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Comparison of lung damage in mice exposed to black carbon particles and 1,4-naphthoquinone coated black carbon particles

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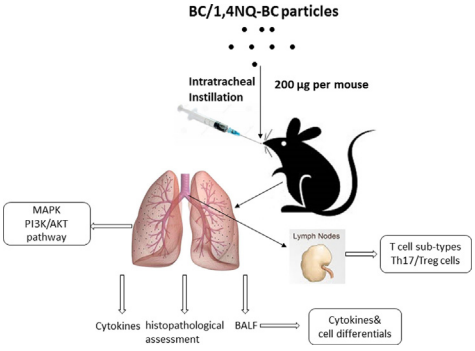
HIGHLIGHTS

- 1,4NQ-BC can enhance the cytokines secretion in BALF than that of BC.
- 1,4NQ-BC can enhance cytokine mRNA expression in lung tissue than that of BC.
- 1,4NQ-BC affected the T cell sub-types in mediastinal lymph nodes.
- MAPK and PI3K/AKT signal pathway play a role in 1,4NQ-BC caused lung injury.

GRAPHICAL ABSTRACT



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ABSTRACT

Black carbon (BC) is a key component of atmospheric particles and has a significant effect on human health. BC can provide reactive sites and surfaces thus absorb quinones which were primarily generated from fossil fuel combustion and/or atmospheric photochemical conversions of PAHs. Oxidation could change the characteristics of BC and increase its toxicity. The comparison of lung damage in mice exposed to BC and 1,4-NQ-coated BC (1,4NQ-BC) particles is investigated in this study. Mice which were intratracheally instilled with particles have a higher expression of IL-1β, IL-6 and IL-33 in bronchoalveolar lavage fluid (BALF). Also, the IL-6, IL-33 mRNA expression in the lung tissue of mice instilled with 1,4NQ-BC were higher than that of mice instilled with BC. The pathology results showed that the lung tissue of mice instilled with 1,4NQ-BC particles have much more inflammatory cells infiltration than that of mice treated with BC. It is believed that the MAPK and PI3K-AKT pathway might be involved in the 1,4NQ-BC particles caused lung damage. Results indicated that 1,4NQ-BC particles in the atmosphere may cause more damage to health.

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1. Introduction

Black carbon (BC), formed by the incomplete combustion of diesel fuels, coal, biofuels and outdoor biomass burning, is the strongest light-absorbing component of particulate matter (PM). It is the second-largest contributor to global warming which is an important constituent of atmospheric PM (Jacobson, 2001; Li et al., 2005). In the past decades, the level of BC in the environment has increased dramatically due to increasing fossil fuel consumption and biomass burning.

BC is a new useful indicator for the adverse effects of traffic-related air pollution (Janssen et al., 2011), since around 75% particles released by mobile diesel engines are comprised of BC (Agency, U.S.E.P., 2010). Furthermore, BC has been categorized as one of several indicators of fine particulate air pollution and also provoke health effects in some community epidemiological studies. The relationship between exposure of BC and cardiovascular effects has been reported in plenty of studies (McCracken et al., 2010; Patel et al., 2010; Smith et al., 2009a; Wittkopp et al., 2016). It has been found that the changes in blood pressure (Delfino et al., 2010; Mordukhovich et al., 2009; Wilker et al., 2010) and variability of heart rate (Adar and Kaufman, 2007; Chuang et al., 2008; Gold et al., 2005; Park et al., 2005a) were affected by the mean concentration of BC in a series analyses.

The BC effects on respiratory system has been investigated in fewer studies (Agency, U.S.E.P., 2010) (Lin et al., 2011; Patel et al., 2010). It has been found that BC exposure was associated with the increase of asthma risk and pulmonary inflammation (Clark et al., 2010; Delfino et al., 2006; Kim et al., 2004). And these results were supported by toxicological studies with the evidence of airway inflammation effects (Godleski, 2002; Saldiva et al., 2002).

The fresh BC is one of the primary pollutants in the atmosphere. When BC undergoes an aging process during transformation after emission into the atmosphere it. BC can provide reactive sites and surfaces, therefore, pollutants can absorb resulting in the alteration of the particle chemical features and toxicity (Li et al., 2013). In addition, polycyclic aromatic hydrocarbons (PAHs) in the atmosphere are mostly generated by vehicle emissions and coal combustion. And these specific contaminants are potentially high carcinogenic and mutagenic (Slezakova et al., 2013). Most quinones found in PM are polycyclic aromatic quinones, such as naphthoquinone, anthraquinone, and phenanthrenequinone (Cho et al., 2004; Walgraeve et al., 2010). Furthermore, these quinones were primarily generated from fossil fuel combustion and/or atmospheric photochemical conversions of PAHs (Eiguren-Fernandez et al., 2008a; Jakober et al., 2007). It has been reported that the concentration of quinones in traffic-related particles was even higher than benzo(a)pyrene (Eiguren-Fernandez et al., 2008b). Toxicological interest of quinones and their reduction products are promoted by their ability to generate ROS (reactive oxygen species) (Cho et al., 2004) which may have health effects on human.

