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Spatial and seasonal heterogeneity of atmospheric particles induced reactive oxygen species in urban areas and the role of water-soluble metals

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ABSTRACT

Adverse health effects are associated with exposure to atmospheric particulate matter (PM), which carry various chemical constituents and induce both exogenous and endogenous oxidative stress. This study investigated the spatial and seasonal variability of PM-induced ROS at four sites with different characteristics in Hong Kong. Cytotoxicity, exogenous and endogenous ROS was determined on a dose and time dependent analysis. Large spatial variation of ROS was observed with fine PM at urban site showing highest ROS levels while coarse PM at traffic site ranks the top. No consistent seasonal difference was observed for ROS levels among all sites. The highly heterogeneous distribution of PM-induced ROS demonstrates the differential capability of PM to produce oxidative stress, and the need to use appropriate metrics as surrogates of exposure instead of PM mass in epidemiologic studies. Several transition metals were found associated with ROS by different degree illustrating the complexity of mechanisms involved.

1. Introduction

Airborne particulate matter (PM) has been reported to be highly associated with human morbidity and mortality in various epidemiological studies. Exposure to high concentrations of PM may result in pulmonary, cardiovascular diseases and also neurodevelopmental effects (Delfino et al., 2005; Morgan et al., 2011; Leiva et al., 2013). Although specific mechanisms involved in it are largely unknown, the intracellular generation of reactive oxygen species (ROS) and the resulting oxidative stress have been hypothesized to play a direct role in pulmonary inflammation leading to subsequent various health effects (Pope and Dockery, 2006; Cachon et al., 2014). ROS is a group of free radicals consisted of superoxide ($O_2^{\cdot -}$), hydroxyl radical (•OH) and other derivatives such as hydrogen peroxide (H_2O_2), which can be formed endogenously during physiological and metabolic processes (Huang et al., 2003), and their concentrations are balanced by protective antioxidant enzymes (Halliwell and Whiteman, 2004). However, excessive generation of ROS can cause significant damages to cellular DNA, lipid, proteins which may lead to increased incidence of cardiovascular and pulmonary diseases (Ciencewicki et al., 2008; Yang and Omaye, 2009; Steenhof et al., 2011). Therefore, it is critical to understand the mechanisms of ROS generation and identify the responsible agents of PM oxidative potential in order to mitigate the adverse health effects.

However, atmospheric PM is a complex mixture of chemical constituents especially in urban environments, with distinct sizes and sources. For example, ambient $PM_{2.5}$, or fine PM, ($d_p < 2.5$ μm) are primarily from fossil fuel combustion and secondary aerosol formation through photo-oxidation of gas precursors etcetera in urban areas (Ning and Sioutas, 2010; Rastogi et al., 2014), while coarse PM (2.5 μm < d_p < 10 μm) arises predominantly from mechanical disruption and attrition processes (Pakbin et al., 2011). Source apportionment investigations have also showed that the chemical composition of both size fractions varied widely depending on the study areas and types of pollution sources (Man and Shih, 2001; Pakbin et al., 2011). Potent ROS inducers in atmospheric PM including organic compounds such as aromatic hydrocarbons and quinones (Eiguren-Fernandez et al., 2010), and

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inorganic components such as transition metals (Ohyama et al., 2007) were reported. The role of transition metals in ROS generation was greatly studied employing surrogate lung fluid under similar physiological conditions of cells (Valavanidis et al., 2005; Vidrio et al., 2009). Further, the involvement of soluble transition metals of PM in ROS generation has been attributed to cardiopulmonary toxicity (DiStefano et al., 2009). Non-redox active metals and elements are also reported to have good correlations with ROS (Zhang et al., 2008; Verma et al., 2009).

There exists a general inconsistency in literature showing a wide variety of correlations between metals and PM-induced ROS among different urban cities (Kunzli et al., 2006), at sites with different source characteristics (Janssen et al., 2014) and with different PM sizes – fine and coarse (Schins et al., 2002) which raise the question of representativeness of considering single component of metals as surrogates for PM oxidative potentials. In addition, the redox activity of PM bound metals was hypothesized to exhibit great heterogeneity that varied by different sources, locations and seasons, yielding the differences in their bioavailability, chemical speciation, and oxidation state (Shi et al., 2003). Such inconsistency across different studies may also have come from the different types of assays used (Li et al., 2004; Miljevic et al., 2010). For example, cell free assays devoid of antioxidants have been used extensively due to its applicability for high throughput screening approach or on-line detection of redox cycling compounds that may be present in PM (Cho et al., 2005; Venkatachari and Hopke, 2008). *In-vitro* assays, however, may provide a more comprehensive assessment due to their physiological relevance with the intracellular generation of ROS (Landreman et al., 2008). Different mechanisms of ROS generation were reported for cellular and non-cell based ROS measurements (Olakanmi et al., 1993; Wang and Joseph, 1999; See et al., 2007), which explained different pathways for free radical generation, suggesting less probability for overlapping of endogenous and exogenous ROS.

