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Overexpression of HO-1 assisted PM2.5-induced apoptosis failure and autophagy-related cell necrosis

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ABSTRACT

Severe smog/haze events accompanied by extremely high concentrations of airborne fine particulate matter (PM2.5) have emerged frequently in China and the potential health risks have attracted ever-growing attention. During these episodes, a surge in hospital visits for acute respiratory symptoms and respiratory diseases exacerbation has been reported to be associated with acute exposure to high-levels of particulate matters. To investigate cell fate determination and the underlying pathogenic mechanisms during severe haze episodes or smog events, we exposed human lung epithelial cells (BEAS-2B) to PM2.5 (0–400 μg/mL) for 24 h and found that high doses of PM2.5 caused cell necrosis and autophagy dysfunction, while co-treatment with the autophagy inhibitor 3-MA could partially reduce PM2.5-induced cell necrosis. Exposure to PM2.5 also increased the expression and mitochondrial transposition of heme oxygenase 1 (HO-1), which consequently reduced the release of cytochrome C from mitochondria to cytosol. Knockdown of HO-1 by siRNA attenuated the mitochondrial accumulation of HO-1, reversed HO-1-induced the reduction of cytochrome C release and promoted PM2.5-induced cell apoptosis. In contrast to necrosis, PM2.5-induced autophagy was independent of HO-1. In conclusion, our results demonstrate that acute exposure to high PM2.5 concentrations causes autophagy-related cell necrosis. The decrease in cytochrome C release and apoptosis by upregulation of HO-1 maybe assist PM2.5-induced autophagy-related cell necrosis. Further, this study reveals dual roles for HO-1 in PM2.5-induced cytotoxicity and presents a possible explanation for the onset of acute respiratory symptoms under extreme particulate air pollution.

1. Introduction

Ambient particulate matter air pollution has become an urgent environmental and public health issue in low- and middle-income countries, including China. In recent years, the adverse health effects of ambient particulate matter exposure, especially fine particulate matter (PM2.5) exposure have gained increasing attention in China due to the frequent occurrence of severe smog/haze episodes (Zhou et al., 2015a). In 2010, around 1.2 million early deaths and loss of 25 million disability-adjusted life-years were attributed to PM2.5 in China (Meng et al., 2016). Numerous epidemiological studies have documented that both short-term and long-term exposure to PM2.5 can increase the risks of pulmonary diseases, coronary diseases and even lung cancer (Burnett et al., 2014; Kim et al., 2015).

During severe smog/haze events, acute exposure to hazardous levels of fine particulate matter air pollution obviously increases emergency ambulance dispatches and hospital visits (Tasmin et al., 2016; Zhang et al., 2016). Most patients visit doctors for the onset of acute respiratory symptoms or the exacerbation of cardiopulmonary diseases triggered by PM2.5 (Zhang et al., 2016; Zhou et al., 2015a). Severe oxidative stress and inflammatory responses are recognized as critical factors in the development of these diseases under those extreme circumstances (Liu et al., 2014; Zhou et al., 2015b). Our previous study also demonstrates that exposure to high concentrations of PM2.5 causes extensive production of reactive oxygen species (ROS), oxidative damage and cells death (under review). But the precise pathogenic mechanisms involved are not fully understood.

Excessive generation of ROS and ensuing oxidative damage are implicated in respiratory cell dysfunction induced by fine particulate matter (Lee et al., 2016; Orrenius et al., 2007). Generally, apoptosis, necrosis and autophagy are the three main modes of cell death (Mughal et al., 2012). Apoptosis is a highly controlled process triggered by

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cytochrome C release from mitochondria, resulting in activation of caspase family proteins, the main effectors of downstream apoptotic events such as cell shrinkage, chromatin condensation, and the formation of apoptotic bodies (Mahdavi et al., 2011). All apoptosis-related products are quickly removed by phagocytic cells, resulting in little damage or local inflammation (Elmore, 2007). Although necrosis was initially considered as an indiscriminant accidental form of cell death by acute cellular injury, recent studies have evidenced that necrosis could be another form of programmed cell death (Mughal et al., 2012). Necrotic cells exhibit swelling and loss of cell membrane integrity, causing the release of cellular contents into the surrounding tissue and initiation of acute inflammatory responses (Degenhardt et al., 2006). Finally, autophagy-related cell death is a well-conserved and highly regulated process of degradation and recycling of unnecessary or dysfunctional components through autophagosome generation and lysosomal degradation (Kroemer et al., 2009; Yang and Klionsky, 2010). Previous studies showed that autophagic cells eventually die through apoptosis or necrosis (Caruso et al., 2006; Nagakannan et al., 2016; Ullman et al., 2008). Particulate matter has been found to trigger cell apoptosis, necrosis, or autophagy depending on exposure conditions (Agopyan et al., 2004; Deng et al., 2014; Su et al., 2016), but the cellular factors determining the specific manner of cell death after acute exposure to severe PM2.5 pollution are still unclear.

Cells under ROS-mediated oxidative stress activate compensatory antioxidant defense systems. Heme oxygenase 1 (HO-1), an important limiting enzyme in heme catabolism involved in the degradation of heme into carbon monoxide (CO), iron and biliverdin, and also acts as an inducible antioxidant defense mechanism in response to various stressors, such as infection, xenobiotic pollution, and ischemia-reperfusion injury (Abdalla et al., 2015; Morse et al., 2009). Cigarette-smoke, diesel exhaust particles and nanomaterials have been shown to increase HO-1 expression in normal human lung epithelial cells (BEAS-2B), and the enhanced HO-1 plays a critical role in antioxidant, anti-inflammation and cytoprotection (Eom and Choi, 2009; Kim et al., 2008; Totlandsdal et al., 2015). Similarly, overexpression of HO-1 and its enzymatic products confer protection against multiple noxious stimuli, including hypoxia, cigarette-smoke, and cadmium, by inhibiting cell apoptosis (Kim et al., 2015; Morse et al., 2009). When apoptosis is inhibited, cells may still die, but through the other two pathways (Bustamante-Marin et al., 2012), inhibition of apoptosis does not necessarily confer systemic benefits to the tissue. However, comparatively little is known about the role of HO-1 in BEAS-2B cell protection against PM2.5-induced toxicity.