The studies about BC (soot) were mainly on its ability of generating ROS and the change of structure during the aging process. Studies on 1,4-naphthoquinone coated BC (1,4NQ-BC) were rare and there were neither studies about its health effects nor the comparison of health effects caused by BC and 1,4NQ-BC. Therefore, in the present study, the lung inflammation and the potential mechanism of damage caused by BC and 1,4NQ-BC have been studied and explored.

2. Materials and methods

2.1. Animals

Female C57BL/6 mice (8–10 weeks old, 18–22 g) were purchased from Vital River Laboratory Technology Co. Ltd. (Beijing, China). All mice were raised under specific pathogen-free condition with a barrier system (temperature: 20–26 °C; relative humidity: 40–70%) and with a 12 h light/dark cycle. Standard mice feed and tap water were available. All animal procedures were approved by the Animal Care Committee of the Peking University according to government guidelines for animal care.

2.2. BC and 1,4-NQ-coated BC

BC and 1,4-naphthoquinone were provided by Professor Jing Shang (College of Environmental Sciences and Engineering, Peking University). Printex U black carbon (UBC), an excellent representative of diesel soot (G. Mul et al., 1998), was obtained from Deussa and utilized as model BC powder (30 nm). 0.05 g 1,4-naphthoquinone and 1 g of BC were mixed and dissolved in dichoromethane. And the mixture was dried at 60 °C for 4 h after rotary evaporation to get 1,4NQ-BC (Li et al., 2015). The particles were suspended in the saline, and then sonicated for 15 min before intratracheal instillation.

2.3. Experimental design

Animals were divided into 4 groups: negative control, vehicle control (saline), and particles treated groups (200 μg of 30 nm BC or 1,4NQ-BC, $n = 7$). Particles were suspended in distilled PBS and instilled 0.06 ml per mouse. The mice were instilled twice a week and repeated for 4 weeks (Bourdon et al., 2012; Chu et al., 2016; Samet et al., 2000; Shwe et al., 2005). Total instillation amount was 1.6 mg. 24 h after last instillation, the body weight of each mouse was measured and animals were sacrificed by cervical dislocation after isoflurane anesthesia.

2.4. Intratracheal instillation

Mice were anesthetized by an intraperitoneal injection of 5% chloral hydrate (0.1 ml/10 g body weight). BC and 1,4NQ-BC were sonicated for 15 min then vortexed immediately prior to intratracheal instillation. The vehicle control group was instilled with 0.06 ml of PBS and the negative control group was left untreated.

2.5. Organ coefficient

Spleen, thymus and mediastinal lymph nodes were separated and weight, and then divided by the body weight, is organ coefficient.

2.6. Bronchoalveolar lavage and cell differentials

Mice were euthanized by anesthesia with 0.1 ml/10 g of 5% chloral hydrate, and lavaged with two separate 0.8 ml aliquots of PBS. At least 1.2 ml bronchoalveolar lavage fluid (BALF) was recovered. Supernatants were collected after centrifugation (300 × *g*, 10 min) and stored at −80 °C for cytokines testing. The cell pellets from lavages were combined and re-suspended in 1 ml of PBS containing 1% BSA. Cells were counted using Counter Star (Shang Hai, China). Supernatants were removed after centrifugation (300 × *g*, 10 min) and cells were smeared to the slides and then stained with Diff Quik (American Scientific Inc., Sewickly, PA). About 300 cells were counted and three kind of cell subtypes (macrophage, lymphocytes, neutrophils) were identified.

2.7. ELISA analysis

Bronchoalveolar lavage fluid was analyzed using commercial enzyme-linked immunosorbant assay (ELISA) kits for IL-1β, IL-6 and IL-33. All of the kits were purchased from Abcam Co. Ltd. (UK). All the procedures were performed according to the manufacturer's instructions.

2.8. RT-PCR analysis

At the Real-time PCR step, 2 μl cDNA was used for quantitative Real-time PCR using SYBR Green Kit (TaKaRa, DRR041A). The reaction mixture was prepared for PCR at 95 °C for 3 min, then subjected to 35 cycles of

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