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RESEARCH ARTICLE

Effect of PM2.5 environmental pollution on rat lung

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Abstract

Particulate matter smaller than 2.5 μm (PM2.5) is a continuing challenge to pulmonary health. Here, we investigated the mechanisms involved in PM2.5 exposure-induced acute lung injury in rats. We analyzed biochemical and morphological changes following a 2-week "real-world" exposure. And then we found that PM2.5 exposure increased the concentrations of total protein, malondialdehyde, hydrogen peroxide, nitric oxide, and soluble elastin in bronchoalveolar lavage fluid, levels of cytokines in blood, and expression of MMP-9 in airways. Further, alveolar macrophage and neutrophil counts increased following PM2.5 exposure, and edema and lung lesions were observed. Our results suggest that PM2.5 exposure can induce oxidative stress and acute inflammatory responses, which can damage the micro-environment and decrease the repair ability of the lung, resulting in tissue damage.

Keywords PM2.5 · Lung · Injury · Inflammation · Oxidative stress · Extracellular matrix

Introduction

Airborne particulate matter (PM) smaller than 2.5 μm (PM2.5) is generated by various sources and is therefore a heterogeneous mixture of various substances. PM2.5 is a continuing challenge to public health, and epidemiological studies have linked PM2.5 to adverse health effects, such as pulmonary and cardiovascular diseases (Beelen et al. 2015; Pope et al. 2009; Shah et al. 2013; Líbalová et al. 2014). The complex mechanisms underlying PM2.5-induced health effects require further study.

Multiple experimental studies have been conducted to elucidate the effects of PM2.5 on human health and the mechanism of its toxicity (Xu et al. 2016; Kim et al. 2017; Riva et al. 2011; Wang et al. 2017). Because the constituents of PM2.5 are known to vary substantially between regions and sources, affecting toxicity, many studies have analyzed PM2.5 composition in different regions, and investigated the function of signaling pathways involved in redox homeostasis and inflammation (Li et al. 2015; Wang et al. 2013; Kumar et al. 2015). However, our knowledge of the molecular and cellular mechanisms underlying PM2.5-associated systemic diseases remains incomplete.

We used a "real-world" PM2.5 system to perform a mouth-nose exposure of rats to environmentally relevant PM2.5 or neutral saline (NS). To elucidate the molecular basis of ambient PM2.5-associated cytotoxicity, we investigated biochemical markers following exposure. We hypothesized that the PM2.5-induced stress response relies on the production of reactive oxygen species (ROS) and regulates the morphological remodeling. The findings on PM2.5-induced pathological changes such as cytotoxicity, leukocyte infiltration, pro-inflammatory cytokines, oxidative stress, damage to the alveolar epithelium-capillary barrier, and vascular permeability will not only contribute to a better understanding of molecular and cellular mechanisms by which PM2.5 elicits its effects, but will also inform the prevention and treatment of air pollution-induced systemic diseases.

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Materials and methods

Animals

Wistar rats that were obtained from Vital River, CP (Beijing, China), were used at 6~8 weeks of age (180~220 g), and they were randomly divided into PM2.5-exposed group and a control group. All studies were performed according to protocols reviewed and approved by the Ethics Committee of Animal Care and Experimentation of the National Institute for Environmental Studies, China. Wistar rats received the same food and water and were kept at the same ambient conditions of temperature, humidity, and noise. After 2 weeks acclimation, all rats were executed according to study design (Fig. 1).

PM2.5 sampling

PM2.5 samples were prepared as described in our previous studies, and the analysis of PM2.5 composition was performed (Ma et al. 2015). Briefly, using a high-volume sampler particle collector equipped with a Hi-Vol PM2.5 inlet (Tisch Environmental, USA) at a constant flow rate of 1.13m^3/min, PM2.5 samples were collected on nitrocellulose filters (Waterman, USA) in the center of the downtown area of ShenYang, China. We confirmed that heavy metals and polycyclic aromatic hydrocarbons are the compositions of PM2.5.

