

## Brief Communication

# Real-world exposure of airborne particulate matter triggers oxidative stress in an animal model

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**Abstract:** Epidemiological studies have shown a strong link between air pollution and the increase of cardio-pulmonary mortality and morbidity. In particular, inhaled airborne particulate matter (PM) exposure is closely associated with the pathogenesis of air pollution-induced systemic diseases. In this study, we exposed C57BL/6 mice to environmentally relevant PM in fine and ultra fine ranges (diameter < 2.5  $\mu\text{m}$ , PM<sub>2.5</sub>) using a "real-world" airborne PM exposure system. We investigated the pathophysiologic impact of PM<sub>2.5</sub> exposure in the animal model and in cultured primary pulmonary macrophages. We demonstrated that PM<sub>2.5</sub> exposure increased the production of reactive oxygen species (ROS) in blood vessels *in vivo*. Furthermore, *in vitro* PM<sub>2.5</sub> exposure experiment suggested that PM<sub>2.5</sub> could trigger oxidative stress response, reflected by an increased expression of the anti-oxidative stress enzymes superoxide dismutase-1 (SOD-1) and *heme oxygenase-1*(HO-1), in mouse primary macrophages. Together, the results obtained through our "real-world" PM exposure approach demonstrated the pathophysiologic effect of ambient PM<sub>2.5</sub> exposure on triggering oxidative stress in the specialized organ and cell type of an animal model. Our results and approach will be informative for the research in air pollution-associated physiology and pathology.

**Keywords:** Air pollution; airborne particulate matter; oxidative stress; reactive oxygen species; animal model

## Introduction

Air pollution is a sustained problem of public health for the general population in urban areas, especially for those who live in areas of intensive traffic or industrial activity. Accumulating evidence has suggested a significant association between exposure to airborne PM and the increase of morbidity and mortality associated with cardiovascular diseases [1-5]. Airborne PM<sub>2.5</sub> is a complex mixture of chemical and/or biological elements, such as metals, salts, carbonaceous material, volatile organic compounds, polycyclic aromatic hydrocarbons and endotoxins, depending upon their natural and/or anthropogenic emission sources [6, 7]. Particularly, airborne PM in fine and ultrafine ranges (diameter < 2.5  $\mu\text{m}$ , PM<sub>2.5</sub>) has been implicated to play a detrimental role in the pathogenesis of the disease. It has been demonstrated that PM<sub>2.5</sub> has an incremental capacity to penetrate the most distal airway units and potentially into systemic circulation with dimin-

ishing sizes [8, 9]. Recent studies from different experimental systems have suggested that the impacts of airborne PM on signaling pathways related to redox homeostasis and inflammation [5, 10-13]. Our study here provides new evidence that "real-world" exposure of environmentally relevant PM<sub>2.5</sub> can trigger oxidative stress in blood vessels in an animal model and in cultured primary pulmonary macrophages.

Our PM exposure system, the "Ohio's Air Pollution Exposure System for the Interrogation of Systemic Effects (OASIS-1)", is a mobile trailer versatile aerosol concentration enrichment system [14-16]. In this system, only PM<sub>2.5</sub> particles, which include fine and ultrafine particles, are concentrated from the ambient air and exposed to the in-house-bred mice [15, 17, 18]. It has been demonstrated that the PM<sub>2.5</sub> particle size and composition distribution did not change before (ambient) and after the VACES system [17, 18]. This system is one of several limited prototypic systems in the United States, which

allows us to use animal models to recapitulate real world exposure to environmental relevant PM<sub>2.5</sub>, and to perform systemic analysis of PM<sub>2.5</sub>-associated physiology and pathology.

### Materials and methods

#### Animals

Male mice of C57BL/6 strain background at six-week-old were purchased from the Jackson Laboratories (Bar Harbor, ME), and were equilibrated for 2 weeks prior to the exposure experiment. The mice were housed in cages with regular chow. The Committees on Use and Care of Animals at the Ohio State University approved all experimental procedures.

