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$PM_{2.5}$ exposure stimulates COX-2-mediated excitatory synaptic transmission via ROS-NF-κB pathway

Ben Li, Lin Guo, Tingting Ku, Minjun Chen, Guangke Li, Nan Sang*

College of Environment and Resource, Research Center of Environment and Health, Shanxi University, Taiyuan, Shanxi 030006, PR China

HIGHLIGHTS

- $PM_{2.5}$ elevated COX-2 expression in primary cultured hippocampal neurons.
- $PM_{2.5}$ increased COX-2 expression and fEPSP amplitude in hippocampal brain slices.
- $PM_{2.5}$ exposure potentiated ROS generation and NF-κB phosphorylation.
- $PM_{2.5}$ disrupted COX-2 mediated synaptic transmission via the ROS-NF-κB pathway.

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ABSTRACT

Long-term exposure to fine particulate matter ($PM_{2.5}$) has been reported to be closely associated with the neuroinflammation and synaptic dysfunction, but the mechanisms underlying the process remain unclear. Cyclooxygenase-2 (COX-2) is a key player in neuroinflammation, and has been also implicated in the glutamatergic excitotoxicity and synaptic plasticity. Thus, we hypothesized that COX-2 was involved in $PM_{2.5}$-promoted neuroinflammation and synaptic dysfunction. Our results revealed that $PM_{2.5}$ elevated COX-2 expression in primary cultured hippocampal neurons and increased the amplitude of field excitatory postsynaptic potentials (fEPSPs) in hippocampal brain slices. And the administration of NS398 (a COX-2 inhibitor) prevented the increased fEPSPs. $PM_{2.5}$ also induced intracellular reactive oxygen species (ROS) generation accompanied with glutathione (GSH) depletion and the loss of mitochondrial membrane potential (MMP), and the ROS inhibitor, N-acetyl-L-cystein (NAC) suppressed the COX-2 overexpression and the increased fEPSPs. Furthermore, the nuclear factor kappa B (NF-κB) was involved in ROS-induced COX-2 and fEPSP in response to $PM_{2.5}$ exposure. These findings indicated that $PM_{2.5}$ activated COX-2 expression and enhanced the synaptic transmission through ROS-NF-κB pathway, and provided possible biomarkers and specific interventions for $PM_{2.5}$-induced neurological damage.

1. Introduction

Haze is a serious environmental issue in China and has become a relatively new threat to human health (Liu et al., 2013; Wang et al., 2015). According to survey data, fine particulate matter ($PM_{2.5}$) is the main contributor to haze pollutants and health hazards and is a complex mixture of various chemical constituents that can vary dynamically with time and place (Cheng et al., 2013). It is widely studied as an important risk factor for neurological diseases and for respiratory and cardiovascular pathology (Weber et al., 2016; Kioumourtzoglou et al., 2016; Zanobetti et al., 2014). It has been suggested that various components of PM, such as ultrafine particles and chemical components, can translocate to the brain and activate adverse biological responses (Block, 2009; Heusinkveld et al., 2016; Solaimani et al., 2016). In fact, epidemiological investigations and laboratory studies show that long-term $PM_{2.5}$ exposure increases the risk of incident ischemic stroke in older people (Qiu et al., 2017), accelerates the loss of both gray matter and

Abbreviation: $PM_{2.5}$, Fine particulate matter; COX-2, Cyclooxygenase-2; ROS, Reactive oxygen species; fEPSP, Field excitatory postsynaptic potential; MMP, Mitochondrial membrane potential; PG, Prostaglandin; AD, Alzheimer's disease; PD, Parkinson's disease; PI3K, Phosphoinositide 3-kinase; AKT, Protein kinase B; ERK, Extracellular signal-regulated kinase; NF-κB, Nuclear transcription factorkappa B; IKKβ, Inhibitor of nuclear factor kappa-B kinase; GSH, Glutathione; CNS, Central nervous system; ChIP, Chromatin immunoprecipitation; CCK-8, Cell counting kit-8; LDH, Lactate dehydrogenase; ACSF, Artificial cerebrospinal fluid; NAC, N-Acetyl-L-cysteine.

