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Differential pulmonary effects of wintertime California and China particulate matter in healthy young mice

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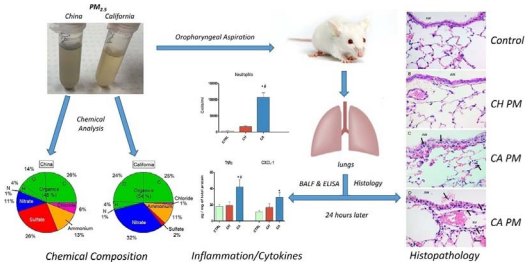
GRAPHICAL ABSTRACT



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ABSTRACT

Airborne particulate matter (PM) is associated with adverse cardiorespiratory effects. To better understand source-orientated PM toxicity, a comparative study of the biological effects of fine PM (diameter ≤ 2.5 μm, $PM_{2.5}$) collected during the winter season from Shanxi Province, China, and the Central Valley, California, United States, was conducted. The overarching hypothesis for this study was to test whether the chemical composition of PM on an equal mass basis from two urban areas, one in China and one in California, can lead to significantly different effects of acute toxicity and inflammation in the lungs of healthy young mice. Male, 8-week old BALB/C mice received a single 50 μg dose of vehicle, Taiyuan PM or Sacramento PM by oropharyngeal aspiration and were sacrificed 24 h later. Bronchoalveolar lavage, ELISA and histopathology were performed along with chemical analysis of PM composition. Sacramento PM had a greater proportion of oxidized organic material, significantly increased neutrophil numbers and elevated CXCL-1 and TNF-α protein levels compared to the Taiyuan PM. The findings suggest that Sacramento $PM_{2.5}$ was associated with a greater inflammatory response compared to that of Taiyuan $PM_{2.5}$ that may be due to a higher oxidice. Male, 8-week old BALB/C mice received a single 50 μg dose of vehicle, Taiyuan PM or Sacramento PM by oropharyngeal aspiration and were sacrificed 24 h later. Bronchoalveolar lavage, ELISA and histopathology were performed along with chemical analysis of PM composition. Sacramento PM had a greater proportion of oxidized organic material, significantly increased neutrophil numbers and elevated CXCL-1 and TNF-α protein levels compared to the Taiyuan PM. The

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findings suggest that Sacramento $PM_{2.5}$ was associated with a greater inflammatory response compared to that of Taiyuan $PM_{2.5}$ that may be due to a higher oxidized state of organic carbon and copper content.

1. Introduction

Particulate matter (PM) air pollution is a worldwide health problem associated with adverse effects on the cardiorespiratory system, such as asthma, COPD, and myocardial infarction. Worldwide air pollution related annual mortalities have been estimated at 7 million (WHO, 2015). PM has a wide variety of physicochemical characteristics that depend on the source and atmospheric aging of the particles. Fine PM, also known as $PM_{2.5}$ ($D_p \leq 2.5$ μm), is especially harmful because it can readily deposit deep in the lung and be retained, irritating lung parenchyma or moving into the blood stream (Churg and Brauer, 1997; Madl et al., 2014; Mannucci et al., 2015).

PM pollution has increased with industrialization and climate change. It is especially prevalent in areas of rapid economic growth fueled by fossil fuels, such as China, or arid regions with geographical/meteorological conditions that trap PM for long periods of time and concentrate it, such as in the large valleys of the Western United States. This paper describes a comparative study of the biological effect of $PM_{2.5}$ from two parts of the world known for high PM air pollution, Shanxi Province in China and the Central Valley in California in the United States. The study was a joint effort to define the influence of the chemical composition of PM from diverse urban sources of these two countries on an equal mass basis in measured biological toxicity to the lungs following acute exposure.

PM was collected in the capital cities of Shanxi Province and the state of California, Taiyuan and Sacramento, respectively, based on the fact that both cities are heavily urbanized, have relatively dry, sunny winters, economies dominated by agriculture and industry, and a long history of unhealthy levels of $PM_{2.5}$, especially during the winter season. Because the economy of Taiyuan is dominated by abundant coal production and combustion, while the economy of Sacramento is largely based on government, transportation, and agriculture, it was expected that the study would provide an opportunity to better understand how PM source influences pulmonary toxicity.

