Environmental Pollution xxx (2017) 1–9

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Diesel exhaust inhalation exposure induces pulmonary arterial hypertension in mice☆

Jing Liu [a, c], Xiaoqing Ye [a, c], Dapeng Ji [a, c], Xiaofei Zhou [b, c], Cong Qiu [b, c], Weiping Liu [a, c], Luyang Yu [b, c, *]

[a] MOE Key Laboratory of Environmental Remediation and Ecosystem Health, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China
[b] Institute of Genetics, College of Life Sciences, Zhejiang University, Hangzhou 310058, China
[c] Research Center for Air Pollution and Health, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China

ARTICLE INFO

Article history:
Received 23 January 2017
Received in revised form
27 October 2017
Accepted 29 October 2017
Available online xxx

Keywords:
Air pollution
Diesel exhaust
Pulmonary arterial hypertension
Vascular remodeling
Inflammatory response

ABSTRACT

Diesel exhaust (DE) is one of the main sources of urban air pollution. An increasing number of evidence showed the association of air pollution with cardiovascular diseases. Pulmonary arterial hypertension (PAH) is one of the most disastrous vascular diseases, which results in right ventricular failure and death. However, the relationship of DE inhalation exposure with PAH is still unknown. In this study, male adult mice were exposed by inhalation to filtered ambient air (negative control), 10% O_2 hypoxia (PAH-phenotype positive control), 350 μg/m^3 particulate matter whole DE, or the combination of DE and hypoxic condition. DE inhalation induced PAH-phenotype accompanied with increased right ventricular systolic pressure (RVSP), right ventricle hypertrophy and pulmonary arterial thickening in a mouse model. DE exposure induced the proliferation of vascular smooth muscle cells (VSMCs) and apoptosis of endothelial cells in pulmonary artery. DE inhalation exposure induced an accumulation of CD45$^+$ lymphocytes and CD68$^+$ macrophages surrounding and infiltrating pulmonary arteriole. The levels of pro-inflammatory cytokines tumor necrosis factor (TNF-α), interleukin-6 (IL-6) and IL-13 produced by T helper 17 (Th17) and Th2 cells were markedly elevated in lung tissues of mice after DE inhalation exposure. Our findings suggest DE exposure induces PAH by activating Th17-skewed and Th2-droved responses, stimulating VSMCs proliferation and inducing endothelial cell apoptosis by the production of multifunctional pro-inflammatory cytokines, especially IL-6 and TNF-α. Considering the adverse impact of air pollution on health care, it is imperative to understand air pollution-induced susceptibility of progressive cardiopulmonary disease, such as PAH, and also elucidate critical mechanistic pathways which mediate pulmonary artery vascular remodeling and may serve as targets for preventive measures.

1. Introduction

Air pollution has become a serious problem in developing countries, such as China. Traffic exhaust emission is one of the main sources of urban air pollution. In China, the total annual vehicle emissions in 2013 have reached 0.37 million tons of fine particulate matter (PM2.5), 7.72 million tons of nitrogen oxides (NO_X), 27.4 million tons of carbon monoxide (CO), and 4.16 million tons of hydrocarbon (HC) (Wu et al., 2016). Compared with petrol engines, diesel engines produce more NO_X and aldehydes but far less CO (Sydbom et al., 2001). Diesel exhaust (DE) significantly contributes to traffic-related air pollution and it contains 20–100 times particulate matter (PM) than the emission from petrol engines (Stevens, 2008). A total of 23% inhalable PM is emitted from vehicle emission and the PM of DE accounts for 63% of total vehicle PM in Beijing, the largest industrial city in northern China (Beijing Environmental Protection Bureau, 2007).

There is a number of evidence that air pollution has adverse effects on human health. Epidemiological studies have reported the association of air pollution with cardiovascular diseases. Increased PM concentrations elevate the risk of stroke, ischemic heart disease

☆ This paper has been recommended for acceptance by David Carpenter.

* Corresponding author. Institute of Genetics and Regenerative Biology, College of Life Sciences, Zhejiang University, Hangzhou 310058, China.
E-mail address: luyangyu@zju.edu.cn (L. Yu).

https://doi.org/10.1016/j.envpol.2017.10.121
0269-7491/

and even arrhythmias not only in those who used to suffer cardiovascular disease before but also healthy people (Sydbom et al., 2001; Bell, 2012; Brunekreef and Holgate, 2002; Pope et al., 2004). It has been found that elevated concentration of PM was positively associated with the increase in hospital emergency room visits for cardiovascular diseases in Tianjin and Beijing, two large industrial cities in northern China (Guo et al., 2009, 2010). A recent study showed that long-term exposure to traffic-related air pollution can result in the increase of arterial blood pressure in European (Fuks et al., 2014). DE inhalation has been implicated in the pathogenesis of cardiovascular functions in some animal studies. For example, inhalation of DE induced a ventricular arrhythmia in a rat model of chronic ischemic heart failure (Anselme et al., 2007). DE inhalation impaired endogenous fibrinolysis and vasomotor function which increased thrombus formation and arterial stiffness in terms of augmentation index (Lundbaeck, 2009). DE exposure increased pulmonary protein leakage and expression of vascular atherogenic markers in male rats (Kodavanti et al., 2013). DE inhalation study in rats showed an anti-oxidant response in the pulmonary and cardiovascular tissues (Kooter et al., 2010).