* Corresponding author. College of Environment and Resource, Shanxi University, Taiyuan, Shanxi 030006, PR China
E-mail address: sangnan@sxu.edu.cn (N. Sang).

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white matter in older women (Casanova et al., 2016), and even damages brain development and clinical cognitive behavior in children (Calderon-Garciduenas et al., 2016). Further studies suggest that oxidative stress, neuroinflammation and synaptic dysfunction are critical events in the progression of various neurodegenerative insults in response to $PM_{2.5}$ exposure. However, the relevant mechanisms of research are rarely reported.

Cyclooxygenase-2 (COX-2) is a key rate-limiting enzyme that converts arachidonic acid (AA) into prostaglandin (PG)H_2, which can be further metabolized to prostanoids, including PGE_2, PGI_2, and thromboxane A_2 (Yagami et al., 2016). These PGs are involved in brain inflammation, and PGE_2 can stimulate the release of glutamate and enhance excitatory synaptic transmission (Sang et al., 2005). Glutamate-mediated excitatory synaptic transmission plays an important role in neural development, differentiation and synaptic plasticity. Thus, COX-2 is not only a key player in neuroinflammation but is also a multifunctional neuronal modulator and has been implicated in the pathogenesis of the central nervous system (CNS) diseases (*e.g.,* stroke, Alzheimer's disease (AD), Parkinson's disease (PD)) (Kaufmann et al., 1996). Meanwhile, experimental evidence indicates that exposure to PM_{10}, cigarette smoke or diesel exhaust particulate elevates the level of COX-2 protein in the brain (Guo et al., 2012; Huang et al., 2013; van Berlo et al., 2010). Prolonged inhalation of $PM_{2.5}$ stimulates the brain inflammatory reaction and promotes the development of early AD-like pathology by inducing a range of inflammatory factors, including COX-2 (Bhatt et al., 2015). However, the role of COX-2 in $PM_{2.5}$-induced inflammation and synaptic damage has not yet been investigated.

Reactive oxygen species (ROS) and nuclear transcription factor kappa B (NF-κB) play a key role in the biological effects of $PM_{2.5}$ (Lakey et al., 2016; Morgan and Liu, 2011). The latest research also shows urban PM induced COX-2/PGE_2 release in human fibroblast-like synoviocytes by activating ROS and NF-κB (Tsai et al., 2017). In the present study, we determined whether and how COX-2-catalyzed PG production participated in $PM_{2.5}$-caused neuroinflammation and synaptic dysfunction. Here, we show that $PM_{2.5}$ elevated COX-2 expression, enhanced PGE_2 release, and increased the amplitude of field excitatory postsynaptic potential (fEPSP) in hippocampal brain slices and that the action was involved in the ROS-NF-κB pathway.

2. Material and methods

2.1. $PM_{2.5}$ collection and characterization

$PM_{2.5}$ was collected according to our previous report (Guo et al., 2012). Briefly, $PM_{2.5}$ were trapped on quartz filters (Φ90 mm, Munktell, Falun, Dalarna, Sweden) using a $PM_{2.5}$ middle-volume air sampler (TH-150CIII, Wuhan, China) at 100 L/min. The sampling points were chosen at Taiyuan (112°21–34′E longitude, 37°47–48′N latitude) in Shanxi Province. Then, $PM_{2.5}$ was transferred to a sterile aqueous suspension by vortexing and sonication. Aqueous suspensions of $PM_{2.5}$ were freeze-dried and stored at −20 °C. The $PM_{2.5}$ extracts were resuspended with sterilized saline and sonicated for 30 min prior to use. The shape, size, and surface morphology of $PM_{2.5}$ were analyzed by high-resolution scanning electron microscope (SEM, Quanta FEG450, Eindhoven, Netherland) and a transmission electron microscope (TEM, Hitachi H-7650, Tokyo, Japan).