To compare the biological effects of the two geographic PM samples, young male BALB/C mice were exposed by oropharyngeal aspiration (50 μg) on an equal mass basis to $PM_{2.5}$ collected from Taiyuan or Sacramento. The PM was collected at both sites during winter since higher air pollution during this season has been associated with increased hospital admissions and the incidence of cardiovascular and respiratory disease (Rodopoulou et al., 2015). Animals were sacrificed 24 h post-exposure to capture peak inflammation, as is well known to occur following gas and particle exposure. Patterns of pulmonary toxicity were assessed by bronchoalveolar lavage (BAL), enzyme-linked immunosorbent assays (ELISA) and histopathologic assessment. In addition, the chemical composition of each PM sample was analyzed to determine if chemical differences could help explain potential differences in pulmonary toxicity.

2. Materials and methods

2.1. Particle collection

Sampling was done during the winter of 2012 in Taiyuan and 2013 in Sacramento to collect sufficient PM mass for toxicological and chemical characterization.

The sampling site in Taiyuan was located on the rooftop of the five story building of the College of Environmental Science and Resources on the Shanxi University campus (N37°47′, E112°34′) in downtown Taiyuan, surrounded by a mixture of residential, commercial and industrial buildings. The sampling site in Sacramento was located on the rooftop of a two story building at the northeast corner of T St. and 13th St. (N38°34′, W121°29′), also surrounded by a mixture of residential, commercial and industrial buildings and within a quarter mile of a major freeway interchange.

In Taiyuan, $PM_{2.5}$ was collected for one day on 3 μm pore size quartz microfiber filters (Whatman) using a high-volume particle collector with a flow rate of 40 cfm (Thermo Anderson, USA). The quartz filters were preheated at 450 °C for 24 h before sampling to eliminate endotoxin. Filters were pre- and post-weighed to calculate the PM mass and then subsequently cut into fragments, placed in a 250 mL conical flask with 30 mL Milli-Q water and sonicated for 10 min (repeated three times for a total of 30 min). The PM extract was filtered through six layers of sterile gauze. These extraction steps were repeated three times. The collected solution was lyophilized to powder and stored at −80 °C until use. Prior to use, the powder was weighed and suspended in Milli-Q water to a concentration of 1 mg/mL.

In Sacramento, $PM_{2.5}$ was collected for seven days with a high-volume sampler system (Tisch Environmental Inc., TE-6070V-2.5-HVS) that was equipped with a PM_{10} size-selective head (Tisch Environmental Inc., TE-6001), operating at a flow rate of 40 cfm and loaded with Teflon coated borosilicate glass microfiber filters (Pall Corporation, TX40H120WW-8 × 10) for collecting $PM_{2.5}$. Glass filters were pre-cleaned via successive sonication in Milli-Q water, dichloromethane and hexane. Field blanks were included. The sample filters were weighed to calculate the PM concentration, placed in Milli-Q water and sonicated for 1 h. The sonication extract was filtered using a 0.2 μm pore size syringe filter. The collected solution (approximately 100 mL) was lyophilized and then resuspended in Milli-Q water to a final PM concentration of 1 mg/mL. Detailed extraction methods can be found in Bein and Wexler (Bein and Wexler, 2015).

The difference in collection times between China and California (1 day vs. 7 days, respectively) was due to China having much higher levels of air pollution. Because the China collection filters had significantly higher areal density (heavier loading) of PM than California filters, the sonification times for the China PM and California PM preparations differed accordingly (30 min vs 60 min, respectively). It is acknowledged that the differences in extraction and sonication times might cause different particle extraction efficiencies between the two PM samples.

2.2. PM suspension preparation

To achieve identical particle concentrations for both CA and CH PM samples prior to oropharyngeal aspiration, extracted PM samples from CA and CH were lyophilized to dryness in order to measure a precise PM mass that was suspended in nanopure water and sonicated for 20 min to derive an identical mass (1 μg/μL) for each PM sample. PM samples were analyzed for endotoxin using a chromogenic Limulus Amebocyte Lysate (LAL) test with a PM sample concentration of 1 mg/mL.

2.3. Hydrodynamic particle size

Following a 20 min bath sonication of each PM suspension, dynamic light scattering (DLS) was used to determine hydrodynamic particle size distribution of each sample immediately prior to oropharyngeal aspiration.