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Inhibition of the WNT/β-catenin pathway by fine particulate matter in haze: Roles of metals and polycyclic aromatic hydrocarbons

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HIGHLIGHTS

- $PM_{2.5}$ was collected from Beijing, Xian and Hong Kong during haze episodes.
- Metals and PAHs of haze $PM_{2.5}$ were characterised.
- Haze $PM_{2.5}$ inhibited WNT pathways and increased inflammation.
- Alveolar destruction was occurred post-exposure to haze $PM_{2.5}$.
- WNT/β-catenin, alveolar destructures and inflammation associate withPAHs.

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ABSTRACT

Air pollution might have a great impact on pulmonary health, but biological evidence in response to particulate matter less than 2.5 μm in size ($PM_{2.5}$) has been lacking. Physicochemical characterization of haze $PM_{2.5}$ collected from Beijing, Xian and Hong Kong was performed. Biological pathways were identified by proteomic profiling in mouse lungs, suggesting that WNT/β-catenin is important in the response to haze $PM_{2.5}$. Suppression of β-catenin levels, activation of caspase-3 and alveolar destruction, as well as IL-6, TNF-α and IFN-γ production, were observed in the lungs. The inhibition of β-catenin, TCF4 and cyclin D1 was observed *in vitro* in response to haze $PM_{2.5}$. The inhibition of WNT/β-catenin signaling, apoptosis-related results (caspase-3 and alveolar destruction), and inflammation, particularly including

Abbreviations: 4′,6-diamidino-2-phenylindole, DAPI; Beijing, BJ; bronchoalveolar lavage fluid, BALF; dimethyl sulfoxide, DMSO; dynamic light scattering, DLS; enhanced chemiluminescence, ECL; enzyme-linked immunosorbent assay, ELISA; fetal bovine serum, FBS; fine particulate matter, $PM_{2.5}$; Hong Kong, HK; horseradish peroxidase, HRP; immunohistochemistry, IHC; inductively coupled plasma-mass spectrometry, ICP-MS; interferon γ, IFN-γ; interleukin 6, IL-6; lymphoid enhancer factor, LEF; one-way analysis of variance, ANOVA; phosphate-buffered saline, PBS; polycyclic aromatic hydrocarbon, PAH; polyvinylidene fluoride, PVDF; Protein ANalysis THrough Evolutionary Relationships, PANTHER; sodium dodecyl sulfate polyacrylamide gel electrophoresis, SDS-PAGE; sulforhodamine B, SRB; T cell factor, TCF; T cell transcription factor-4, TCF4; tumor necrosis factor α, TNF-α; World Health Organization, WHO; Xian, XA.

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caspase-3 and alveolar destruction, were more highly associated with polycyclic aromatic hydrocarbons in haze $PM_{2.5}$. In conclusion, decreased WNT/β-catenin expression modulated by haze $PM_{2.5}$ could be involved in alveolar destruction and inflammation during haze episodes.

1. Introduction

Rapid industrialization and urbanization in developing countries, such as China and the Southeast Asian countries, have led to an increase in air pollution. Particulate pollution is a serious environmental issue that is affecting air quality, climate and, in particular, human health (Institute, 2013). The 2010 Global Burden of Disease study reported that outdoor air pollution in the form of fine particles (≤2.5 μm in aerodynamic diameter; $PM_{2.5}$) is an important public health risk, contributing annually to 3.2 million premature deaths worldwide and 76 million years of healthy life lost (Murray et al., 2014). In China, the Global Burden of Disease analysis reported 1.2 million premature deaths and 25 million healthy years of life lost in 2010, which were strongly associated with outdoor air pollution (Murray et al., 2014). Additionally, approximately 800 million people experienced an extremely severe and persistent haze pollution event at the beginning of 2013. However, the consequent health impact resulting from haze episodes remains unclear.

