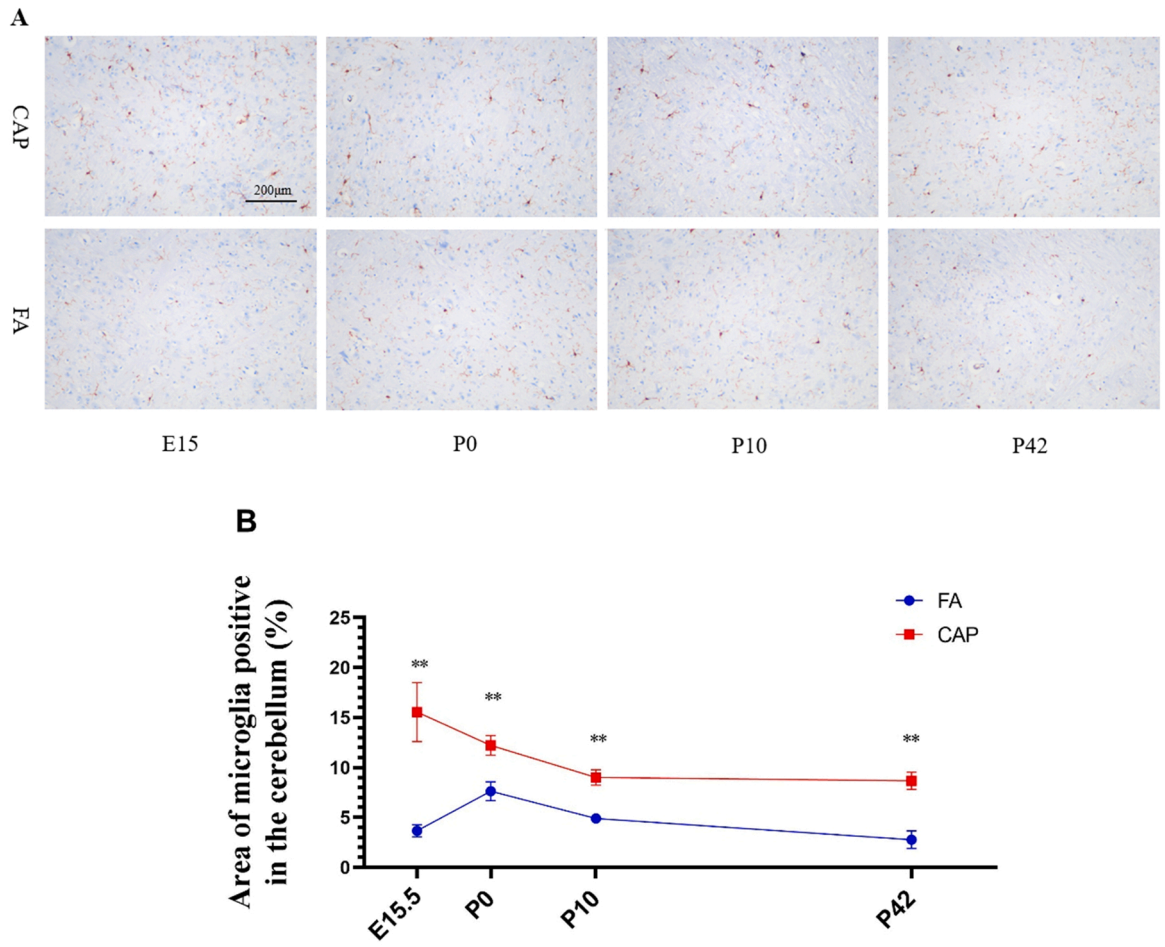

Fig. 4. Difference of microglia presence in cerebellum between FA and CAP by immunohistochemistry. (A) Representative photomicrographs of immunohistochemical staining of microglial cells in the cerebellum at different stages of offspring, 200 × . (B) Statistical comparison of microglia percentage in the cerebellum area. n = 6, * * $p < 0.01$.