#### Exposure to ambient PM<sub>2.5</sub>

Animals were exposed to concentrated ambient PM<sub>2.5</sub> or filtered air (FA) for a total duration of 10 weeks in a mobile trailer "Ohio's Air Pollution Exposure System for the Interrogation of Systemic Effects (OASIS)-1" composed of the mid-western regional background in Columbus, OH, on the Ohio State University campus. The concentrated PM<sub>2.5</sub> in the exposure chamber was generated using a versatile aerosol concentration enrichment system [14, 15]. The mice were exposed to PM<sub>2.5</sub> at nominal 10 times of ambient concentrations for 6 hours per day, 5 days per week (Monday to Friday) for a total period of 10-weeks [4, 16]. The control mice were exposed to an identical protocol in filtered air chambers in which a high-efficiency particulate-air (HEPA) filter positioned in the inlet valve position to remove all the PM<sub>2.5</sub>.

#### Primary macrophage isolation, culture, and *in vitro* exposure to PM<sub>2.5</sub>

C57BL/6 mice at 3-month of age were anesthetized with pentobarbital. A 5ml-syringe with a blunt needle containing 1.5 ml PBS (Ca/Mg free) was inserted into the lung through the trachea for flushing off the bronchoalveolar macrophages. Isolated macrophages were cultured in FG12 media supplemented with L-glutamine and 10% fetal bovine serum. For *in vitro* PM<sub>2.5</sub> exposure experiment, PM<sub>2.5</sub> particles were collected at the time when the mice were exposed. The stock PM<sub>2.5</sub> solution (5 mg/ml in PBS) was stored in -80 °C. The frozen PM<sub>2.5</sub> aliquots were thawed and briefly sonicated before adding into

macrophage cell culture media for the *in vitro* experiment. Macrophages were treated with PM<sub>2.5</sub> at the concentration of 50 µg/ml, and the same volume of PBS was added as the control.

#### Quantitative real-time reverse-transcription (RT)-PCR analysis

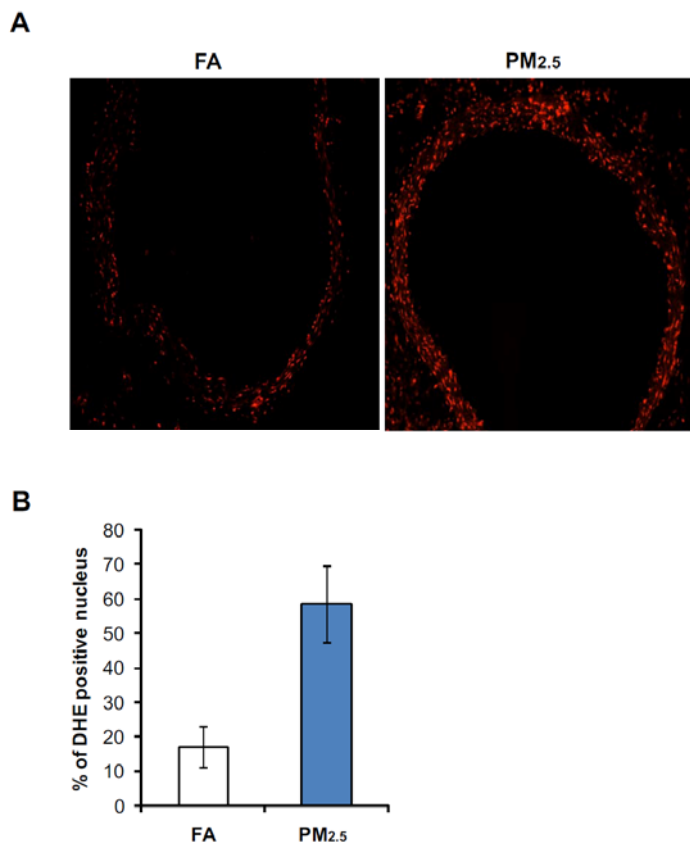
Total cellular RNA was prepared using TRIzol reagent as instructed by the manufacturer (Invitrogen Corp.). Total RNA was reverse-transcribed to cDNA using a random primer (Applied Biosystems). The quantitative real-time PCR analysis was performed with a Stratagene MX3000P Real-Time PCR System (Stratagene) following the standard procedure [19]. Real-time PCR primer sequences are: *Heme oxygenase 1* (HO-1): 5'-CACGCATATACCCGCTACCT-3' and 5'-CCAGAGTGTTTCATTCGAGA-3'; superoxide dismutase 1 (SOD-1): 5'-GCGGTGAAC CAGTTGTGTTGTC-3' and 5'-CAGTCACATTGCCCA GGTCTCC-3'. Fold changes of mRNA levels were determined after normalization to internal control  $\beta$ -actin RNA levels.

