Research Article

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Exposure to fine airborne particulate matters induces hepatic fibrosis in murine models

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Background & Aims: Hepatic fibrosis, featured by the accumulation of excessive extracellular matrix in liver tissue, is associated with metabolic disease and cancer. Inhalation exposure to airborne particulate matter in fine ranges ($PM_{2.5}$) correlates with pulmonary dysfunction, cardiovascular disease, and metabolic syndrome. In this study, we investigated the effect and mechanism of $PM_{2.5}$ exposure on hepatic fibrogenesis.

Methods: Both inhalation exposure of mice and *in vitro* exposure of specialized cells to $PM_{2.5}$ were performed to elucidate the effect of $PM_{2.5}$ exposure on hepatic fibrosis. Histological examinations, gene expression analyses, and genetic animal models were utilized to determine the effect and mechanism by which $PM_{2.5}$ exposure promotes hepatic fibrosis.

Results: Inhalation exposure to concentrated ambient $PM_{2.5}$ induces hepatic fibrosis in mice under the normal chow or high-fat diet. Mice after $PM_{2.5}$ exposure displayed increased expression of collagens in liver tissues. Exposure to $PM_{2.5}$ led to activation of the transforming growth factor β-SMAD3 signaling, suppression of peroxisome proliferator-activated receptor γ, and expression of collagens in hepatic stellate cells. NADPH oxidase plays a critical role in $PM_{2.5}$-induced liver fibrogenesis.

Conclusions: Exposure to $PM_{2.5}$ exerts discernible effects on promoting hepatic fibrogenesis. NADPH oxidase mediates the effects of $PM_{2.5}$ exposure on promoting hepatic fibrosis.

Keywords: Air pollution; Hepatic fibrosis; $PM_{2.5}$.

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Abbreviations: PM, ambient particulate matter; $PM_{2.5}$, PM with aerodynamic diameter less than 2.5 μm; FA, filtered air; OASIS, Ohio's Air Pollution Exposure System for the Interrogation of Systemic Effects; PPAR, peroxisome proliferator-activated receptor; TGFβ, transforming growth factor β; HSC, hepatic stellate cells; *p47phox*, Neutrophil cytosolic factor 1; NOX, NADPH peroxidase; ROS, reactive oxygen species.

Introduction

Recent studies indicated that exposure to fine ambient particulate matter (aerodynamic diameter <2.5 μm, $PM_{2.5}$) is a risk factor for pulmonary and cardiovascular diseases as well as metabolic syndrome [1–3]. Traffic-related airborne $PM_{2.5}$ is a complex mixture of particles and gases from gasoline and diesel engines, together with dust from wear of road surfaces, tires, and brakes [4,5]. Airborne $PM_{2.5}$ demonstrates an incremental capacity to penetrate into the distal airway units and potentially enter the systemic circulation with diminishing sizes. It has been suggested that the cytotoxic effects of $PM_{2.5}$ are more associated with $PM_{2.5}$ as a complex other than single or a few components of $PM_{2.5}$ particles [6]. The particle sizes, charges, and combined effects of individual components of $PM_{2.5}$ are all crucial to the adverse health impact of $PM_{2.5}$ exposure. Studies from our group and others suggested that $PM_{2.5}$ exposure triggers a variety of maladaptive signaling pathways in the lung, blood vessels, liver, and adipose tissues that are associated with endoplasmic reticulum (ER) stress, oxidative stress, and inflammatory responses [1,7–12]. Moreover, we recently demonstrated an important finding that inhalation exposure to $PM_{2.5}$ causes a non-alcoholic steatohepatitis (NASH)-like phenotype and depletion of hepatic glycogen storage in animals [1]. Through both *in vivo* and *in vitro* analyses, we revealed the signaling pathways through which $PM_{2.5}$ exposure promotes NASH-associated activities and impairment of hepatic glucose metabolism. We identified the disruption of hepatic lipid/glucose homeostasis, lobular and portal inflammation, as well as mild hepatic steatosis in the liver of the mice exposed to $PM_{2.5}$ for 10 weeks [1]. However, the pronounced effect of $PM_{2.5}$ exposure on modulating hepatic pathways associated with liver fibrogenesis has not been characterized.

