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Effects of non-protein-type amino acids of fine particulate matter on E-cadherin and inflammatory responses in mice

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HIGHLIGHTS

- $PM_{2.5}$ was collected from Beijing, Xian and Hong Kong during haze episodes.
- OC, EC and free amino acids of $PM_{2.5}$ were characterised.
- BALB/c mice were received an aspiration exposure of $PM_{2.5}$.
- $PM_{2.5}$ caused pulmonary inflammation and E-cadherin inhibition.
- Non-protein type amino acids were associated with inflammation.

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ABSTRACT

Exposure to particulate matter less than 2.5 μm ($PM_{2.5}$) in size is an urgent issue for the protection of human health. Chemicals with $PM_{2.5}$ collected during a period of intensive haze episodes in Beijing (BJ), Xian (XA) and Hong Kong (HK) were characterised for organic carbon (OC), elemental carbon (EC), total carbon (TC) and free amino acids. BALB/c mice underwent aspiration exposure of 50 or 150 μg of $PM_{2.5}$/mouse (BJ, XA and HK) on days 1 and 7 and were euthanised on day 14. The effects of these exposures on E-cadherin and inflammatory responses in the mouse lungs were analysed. The $PM_{2.5}$ chemicals consisted of significant amounts of OC: 36.6 ± 17.2 μg/m^3 for BJ, 38.8 ± 3.8 μg/m^3 for XA and 7.2 ± 1.4 μg/

Abbreviations: Ala, alanine; Asp, aspartic acid; BJ, Beijing; BALF, bronchoalveolar lavage fluid; Cys, cysteine; EC, elemental carbon; ECA, ethanolamine; EA, ethylamine; Gal, galactosamine; Glu, glutamic acid; Gly, glycine; He, helium; His, histidine; HK, Hong Kong; IFN-γ, interferon γ; IL-10, interleukin 10; IL-2, interleukin 2; IL-4, interleukin 4; IL-6, interleukin 6; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; MA, methylamine; OC, organic carbon; Orn, ornithine; O_2, oxygen; $PM_{2.5}$, particulate matter less than 2.5 μm in aerodynamic diameter; Phe, phenyalanine; Pro, proline; ROS, reactive oxygen species; Ser, serine; TOR, thermal optical reflectance; Thr, threonine; TC, total carbon; Tyr, tyrosine; Val, valine; XA, Xian; β-Ala, β-alanine; γ-Aba, γ-aminobutyric acid.

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m^3 for HK. A total of 23 free amino compounds for the $PM_{2.5}$ samples were analysed: 4075 ± 1578 pmol/m^3 for BJ, 4718 ± 2190 pmol/m^3 for XA and 1145 ± 213 pmol/m^3 for HK. Exposure to $PM_{2.5}$ resulted in the suppression of E-cadherin levels in the lung tissues and increased IFN-γ, IL-2, IL-4, IL-6 and IL-10 in the bronchoalveolar lavage fluid. The alterations in E-cadherin, IFN-γ, IL-6 and IL-10 were associated with OC, TC and some amino acids, particularly non-protein-type amino acids. These data emphasised the deleterious health effects of $PM_{2.5}$.

1. Introduction

Day-to-day variations in air pollution have been linked to increased risks of cardiopulmonary morbidity and mortality (Brook et al., 2010). Individuals who have pre-existing cardiopulmonary disease are more sensitive to changes in pollutant levels (Chuang et al., 2005). Epidemiological studies have demonstrated an association between the incidence of cardiopulmonary disease and the inflammatory effects of pulmonary exposure to particulate matter less than 2.5 μm in aerodynamic diameter ($PM_{2.5}$) (Gong et al., 2008; Riedl et al., 2012; Thurston et al., 1997). However, the different physicochemistry of various forms of $PM_{2.5}$ can have different effects on human health. The formation of primary particles involves the atmospheric cooling of combustion-derived organic vapours, followed by their sublimation into primary particles (Lipsky and Robinson, 2006). Incomplete combustion generates more vapour, which allows for the formation of additional primary particles (Chuang et al., 2013a). The combustion-derived carbonaceous core can act as a platform for the intermixing or intercalation of inorganics and organics (Murr, 2008). However, the specific components responsible for the effects on inflammatory responses remain unclear.