#### Dihydroethidium (DHE) fluorescence staining of liver tissue

DHE (Sigma-Aldrich), an oxidative fluorescent dye, was used to detect superoxide (O<sub>2</sub><sup>-</sup>) in segments of frozen carotid artery as described previously [20]. Briefly, fresh segments of the common carotid artery were frozen in OCT compound, and transverse sections (10 µm) were generated with a cryostat and placed on glass slides. Sections were then incubated in chamber with 10 µmol/L DHE (Molecular Probes) for 30 minutes at room temperature. Images were obtained with a fluorescent microscope. The excitation wavelength was 488 nm, and emission fluorescence was detected with the use of a 585 nm filter.

### Results and discussion

To investigate the pathophysiological impact of airborne PM<sub>2.5</sub> exposure, in-house bred C57BL/6 mice were exposed to concentrated ambient PM<sub>2.5</sub> for 10 weeks in the mobile trailer "OASIS-1" exposure system located in Columbus (Ohio State University campus), Ohio, where most of the PM<sub>2.5</sub> was attributed to long-range transport [4, 16]. The State of Ohio is one of many "perfect" states to study the effects of PM<sub>2.5</sub> on human health, since "Ohio has a seri-

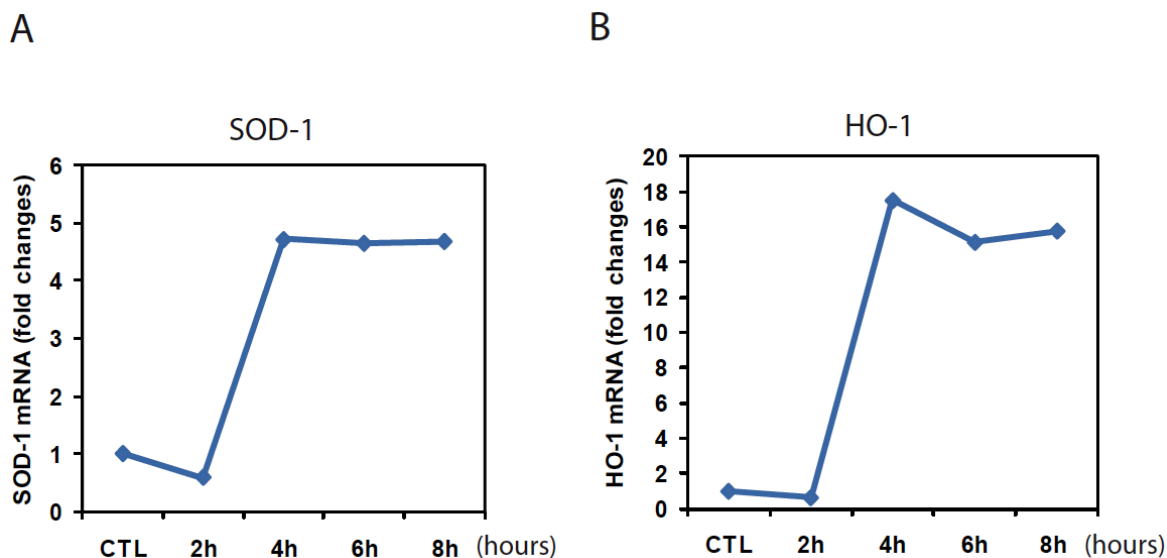


**Figure 1.** Exposure to PM<sub>2.5</sub> cause increased ROS in mouse blood vessel. (A-B) DHE staining of aortic tissue sections from the mice exposed to PM<sub>2.5</sub> or FA for 10 weeks. Frozen aortic tissue sections were stained with DHE (10 μmol/L). The oxidative red fluorescence was analyzed by a fluorescent microscope. (C) DHE signals were quantified by counting the number of positive stained nuclei in 10 random fields. Microscopic interference contrast was used to exclude positive signals from non-cell origin. The percentages of DHE-positive nuclei (compared to total nuclei) were shown. Data are shown as mean ± SEM for 6 animals per group.