PM2.5 administration

Using the Mouth-nose Exposed system (Beijing Huironghe Technology Co., Led, China), the experimental group animals were exposed to concentrated PM2.5 at nominal 10 ambient concentrations of 750 μg/m^3 for 4 h per day, 5 days per week for a total of 2 weeks, and the control group animals were treated with similar condition. Twenty-four hours following the last exposure, animals were euthanized and BAL fluid and tissue samples were collected for further analysis.

Lung harvesting, tissue processing, and H&E staining

The lungs were inflated and fixed with 10% neutral formalin overnight at room temperature. Lung tissue was dehydrated with increasing ethanol (EtOH) concentrations and then embedded in paraffin. Five-micrometer-thick paraffin sections were stained with hematoxylin and eosin (H&E). Five-micron sections were placed on slides and stained with H&E in order to observe cell morphology.

Transmission electron microscopy insection

We performed the transmission electron microscopy insection as previous described (Wang et al. 2013). Two apexes of the left lungs were selected and immersed in 2.5% glutaraldehyde

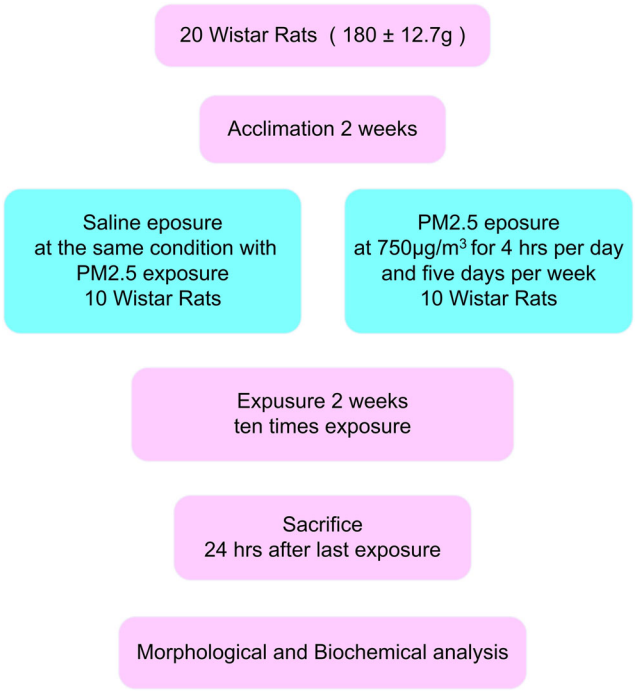


Fig. 1 Study design

at 4 °C. After washing with phosphate buffer solution sufficiently, they were fixed with 1% osmium tetroxide, dehydrated in a graded series of ethanol, embedded in araldite and polymerized for 24 h at 37 °C. Ultrathin sections (50–60 nm) were cut with ultramicrotome (LKB-I, Sweden). Thin sections were mounted directly on copper grids, stained with lead citrate, and observed with H-7650 electron microscopy (HITACHI, Japan).

Bronchoalveolar lavage fluid collection and cell count

Following removal of the lung's lower right lobe, bronchoalveolar lavage (BAL) fluid was collected by flushing 5 × 5 ml of phosphate buffered saline (PBS) containing 0.1 mM EDTA into the lung via a tracheal cannula. The pooled BAL fluid was centrifuged at 500×*g* at 4 °C for 5 min. Pelleted cells were then resuspended in 2 ml of PBS. Total cell number was counted by hemocytometer and a differential cell count was performed by cytospin staining with Diff-Quik (Siemens, Newark, China).

BAL fluid protein

Increase in BAL fluid protein concentration was taken as a measure of increased permeability of alveolar-capillary barriers. Total protein concentration in the supernatant following BAL fluid centrifugation was determined using the BCA Protein Assay kit (Thermo Fisher Scientific, MA, USA).