2.2. Primary hippocampal neuron culture and $PM_{2.5}$ exposure

According to the previously reported protocol (Li et al., 2015), hippocampus tissue was separated from postnatal day 1 mice (C57BL/6, Hebei medical university, China), incubated in sterile trypsin with shaking (37 °C, 10 min) and then separated into individual cells by mechanically triturating. The cells were slowly centrifuged to the bottom (1000 rpm/min, 8 min) and resuspended with neurobasal/B27 medium (Invitrogen, USA) supplemented with 1.25 μM glutamine, 5 μg/mL insulin, 25 μM glutamate and penicillin/streptomycin. Half to a third of the medium was replaced every three days with neurobasal medium without glutamate. The cells (5×10^5) were plated into poly-L-lysine-coated 35-mm culture dishes for western blot analysis. The cells (2×10^4) were plated on poly-L-lysine-coated 96-well plates for the other experiments. The control group was incubated in neurobasal/B27 medium, and the other groups were treated with $PM_{2.5}$ for 24 h in the absence or presence of the ROS inhibitor NAC (0.5 mM, Sigma-Aldrich, Italy) or the NF-κB inhibitor, SC-514 (50 μM, Cell Signal, USA).

2.3. Cell counting Kit-8 (CCK-8) and lactate dehydrogenase (LDH) release assays

Cell viability was assessed by the CCK-8 assay (Dojindo, Japan) (Zhou et al., 2016). Briefly, 20,000 neuron cells per well were seeded in 96-well plates and exposed to different concentrations of $PM_{2.5}$ for 24 h. Then, the $PM_{2.5}$ suspension was replaced with an equal volume of fresh medium containing 10% CCK-8, followed by 3-h incubation at 37 °C. The absorbance was determined at 450 nm on a micro-plate reader (Multiskan MK3, Thermo Fisher Scientific, USA).

Lactate dehydrogenase (LDH) was an indicator of cell viability and membrane integrity and was detected to assess the extent of cellular damage (Zhou et al., 2016). Briefly, culture medium was centrifuged at 1000 rpm for 5 min and then incubated with the prepared reagent mixture at room temperature for 20 min according to the manufacturer's protocol (Jiancheng Biotechnology, Nanjing, China). The absorbance was determined at 450 nm on a micro-plate reader (Multiskan MK3, Thermo Fisher Scientific, USA).

2.4. Hippocampal slices preparation and electrophysiological recordings

Hippocampal slices from mouse were prepared as previously described protocols (Li et al., 2015). Briefly, the mice were sacrificed, and the brains were separated and kept in cold oxygenated artificial cerebrospinal fluid (ACSF) for 1–2 min to protect the brain activity. The slices (350–400 μm) were prepared with a vibrating microtome (Leica VT1200S, Nussloch, Germany), then transfered to oxygenated ACSF at 34 °C for 0.5–1 h to restore the cutting damage, and then incubated at room temperature for 1 h to prepare for recording. Field EPSP recordings were made in response to stimulation of the schaffer collateral at a frequency of 0.05 Hz using an Axoclamp-2B patch-clamp amplifier (Molecular Devices, Silicon Valley, USA) in bridge mode. Recording pipette was pulled from borosilicate glass with a micropipette puller (Sutter Instruments, Novato, USA), filled with ACSF and placed in the stratum radiatum of the CA1 region of the hippocampus. The control group was perfused with ACSF, and the treatment group were perfused with NS398 (20 μM), NAC (0.5 mM) or SC-514 (50 μM) for 30 min prior to $PM_{2.5}$.

2.5. Western blot analysis

The hippocampal neurons were lysed in lysate buffer for 20 min, and the proteins were extracted and quantified according to our previously reported protocols (Li et al., 2015). Briefly, 30–50 μg of total protein was dispensed and mixed with 1/4 vol sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) loading