In our current study, we examined the mode of cell death to investigate the underlying pathogenic mechanisms during severe haze episodes or smog events. We also detected the expression of HO-1 in BEAS-2B epithelial cells under acute exposure to high levels of PM2.5, which helped to elucidate the role of HO-1 in PM2.5-induced cell death. Our results would provide a plausible explanation for PM2.5-induced acute respiratory syndromes during severe smog/haze episodes.

2. Methods and materials

2.1. PM2.5 sampling, extraction and cell treatment

Details on PM2.5 collection, extraction, and characterization were well described in our previous articles (Zhou et al., 2016). Briefly, PM2.5 was collected with glass fiber filters in Wuhan (Hubei Province, China), then extracted and stored at − 20 °C until use. Prior to cell treatment, fine particulate matter was re-suspended in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) containing 2% fetal bovine serum (FBS, Gibco) and sonicated for 30 min. The normal human bronchial epithelial cell line BEAS-2B was cultured in DMEM containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C under 5% CO_2 atmosphere. For exposure assays, BEAS-2B cells were treated with PM2.5 (0, 25, 50, 100, 200 or 400 μg/mL) for 24 h. For inhibition experiments, the autophagy inhibitor 3-methyladenine (3-MA) (5 mM, Sigma, USA) or the HO-1 inhibitor Sn (IV) Protoporphyrin IX dichloride (SnPP, 20 μM, Frontier Scientific, USA) was co-treated with particulate matter for 24 h.

2.2. Flow cytometry

After exposure to PM2.5, BEAS-2B cells were trypsinized and collected for apoptosis and necrosis detection using Annexin V/PI staining. Harvested cells were washed with 1 mL PBS, centrifuged at 1000 × *g* for 5 min, and resuspended in 195 μL Annexin V-FITC binding buffer (Beyotime, China). The suspension was labeled with Annexin V (5 μL, Beyotime, China) and PI (10 μL, Beyotime, China) in darkness at room temperature for 15 min and stored at 4 °C in the dark until FACS analysis (Becton Dickinson, USA).

2.3. Transmission electron microscopy (TEM)

Treated cells were harvested and immediately fixed in 3% glutaraldehyde overnight at 4 °C, followed by 3 times washes with 0.1 M PBS and 2 h post-fixation in osmic acid at room temperature. After another 3 washes with 0.1 M PBS, the cells were dehydrated in a graded alcohol series (50%, 70%, 80%, 90% and 100%) and embedded in epoxy resin. Then ultrathin serial sections (50 nm) were cut from the embedded samples, stained with uranyl acetate and lead citrate, and prepared for TEM (Hitachi H-7650, Japan).

2.4. Confocal microscopy

Cells (0.5×10^4 per dish) were plated into 35-mm dishes and exposed to PM2.5 (400 μg/mL). After 24 h of treatment, cells were washed with PBS and loaded with MitoTracker probe (red) (200 nM, ThermoFisher, USA) at 37 °C for 30 min. Cells were washed to removed excess probe and fixed with 1 mL 10% paraformaldehyde at room temperature for 15 min in the dark. Fixed cells were blocked with Immunol Staining Blocking Buffer containing Triton X-100 (Beyotime, China) overnight at 4 °C. Subsequently, cells were incubated with primary antibody against HO-1 (1:150, Abcam) at 4 °C for about 8 h, followed by 3 washes with PBS and 1 h incubation with secondary antibodies conjugated with Alexa Fluor 488 or 546 (1:1000, Beyotime, China). Nuclei were counterstained with 4′, 6-diamidino-2-phenyindole, dilactate (DAPI) (5 μg/mL, Sigma, USA) for 7 min. Finally, cells were inspected using a confocal microscopy (Carl Zeiss, Germany).

2.5. Immunofluorescence microscopy

For immunofluorescence analysis, cells (1×10^4 per well) were seeded in 6-well plates containing coverslips and exposed to PM2.5 as indicated. After 24 h treatment, culture medium was removed and cells were incubated with the specific autophagolysosome marker monodansylcadaverine (MDC) (50 μM, Sigma) at 37 °C for 30 min. After 3 washes with PBS, cells were stained with PI (1:500 dilution, Beyotime) at 37 °C for another 30 min. The stained cells were observed by fluorescence microscopy (Leica DMI3000B, Germany).

2.6. siRNA transfection

To silence *HO-1* expression, cells (2×10^5 per well) were plated in 6-well plates and transiently transfected with 70 nM of small interfering oligonucleotide (siRNA) against *HO-1* (sc-35554, Santa Cruz Biotechnology, USA) or control non-specific oligonucleotide (sc-37007, Santa Cruz Biotechnology) using lipid-based transfection system (Lipofectamine 3000, ThermoFisher) according to the manufacturer's protocol. 24 h later, the silencing efficiency of *HO-1* siRNA was evaluated by RT-PCR using primers pairs 5′-TCTTGGCTGGCTTCCTTACC-3′ and 5′-GGATGTGCTTTTCGTTGGGG-3′ for *HO-1* and 5′-