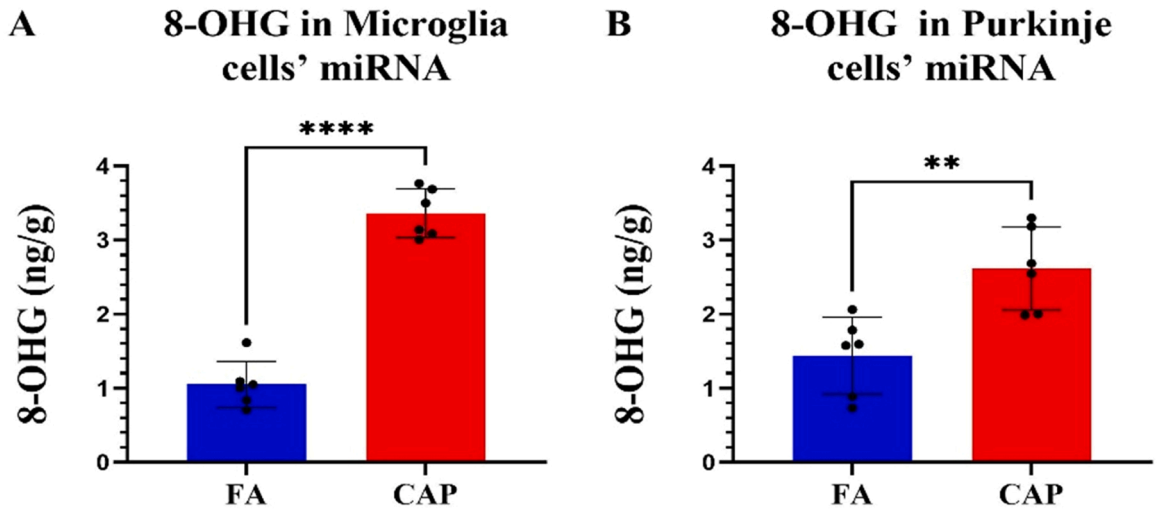

Fig. 5. Concentrations of 8-OHG modification in the miRNA from microglia and Purkinje cells. * * $p < 0.01$, * ** * $p < 0.0001$. n = 6 offsprings from independent breeders per group.

2021), with only female offspring showing increased behavioral anxiety. Animal experiments on anxiety-like responses in offspring mice prenatally exposed to $PM_{2.5}$ are still relatively scarce. Further investigations are needed to confirm the effects and reveal the underlying mechanisms. Evaluation using the Morris water maze clearly demonstrated the deleterious effects induced in the offspring after gestational exposure, including impairment of spatial memory capacity and cognitive abilities, which was consistent with previous studies (Zheng et al., 2018). In