Liver fibrosis and cirrhosis are the advanced stages of chronic liver injuries caused by chronic hepatitis viral infection, obesity, alcoholism, or autoimmune diseases. Recent studies showed that $PM_{2.5}$ exposure activates Kupffer cells in murine liver tissues, indicating that $PM_{2.5}$ represents a risk factor for NAFLD progression [1,10,13]. In this study, we used a "real-world" $PM_{2.5}$

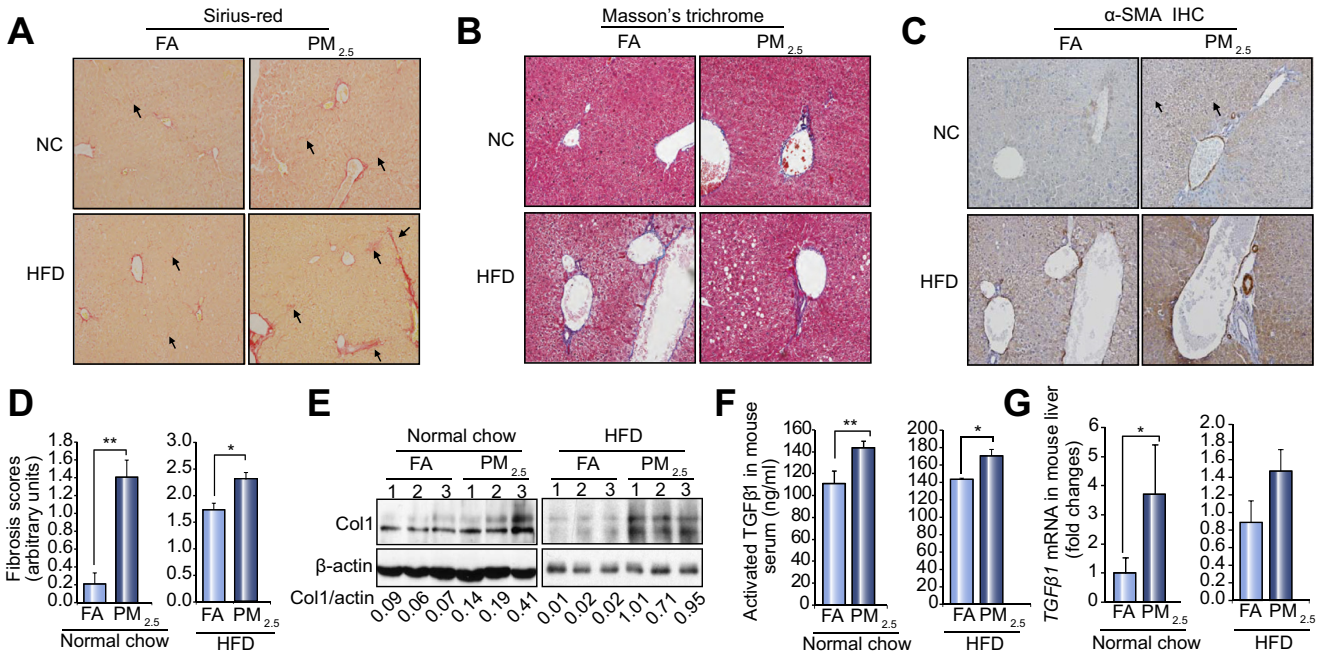

Fig. 1. $PM_{2.5}$ exposure induces hepatic fibrosis in mouse liver. (A and B) Sirius-red staining of hepatic collagen deposition (A) and Masson's trichrome staining of collagen fiber (B) in formalin-fixed liver tissue sections from C57BL/6 mice under the normal chow (NC) or high-fat diet (HFD) exposed to FA or $PM_{2.5}$ for 10 weeks. Magnifications: 200×. The arrows point out areas of hepatic fibrosis. (C) Immunohistochemical (IHC) staining of the hepatic stellate cell surface marker α-SMA in the liver tissue sections from the NC or HFD fed mice exposed to FA or $PM_{2.5}$ for 10 weeks. Magnifications: 200×. (D) Hepatic fibrosis grades of the mice exposed to $PM_{2.5}$ or FA for 10 weeks. Hepatic fibrosis grades were determined based on the histological analyses of Sirius-red staining of collagens, according to the Scheuer scoring system for fibrosis and cirrhosis [16,17]. Data are shown as mean ± SEM (n = 8 FA- or 9 $PM_{2.5}$-exposed animals). (E) Immunoblotting analysis of collagen I (Col1) in the liver tissue from the mice exposed to $PM_{2.5}$ or FA. The values below gel images represent quantification of Col1 protein signal intensities after normalization to those of β-actin. (F) Serum levels of activated TGFβ1 (determined by ELISA) in the mice exposed to $PM_{2.5}$ or FA. For A-B, each bar denotes the mean ± SEM (n = 3). (G) Quantitative real-time PCR (qRT-PCR) analysis of expression levels of the *Tgfβ1* mRNA in the livers of the mice exposed to $PM_{2.5}$ or FA for 10 weeks. Fold changes of mRNA levels were shown by comparing to the FA-exposed control mice. From B-G, $^{*}p$ <0.05; $^{**}p$ <0.01. (This figure appears in colour on the web.)

exposure system, "Ohio's Air Pollution Exposure System for the Interrogation of Systemic Effects (OASIS)", to perform whole-body exposure to mice of environmentally relevant $PM_{2.5}$. We demonstrate that exposure to $PM_{2.5}$ causes a discernible phenotype of hepatic fibrosis in animals. Through both *in vivo* and *in vitro* analyses, we reveal the signaling pathways through which $PM_{2.5}$ exposure promotes hepatic fibrogenesis-associated activities. The information from this work has important implications in the understanding and treatment of air pollution-induced liver diseases.

Materials and methods

Animal experiments