Pulmonary arterial hypertension (PAH) is one of the most lethal cardiovascular diseases characterized by a sustained and marked elevation of pulmonary artery pressure (Schermuly, 2005), which results in right ventricular failure and death (Simonneau et al., 2009). The pathogenesis of PAH involves marked thickening of pulmonary arterial and an increase in right ventricular systolic pressure (RVSP). The proliferation of vascular smooth muscle cells (VSMCs) in pulmonary artery has been considered as one of the major causes for progressive pulmonary arterial remodeling (Satoh et al., 2014). Recently, some epidemiological studies showed a relationship between air pollution and elevation of pulmonary arterial pressure. A study reported that higher daily PM levels are related to increased pulmonary artery and right ventricular diastolic pressure in patients with severe heart failure (Rich et al., 2008). Another study found that chronic exposure of children to elevated PM2.5 was associated with increased mean pulmonary arterial pressure (Calderon-Garciduenas et al., 2007). An animal study showed that co-exposure to antigen and urban ambient PM2.5 induced severe pulmonary arterial thickening and increased RVSP in mice (Park et al., 2015). However, the relationship of DE inhalation exposure with PAH is still unknown. In this study, we examined the effects of DE inhalation exposure on the development of PAH in male mice model. We further assessed the pulmonary vascular remodeling and inflammatory response to explore the mechanism(s) involved in DE-induced PAH.

2. Materials and methods

2.1. Generation of DE

DE was generated by a 0.2-L diesel engine (TP168F; Tuopu Motors Ltd., Chongqing, China) using low-sulfur NO.0 diesel fuel under a steady-state condition with 3000 rpm engine speed for 5 h/day. The exhaust was immediately diluted with filtered ambient air (FA) by a ratio of 1:10. The exposure air was supplied after passing through the dilution system. The control chamber received clean FA filtered by a high efficiency particulate air (HEPA) filter. The total suspended particulates (TSP) were collected on 47-mm Pallflex filters and TSP mass concentrations were measured using the standard filter weighing method. The filter based TSP mass data were utilized for monitoring and controlling TSP concentrations during exposures. Chambers were monitored continuously for the concentrations of NO, NO_2, CO and SO_2 using gas analyzers (Thermo Fisher Scientific, USA). The exposure targets were set to achieve DE TSP concentrations of 350 μg/m^3 and the air conditions in exposure chambers were shown in Table 1.

Table 1
Air condition in exposure chamber.

	FA	DE
Air velocity	40 L/min	40 L/min
Dilution ratio	NA	10
TSP (μg/m^3)	12.77 ± 9.46	345 ± 33.78
NO (μg/m^3)	2.67 ± 0.58	466.67 ± 152.75
NO_2 (μg/m^3)	4.33 ± 2.08	500 ± 0.00
CO (mg/m^3)	1.33 ± 0.29	26.67 ± 4.71
SO_2 (μg/m^3)	4.00 ± 1.00	14.33 ± 1.15

2.2. Animal exposure

Male 8-week-old CD-1 mice obtained from Shanghai Laboratory Animal Center, Chinese Academy of Sciences (Shanghai, China) were housed in stainless-steel wire-mesh cages in whole-body-exposure chambers that were maintained at an air flow of 40 L/min at a temperature of 22–25 °C with 50–60% humidity, with a controlled 12-h light/dark cycle. All the animal procedures were approved by Zhejiang University Animal Care and Use Committee. For negative control group, mice were maintained in chamber received clean FA for 7 days (24 h/day) (n = 10 for each group). For DE group, mice were maintained in chamber supplied DE (350 μg/m^3 TPS) for 5 h/day and clean FA for 19 h/day throughout the 7-day exposure (n = 10 for each group). For the hypoxic group (PAH-phenotype positive control), mice were maintained in an acrylic chamber with a non-recirculating gas mixture of 10% O_2 and 90% N_2 for 14 days (24 h/day) as previously described (Satoh et al., 2009) (n = 10 for each group). For the combined exposure group, mice were first exposed to DE for 7 days (5 h/day DE+ 19 h/day FA) and subsequently maintained in hypoxic condition for 14 days (24 h/day) (n = 10 for each group). Three independent exposure experiments were conducted for RVSP measurement, heart tissues

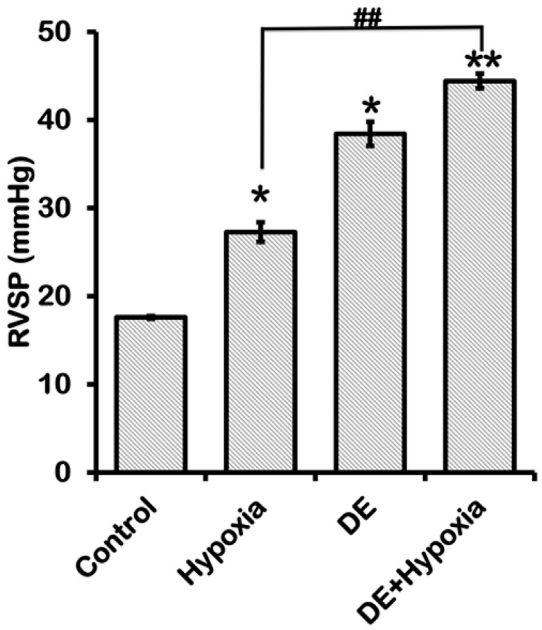

Fig. 1. Effects of DE inhalation exposure on RVSP. (A) FA (controls); (B) hypoxic condition; (C) DE (350 μg/m^3 TPS); (D) the combination of DE and hypoxic condition. Values represent as mean ± SE from 10 mice per group. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with FA control. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared Hypoxic+DE with Hypoxic or DE.

Please cite this article in press as: Liu, J., et al., Diesel exhaust inhalation exposure induces pulmonary arterial hypertension in mice, Environmental Pollution (2017), https://doi.org/10.1016/j.envpol.2017.10.121