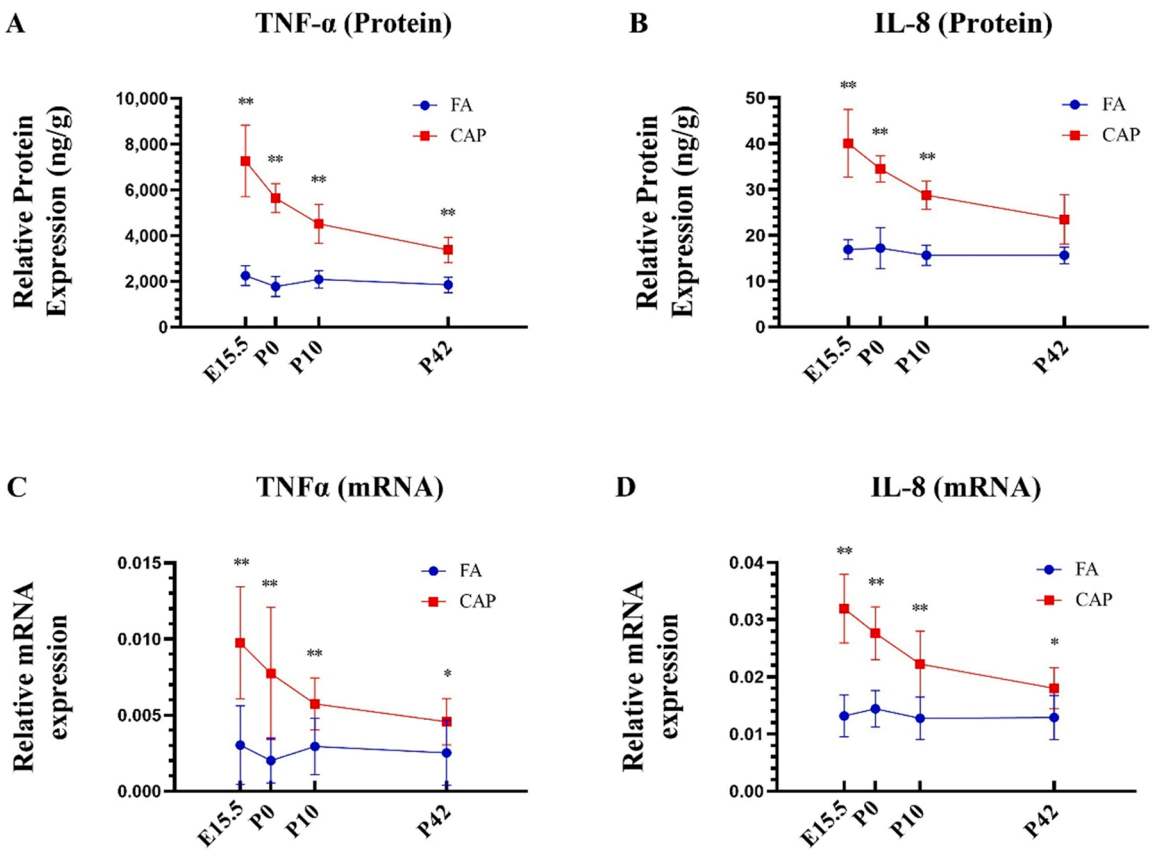

Fig. 6. Effects of $PM_{2.5}$ exposure on the expression of TNF-α and IL-8 in cerebellum.

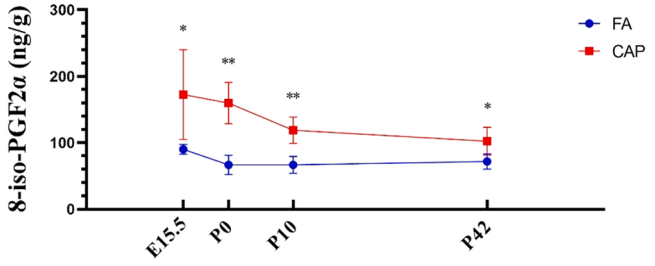

Fig. 7. Comparison of 8-iso-PGF2α between CAP and FA groups. * $p < 0.05$, * * $p < 0.01$, n = 6 offsprings from 3 to 4 breeders per group.

order to explore the underlying mechanisms, we innovatively further investigated the aseptic inflammatory response, microglia inflammatory injury and o8g oxidative stress index of Purkinje cells and microglia miRNA in the cerebellum of offspring mice at various stages of growth (embryonic day 15.5th, postnatal days 0, 10th and 42nd), a study design that has rarely been conducted before. We found that there were aseptic inflammatory reactions characterized by cerebellar oxidative stress, including an increase in chemokine TNF-α, IL-8 and lipid peroxidation product 8-iso-PGF2α. Compared with the control group, overall miRNA-specific (8-OHG) oxidative damage of cerebellar microglia and Purkinje cells in 6-week-old postnatal rats was found. All these oxidative stress characteristics were most remarkable in the embryonic stage and gradually relieved after birth.

The effects of $PM_{2.5}$ on spatial memory and cognitive abilities may be due to $PM_{2.5}$ activating microglia, producing neuroinflammation, thereby damaging neurons and synaptic function (Spangenberg and Green, 2017). Severe inflammation has been observed in the rodent brain after exposure to high concentrations of $PM_{2.5}$ and diesel engine exhaust (Patchin et al., 2016). In vitro experiments have shown that $PM_{2.5}$ exposure reduces the survival rate of neuronal cells in a dose-dependent manner, leading to microglia activation and neuronal damage (Roqué et al., 2016). The activation of microglia also plays a vital role in mouse pruning and the development of the Purkinje cell layer after birth (Beckinghausen and Sillitoe, 2019). While the normal development of Purkinje cells in the cerebellum is related to motor coordination (Neveu and Arenas, 1996), some researchers have pointed out that due to environmental endocrine disruptors, the branching area of Purkinje cell dendrites may shrink (Saywell et al., 2014). Abnormal Purkinje cells make mice hesitate when passing the balance beam, increase the number of hind paw slips, and reduce staying time on the rotating rod, which harms sport behavior (Glinoer, 1997; Hartmann et al., 2014; Zhu et al., 2016). Our results are consistent with previous animal studies, showing that ambient $PM_{2.5}$ exposure significantly increases microglia cells and induces oxidative stress in the cerebellum to influence cognition, and such effects are gradually erased with the development of the mice.