Haze is a weather phenomenon, mainly produced by combustion-derived pollutants, that leads to poor visibility because of the mixture of dust, moisture, smoke and vapor in the atmosphere (Sun et al., 2006b). Notably, epidemiological and clinical evidence has shown that haze pollutants contributed to 24% of respiratory admissions (Thurston et al., 1994) and a 30% increase in outpatient attendance (Emmanuel, 2000). The lung is the main portal of entry for aerial detritus, and as such, it is a critical organ for whole body defense by the clearance of deposited foreign materials. Alveolar epithelial cell apoptosis is an important step toward alveolar destruction in response to $PM_{2.5}$ (Farina et al., 2011). WNTsignaling has been reported to be a crucial mechanism controlling cell differentiation. The deregulation of WNT signaling regulates lung epithelial injury and repair processes (MacDonald et al., 2009). The central mediator of the WNT pathway is β-catenin, which is a transcription cofactor with T cell factor (TCF) and lymphoid enhancer factor (LEF) (Cadigan and Nusse, 1997). Libalova and colleagues (2012) suggested that exposure to $PM_{2.5}$ was related to WNT pathway expression in human embryonic lung fibroblasts (Libalova et al., 2012). However, little is known about the effects of WNT/β-catenin signaling in response to $PM_{2.5}$ exposure.

Haze occurs frequently in many cities in China, and it has been associated with pulmonary hospital admissions (Zhang et al., 2014). Xu and colleagues emphasized the importance of haze episodes in China (Xu et al., 2013), and the health impact of this type of pollution is an urgent issue in Asia. The respiratory system is a target organ for air pollutants, and short-term, high-intensity exposure to haze $PM_{2.5}$ could cause distal lung injury. Here, we elucidated the potential proteomic-driven mechanisms in response to $PM_{2.5}$ exposure in a haze episode *in vivo*, and the WNT/β-catenin signaling pathway was examined *in vitro*. Additionally, the effects of metals and polycyclic aromatic hydrocarbons (PAHs) on the consequent bioreactivity were determined.

2. Materials and methods

2.1. Particle collection and physicochemical characterization

Two haze-influenced cities in China, Beijing (BJ) and Xian (XA), and one non-haze-influenced city, Hong Kong (HK), were selected to investigate the health effects caused by haze $PM_{2.5}$ exposure. $PM_{2.5}$ was collected for seven days during a haze air pollution episode from January 26, 2013, to February 1, 2013, using mini-volume samplers equipped with two $PM_{2.5}$ impactors operated at flow rates of 5 L/min (Airmetrics, USA). The $PM_{2.5}$ samples were collected onto 47-mm Teflon substrates for 24 h and were equilibrated in 50% ± 5% relative humidity to obtain their gravimetric mass concentrations.

2.2. Physicochemical characterization

One set of the $PM_{2.5}$ substrates was removed using two-stage sonication in methanol, followed by drying with a nitrogen stream. The samples were then resuspended in dimethyl sulfoxide (DMSO) (<0.01% vol in phosphate-buffered saline [PBS]) at 50 and 150 μg/mL. The hydrodynamic diameters of the $PM_{2.5}$ samples were determined using dynamic light scattering (DLS; Malvern Zetasizer Nano-ZS, Worcestershire, UK). The zeta potential of the samples was determined using a Zetasizer (Malvern Zetasizer Nano-ZS, Worcestershire, UK). The other set of the $PM_{2.5}$ substrates was analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) to identify 16 metals, according to our previous method: aluminum (Al), cesium (Cs), cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), nickel (Ni), lead (Pb), vanadium (V), zinc (Zn), chromium (Cr), manganese (Mn), molybdenum (Mo), selenium (Se), strontium (Se) and titanium (Ti) (Chuang et al., 2011a). The concentrations of 15 PAH compounds were reported previously (Lee et al., 2014).

2.3. Animals

The animal experiments were performed in accordance with the guidelines of the ethics review committee of the Laboratory Animal Center at Taipei Medical University, Taiwan (Approval No. LAC-101-0003). Female six-week-old BALB/c mice were obtained from BiOLASCO (Taipei, Taiwan). The mice were provided free access to water and laboratory rodent chow. The mice's body weights were between 16 and 19 g during the experimental period. The mice were randomly divided into 7 groups (n = 6 per group), as shown in Fig. 1. On days 0 and 7, the mice in the exposure groups received an intratracheal instillation of 50 or 150 μg/kg of $PM_{2.5}$/mouse in PBS (<0.01% DMSO) under light anesthesia induced by isoflurane (Abbott Laboratories, Illinois, UK), whereas those in the control group received the same volume of vehicle PBS (<0.01% DMSO). On day 14, the animals were euthanized, and bronchoalveolar lavage fluid (BALF) and lung tissues were collected. The lung tissues were excised and snap-frozen or fixed in 4% (m/v) paraformaldehyde in PBS at 21 cm H_2O of pressure for histological analyses.

The doses applied in the present study were relevant for human