C57BL/6 male mice of six-weeks-old were purchased from the Jackson Laboratories (Bar Harbor, ME), and were equilibrated for two weeks prior to experimental enrollment. The mice were housed in cages with regular chow or a high-fat diet (Teklad TD 88137, 42% calories from fat) in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited animal housing facility. All the animal experiments were approved by the Ohio State University and the Wayne State University IACUC committee and carried out under the institutional guidelines for ethical animal use.

Exposure of animals to ambient $PM_{2.5}$

Mice were exposed to concentrated ambient $PM_{2.5}$ or filtered air (FA) in the OASIS in Columbus, OH, where most of the $PM_{2.5}$ component is attributed to long-range transport [10]. The concentrated $PM_{2.5}$ was generated using a versatile aerosol concentration enrichment system (VACES) as we described previously [14]. Mice under the normal chow or high-fat diet were exposed to concentrated $PM_{2.5}$ for six hours per day, five days per week for 10 weeks or 9 months [1,10]. The control (FA) mice were exposed to an identical protocol with the exception of a high-efficiency particulate-air filter positioned in the inlet valve to remove all of the $PM_{2.5}$ in the filtered air stream. Mice deficient in the cytosolic subunit of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (*p47phox*$^{-/-}$) and wild-type control mice of C57BL/6 strain background (both from Jackson Laboratories) were exposure to FA or $PM_{2.5}$ beginning at the age of three weeks for a duration of 10 weeks.

Histological scoring for hepatic fibrosis

Paraffin-embedded mouse liver tissue sections (5 μm) were subjected to Sirius-red or Masson's trichrome staining for hepatic fibrosis. The histological analysis of liver fibrosis were as described previously [15,16]. Each section was examined by a specialist who was blinded to the sample information. Hepatic fibrosis were scored according to the modified Scheuer scoring system for fibrosis and cirrhosis [16,17]. The fibrosis stage scores were based on the 0–4 stage system: 0, none; 1, zone 3 perisinusoidal fibrosis; 2, zone 3 perisinusoidal fibrosis plus portal fibrosis; 3, perisinusoidal fibrosis, portal fibrosis, plus bridging fibrosis; and 4, cirrhosis.

Statistics

Experimental results are shown as mean ± SEM (for variation between animals or experiments). All *in vitro* experiments were repeated with biological triplicates at least three times independently. The data were analyzed and compared by paired, two-tailed Student's *t* tests. Multiple comparisons were compared with ANOVA and proceeded by ad hoc statistical test when necessary. Statistical tests with p <0.05 were considered significant.

For a full description of materials and methods used in this work, see Supplementary data.