In the present study, we compared the chemical based cell free method and *in-vitro* probe based method for exogenous and endogenous ROS activity of size fractionated PM from different seasons and environments within the urban area of Hong Kong, aiming to better understand the spatial and seasonal heterogeneity of PM-induced ROS and the relative abundance of oxidative potential of PM in different size fractions. By linking the water-soluble metals with ROS activities, the study also provides evidences of inconsistency in using PM chemical characteristics as surrogate of PM toxicity for epidemiologic studies.

2. Experimental methodology

2.1. PM sample collection

The weekly time integrated particle samples were collected from 12 November 2012 to 10 December 2012 (typical Winter season) and 8 April 2013 to 13 May 2013 (Spring to Summer seasons, designated as Summer in the present study) with each sample lasting for seven days. The Personal Cascade Impactor Sampler (SKC Inc., Eighty-Four, PA) loaded with 25 mm and 37 mm Teflon filter (PALL Life Sciences, Ann Arbor, MI) were run throughout the week at flow rates of 9 liters per minute to collect coarse (PM_{10}-$PM_{2.5}$) and fine PM ($PM_{2.5}$), respectively. The flow rates were checked and calibrated by a Gillian airflow calibrator (Sensidyne Inc, Clearwater, FL) regularly before and after the sampling period. Teflon filters are pre-cleaned in methanol by sonication for 20 min and air dried under standard fume hood condition prior to the field sampling. For on-site filter reloading between sampling intervals, the sampler was cleaned with methanol three times and dried before loading the filters. The samplers were located at 4 sites in Hong Kong – Hung Hom (22°18′14″N, 114°10′50″E), Sha Tin (22°22′59″N, 114°11′25″E), Kwai Chung (22°21′30″N, 114°7′50″E) and Kowloon Tong (22°20′8″N, 114°10′28″E) as shown in Fig. 1 and detailed description of the sites was given in Table 1. A total of 32 sets of samples were collected, and each set was loaded with both fine and coarse PM. Filter samples were stored at −20 °C in plastic petri dishes sandwiched with baked aluminum foil, and sealed with Teflon tape until further analysis.

2.2. Gravimetric and metals analysis

2.2.1. Gravimetric measurements

Gravimetric analysis was performed by a high-precision (±1 μg) microbalance (ME-5, Sartorius Inc., Germany). Filters were equilibrated in a temperature (21 ± 2 °C) and humidity (35 ± 5% RH) controlled room for 24 h before weighing. The filter weighing was repeated at least three times to ensure the uncertainty of weight within ±3 μg and PM gravimetric concentration was determined by the pre- and post- weight of the filters.

2.2.2. Sample extraction

The sample extraction procedures followed previous work (Jiang et al., 2014). PTFE filters were cut in half prior to the sample extraction. One half of each filter was soaked in a 15 ml metal-free centrifuge tube with 8 ml of Milli-Q water. Water extraction was carried out using a multi-tube vortex mixer (Model X-2500, VWR). After 12 h of vortex, extracts were filtered with 0.22 μm filter membranes and divided into two subsamples for water-soluble metals and reactive oxygen species analysis, respectively. For metal analysis' subsample, HCl was added at final concentrations of 2%. Samples were stored at −20 °C prior to analysis. Extraction and preservation of samples are commonly used standard procedures (Kleeman et al., 2000; Rees et al., 2004; Ning et al., 2007; Pakbin et al., 2011), which we have adopted for consistency.

2.2.3. Water-soluble metals determination

Total of 12 metals were determined including 9 trace metals and 3 base metals in the study. Multi-element standard solutions were purchased from Fluka (Mo, USA). Standard mixtures containing all analytes were prepared by diluting the standard solution with Mill-Q water (ELGA, VWS Ltd., England & Wales) to concentrations ranging 0.0005–5.0 mg L^{-1}. All standard solutions were stored in a fridge at 4 °C. HCl fuming (37%) was purchased from Merck Millipore (Germany). Argon with purity ≥ 99.995% used in Inductively Couple Plasma (ICP) was purchased from Hong Kong Linde Ltd. Al, Cd, Cr, Cu, Pb, Mn, Ni, V, and Zn were determined using ICP-Mass Spectrometry (ICP-MS) with a 7500cx system (Agilent, USA) using the following parameters: Sample pump rate 1.0 rps, nebulizer pump rate 0.2 rps, RF power (W) 1600, and carrier flow 0.93 L min^{-1}. Three base elements (Na, Fe, Ca) were detected using ICP-Optical Emission Spectrometry (ICP-OES). The analysis was processed in an Optima 2100 DV system (Perkin Elmer, USA) with ion lenses. Instrumental parameters were set for a plasma flow of 15 L min^{-1}, Nebulizer flow 0.8 L min^{-1}, auxiliary flow 0.3 L min^{-1}. RF power was set at 1300 Watt and flow rate of pump at 1.00 ml min^{-1}. Detailed analytic procedures for ICP-MS and ICP-OES analyses were described in Jiang et al (2014).

2.3. Reactive oxygen species and cell toxicity analysis

2.3.1. Macrophage cell culture

RAW 264.7 murine monocytic-macrophage cell line (ATCC, Manassas, VA, USA) was cultured in DMEM (Dulbecco's Modified Eagle's Medium) (Invitrogen, USA) supplemented with 10% heat in activated FBS (Fetal Bovine Serum) (Gibco, USA). The cells were