Airborne particles and atmospheric droplets consist of organic nitrogen, which constitutes 20–80% of all nitrogen (Barbaro et al., 2011). One of the forms of organic nitrogen previously measured in the atmosphere is amino acids, which represent a significant component of these particles (Barbaro et al., 2011; Zhang and Anastasio, 2003). Biologically, some free amino acids, such as methionine, interact with reactive oxygen species (ROS) to form methionine sulphonamide, thereby scavenging the ROS (Roche et al., 2008). Notably, amino acids have been associated with allergic-inflammatory responses (Meyers et al., 1979; Niese et al., 2010). Although many studies have focused on understanding the atmospheric concentrations and biological mechanisms of amino acids, little is known about the effects of inhaling the free amino acids of $PM_{2.5}$ on the lung environment. To maintain normal gas exchange in the pulmonary regions, foreign substances must be identified and removed without undue inflammation. Consequently, lung defence involves a wide array of mechanisms, ranging from the nostrils to the alveoli, to remove inhaled particles. Alveolar epithelial cells actively participate in host defence, to protect underlying tissues from desiccation, toxic challenge and physical trauma. The airway epithelium also plays an important role in the regulation of immune-inflammatory responses after exposure to $PM_{2.5}$. Nawijn et al. (2011) indicated that E-cadherin associated with the structural and immunological functions of the airway epithelium through the regulation of epithelial junctions and inflammation (Nawijn et al., 2011). Pulmonary inflammation could be associated with E-cadherin regulation and free amino acids of $PM_{2.5}$ after exposure to haze episodes; however, their associations remain poorly understood.

Haze is an atmospheric phenomenon in which dry particulate pollutants reduce visibility. Epidemiological studies have shown that haze episodes increase the risk of cardiovascular and pulmonary diseases (Tie et al., 2009). Previous studies have shown that haze episodes are important public health issues that might contribute to 24% of respiratory admissions in China (Thurston et al., 1994). Pulmonary exposure to air pollution is associated with respiratory illnesses in China during haze episodes (Tie et al., 2009; Zhang et al., 2014). The potential adverse health effects of exposure to haze $PM_{2.5}$ might be related to the mechanism of the sedimentation of particles in the lung, leading to pulmonary-to-systemic inflammatory responses. However, the associations among the chemical compounds in haze $PM_{2.5}$, such as free amino acids, and inflammatory responses remain unclear. To investigate the possible health effects during haze episodes, $PM_{2.5}$ was collected in two haze-affected cities, Beijing (BJ) and Xian (XA), and in a control city, Hong Kong (HK), and their chemical composition and bioreactivity were determined.

2. Materials and methods

2.1. Particle collection and preparation

To determine the effects of $PM_{2.5}$ during haze episodes, two severely haze-influenced cities (BJ [39°59′10.78″ N, 116°23′09.25″ E] and XA [34°13′49.36″ N, 108°52′58.59″ E]) and a mild haze-influenced control city (HK [22°18′11.49″ N, 114°11′00.17″ E]) in China were selected for this study. The particle collection process was described previously (Lee et al., 2014). Briefly, 24-h $PM_{2.5}$ samples were collected during a haze air pollution episode from 26 January 2013 to 1 February 2013 using mini-volume samplers (Airmetrics, Oregon, USA). Methanol $PM_{2.5}$ extracts were prepared as previously described, using two-stage sonication in methanol (Chuang et al., 2013b). The extract was then dried using a pure nitrogen stream. The residues were then resuspended in dimethyl sulfoxide (DMSO) [<0.01% vol in phosphate buffered saline (PBS)] at 0, 50 and 150 μg/ml. The mass concentrations were previously reported (Lee et al., 2014).

2.2. Organic carbon/elemental carbon

Organic carbon (OC) and elemental carbon (EC) were determined from each filter by thermal optical reflectance (TOR), following the IMPROVE protocol with a DRI Model 2001 Thermal/Optical Carbon Analyzer (Atmoslytic Inc., Calabasas, CA, USA) (Chow et al., 2004). Briefly, this procedure produced four OC fractions in a pure helium (He) atmosphere: a pyrolysed carbon fraction (OP, determined when reflected laser light attained its original intensity after oxygen [O_2] was added to the combustion atmosphere) and three EC fractions in a 2% O_2/98% He atmosphere. The IMPROVE OC is operationally defined as OC1 + OC2 + OC3 + OC4 + OP, whereas the EC is defined as EC1 + EC2 + EC3 − OP. The method detection limits (MDLs) for the carbon analysis were 0.8 and 0.4 μgC cm^{-2} for the OC and EC, respectively, with precision better than 10% for total carbon (TC).

2.3. Free amino compounds

Twenty-three free amino compounds (protein type amino acids: Alanine [Ala], Aspartic acid [Asp], Cysteine [Cys], Glutamic acid [Glu], Glycine [Gly], Histidine [His], Isoleucine [Ile], Leucine