ous and widespread air pollution problem, and had failed to meet the National ozone and particulate matter annual standards” [21]. The ambient PM<sub>2.5</sub> in Columbus is representative of regional background PM<sub>2.5</sub> of the megalopolis that extends from Detroit to Cincinnati. Traffic-related PM<sub>2.5</sub> is a complex mixture of particles and gases from gasoline and diesel engines, together with dust from wear of road surfaces, tires, and brakes [6, 7]. The composition analysis indicated that the distribution of concentrated PM<sub>2.5</sub> collected from the exposure system can truly reflect that of non-concentrated PM<sub>2.5</sub> present in the ambient air (data not shown) [16]. During the exposure period, the mean PM<sub>2.5</sub> concentration inside the exposure chamber was 74.6 μg/m<sup>3</sup>. Because the mice were exposed 6 hours a day, 5 days a week, the equivalent PM<sub>2.5</sub> concentration to which the mice were exposed in the chamber “normalized” over the 10-week period was 11.6 μg/m<sup>3</sup>. These calculations have taken into account non-exposed time and weekends. The equivalent PM<sub>2.5</sub> exposure concentration in this study is well within the annual average PM<sub>2.5</sub> National Ambient Air Quality Standard of 15.0

μg/m<sup>3</sup> [21]. As controls, the mice were exposed to an identical protocol with the exception of HEPA filters installed in the PM chambers to remove all the PM<sub>2.5</sub>.

PM<sub>2.5</sub> has been shown to stimulate generation of reactive oxygen species (ROS) in the cells due to its features of small diameters and high surface area [4, 5]. To test whether PM<sub>2.5</sub> exposure can trigger ROS production *in vivo*, we examined the redox states in the blood vessels of mice exposed to PM<sub>2.5</sub> or filtered air (FA). To evaluate the *in situ* levels of superoxide (O<sub>2</sub><sup>-</sup>), we stained frozen mouse blood vessel tissue sections with DHE, an oxidative fluorescent dye. DHE staining showed that O<sub>2</sub><sup>-</sup> production was significantly increased in the aorta endothelium of PM<sub>2.5</sub>-exposed mice compared to that in FA-exposed mice (**Figure 1A-B**). O<sub>2</sub><sup>-</sup> activity in the aortic segments of mice exposed to PM<sub>2.5</sub> was approximately 3.5-times higher than that of the FA-exposed controls (**Figure 1B**). To further evaluate the effect of PM<sub>2.5</sub> on triggering oxidative stress, we exposed mouse primary macrophages to PM<sub>2.5</sub> collected from the “OASIS-1” system at the time when the mice were ex-



**Figure 2.** Expression of the mRNAs encoding the anti-oxidative enzymes SOD-1 and HO-1 was increased in the primary macrophages after PM<sub>2.5</sub> treatment. (A-B) Quantitative real-time RT-PCR analysis of the expression of mRNAs encoding SOD-1 and HO-1 in mouse primary macrophages in response to *in vitro* exposure to PM<sub>2.5</sub>. Primary macrophages were isolated from wild-type C57BL/6 mice. The primary macrophages were incubated with PM<sub>2.5</sub> particles (50 µg/mL), which were collected by the “OASIS-1” system at the time when the mice were exposed, for 2, 4, 6 or 8 hours. The macrophages were treated with the vehicle buffer PBS as the control (CTL). Fold changes of mRNA levels were determined after normalization to internal control  $\beta$ -actin RNA levels.

posed. Quantitative real-time RT-PCR analysis showed that expression of anti-oxidant enzymes, including SOD -1 and HO-1, was significantly increased in the primary macrophages after 4 hours of the PM<sub>2.5</sub> treatment (**Figure 2A-B**). Taken together, the results obtained with our “real-world” PM exposure system indicated that PM<sub>2.5</sub> is a trigger of oxidative stress *in vivo* and *in vitro*.

It has been proposed that several proposed biological pathways are involved in PM-associated adverse cardiovascular outcomes [5, 12, 22, 23]: (1) systemic inflammatory response induced by PM exposure that impacts blood vessels; (2) extra-pulmonary translocation of fine and ultrafine particles that activate vasculature; (3) ROS generated by particles that result in cardiovascular dysfunction; (4) central nerve system manipulation of cardiovascular function following PM exposure. Our study indicated that exposure to PM<sub>2.5</sub> indeed caused oxidative stress in blood vessels and in primary macrophages of the mouse model. Furthermore, our results also suggested that the “real-world” PM<sub>2.5</sub> exposure with this mouse model is a useful approach for investigating the pathophysiologic impact of PM<sub>2.5</sub> on the cardiovascular

system. Future studies with this system should provide significant insights into the role and mechanism of PM<sub>2.5</sub> on air pollution-associated physiology and pathology.

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