Oxidative stress and inflammation are believed to be the most important contributors to the adverse effects of air pollution on the respiratory and cardiovascular systems (Møller et al., 2014). The fact that exposure to air pollution leads to microglia activation, oxidative stress, and neuroinflammation provides biological plausibility and potential underlying mechanisms for the observed association between exposures and risks of neurodevelopmental diseases (Kraft and Harry, 2011). In addition, microglia stimulated by $PM_{2.5}$ exposure could release more pro-inflammatory cytokines, such as IL-6 and IL-1β And TNF-α (Brás et al., 2020). TNF-α, a key pro-inflammatory cytokine, has been considered to cause axonal degeneration and affect the formation of neural synapses (Olmos and Lladó, 2014) Lipid peroxidation is one of the major outcomes of oxidative stress following free-radical-mediated injury, leading to the generation of various end products (Vazzana

et al., 2013). Studies have confirmed that the increase in lipid peroxidation is related to the decline of cognitive ability. 8-iso-PGF2α is increased in Alzheimer's disease (AD) and may mediate neuronal response to oxidative stress (Loffredo et al., 2020). Our present findings are consistent with these previous study results (Allen et al., 2017), suggesting that exposure to CAP via inhalation leads to impaired cognition in offspring likely through the inflammatory response and oxidative stress.

Previous studies indicate that air pollution exposure generates oxidatively damaged DNA, as well as RNA, by promoting a milieu of oxidative stress and inflammation (Møller et al., 2014). Compared with age-matched groups, the levels of mRNA oxidation measured by immunoprecipitation were higher in the frontal cortex of AD patients (Kong and Lin, 2010). Cioffi et al. summarized that neurodegenerative disorders (Cioffi et al., 2021), including AD (Zhang et al., 1999), amyotrophic lateral sclerosis (ALS) and dementia with Lewy bodies (Chang et al., 2008), were characterized by oxidation of neuronal mRNA. The oxidation status of miRNA, as a posttranscriptional regulator targeting mRNA, was also found to be related to the redox state of cells (Simms and Zaher, 2016). We extracted miRNA from microglia and Purkinje cells of 6-week-old offspring and explored the content of 8-hydroxyl deoxy guanine (8-OHG), one of the widely used biomarkers to assess oxidative stress. It is reactive oxygen species (ROS)-induced oxidation product of RNA base, formed by hydroxyl radical (·OH)-mediated damage to guanosine. The 8-OHG level was higher in the CAP group than that in the FA group for both microglia and Purkinje cells. Our results suggested that 8-OHG levels in microglia and Purkinje cells might provide information on oxidative stress mechanisms for the impacts of prenatal $PM_{2.5}$ exposure on mouse offspring neurodevelopment.

There are several strengths in the current studies. Our research simulated "real-world" $PM_{2.5}$ pollution exposure in pregnant mice, and the cytokine indicators were measured at different growth stages in offspring mice. Combining animal behavior, pathology, and molecular biology, this work explored the adverse effects and potential mechanisms of $PM_{2.5}$ exposure on the cognitive impact of offspring mice during gestation, and provided some insight and reference for future studies of $PM_{2.5}$ on human health and systemic disease development. Nevertheless, a few questions need to be addressed by future studies. The intermediate steps linking the presence of $PM_{2.5}$ in pregnant mice and oxidative stress in the embryonic brain are still unclear. The identification of the direct downstream effector of $PM_{2.5}$ particles will deepen the understanding of environmental toxicology and, more importantly, guide the way of early clinical intervention aiding pregnancy. As a recent finding of miRNA modification, 8-OHG has been shown to skew the targeting spectrum of miRNA (Spangenberg and Green, 2017). Given that the global 8-OHG level is elevated in CAP offspring, the key miRNAs with the heaviest modification need to be screened out, as well as their dysregulated targets. Knowledge of 8-OHG writer and eraser will also potentially provide clinical targets for neurodevelopmental disorders and others, although it is completely unexplored yet. Mechanistically, the causal network between 8-OHG modification, microglia proliferation, and local inflammation should be further investigated by means of molecular biology in future in vitro and in vivo studies. Further animal studies screening specific sites of miRNA oxidative modification are warranted, which provides a new idea for the study of miRNA oxidative modification in brain developmental defects[52].

5. Conclusion

This study demonstrate that exposure to $PM_{2.5}$ during pregnancy impaired cognitive and motor ability in the offspring mice, which might be mainly mediated by persistent brain damage of aseptic inflammatory response and cerebellar oxidative stress. Furthermore, oxidative stress injury biomarkers of miRNA in microglia and Purkinje cells could modify the oxidative stress pathway, and age appeared to be a significant susceptibility factor with the very young being more affected. Our findings may have potential implications for public health and environmental policy protecting the public, especially pregnant women and those of early ages, from air pollution damage.

CRediT authorship contribution statement

Jiajia Zhang, Yingying Yang: Data curation, Methodology, Formal analysis, Writing – original draft. **Zahraa S. Al-Ahmady, Wenchong Du** and **Zehuan Liao:** Methodology, Writing – review & editing. **Jinjin Duan:** Investigation. **Qinghua Sun, Zhiyun Wei** and **Jing Hua:** Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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