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Reactive oxygen species (ROS) activity of ambient fine particles ($PM_{2.5}$) measured in Seoul, Korea

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ABSTRACT

Substantial increase in level of particulate matter has raised concerns in South Korea recently. Ambient particulate matter is classified as Group I carcinogen (IARC, 2013) and multiple epidemiological studies has demonstrated adverse health effects due to exposure of particulate matter. Fine particulate matter ($PM_{2.5}$) which has a diameter < 2.5 μm is likely to penetrate deeply into lung and is known to be eliciting adverse health effects. A number of epidemiological studies have been conducted on adverse health effects of PM-related diseases and mortality rate, yet particulate matter (PM)-induced reactive oxygen species (ROS) activity at the cellular level has not been actively studied in Korea. This study assessed PM-induced oxidative potential by exposure of collected ambient $PM_{2.5}$ samples to the rat alveolar macrophage cell line. The characteristics of $PM_{2.5}$ in Korea were further characterized by linking chemical constituents and contributing sources to ROS. $PM_{2.5}$ mass concentration during the cold season was relatively higher than mass concentration during the warm season and chemical constituents except for Secondary Organic Carbon (SOC) and SO_4^{2-} which both showed similar trends in both the cold and cold seasons. The concentration of crustal elements was especially high during the cold season which can be an indication of long range transport of Asian dust. Water soluble organic carbon and water soluble transition metals (Cr and Zn) were also shown to be correlated to oxidative potential and metals such as As and V were shown to have a high contribution to ROS activity according to stepwise multiple linear regression. Principal Component Analysis (PCA) results identified six factors that can be interpreted as soil, mobile, industry, secondary inorganic aerosol, secondary organic aerosol and oil combustion. Moreover, through Principal Component Regression (PCR), industry, soil, mobile and SIA were shown to be statistically significant sources in a relation to ROS activity.

1. Introduction

The increase of airborne particulate matter has raised concerns for adverse health effects. Sizes, sources and chemical constituents of particulate matter are important factors that can alter the impacts on health effects. While coarse particles can be eliminated from the upper respiratory tract, fine particulate matter ($PM_{2.5}$) which has a diameter of < 2.5 μm is more likely to penetrate deeply into the lungs (Araujo and Nel, 2009) and shows more adverse health effects in comparison to coarse particulate matter (Schwartz et al., 1996; McDonnell et al., 2000). A number of studies have reported effects of ambient particulate matter to mortality and morbidity due to respiratory disease and cardiovascular disease (Atkinson et al., 2014; Heo et al., 2014; Pope et al., 2004; Schwartz et al., 1996). The International Agency for Research on Cancer (IARC) classified particulate matter as Group 1 carcinogen and with the indication of carcinogenicity, PM-induced inflammation has been studied in various fields (IARC, 2013).

While the mechanism of $PM_{2.5}$ causing adverse health effects are still unclear, many studies have shown reactive oxygen species (ROS) to be highly associated with PM-induced negative health effects (Valavanidis et al., 2013; Schwarze et al., 2010). In recent studies, toxicological effects of ROS leading to systemic inflammation and disease generation have been discovered. ROS is known to be generated as a byproduct of energy metabolism pathway of aerobic organism. Exposure to the environmental factors such as PM causes an increase in the level of ROS through exogenous or endogenous cellular processes (Michael et al., 2013; de Kok et al., 2006). Inflammation can be driven by oxidative stress, a state when the level of ROS exceeds antioxidant

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capacity (Ray et al., 2012). Moreover, various signaling molecules such as cytokines and chemokines are released or recruited by the inflammatory response leading to systemic inflammation (Hiraiwa and van Eeden, 2014). Since alveolar macrophage is the first cell line to encounter pollutants and initiate pro-inflammatory cascades (Brook et al., 2010), this study focused on ROS activity of alveolar macrophages after exposure to fine particulate matter.

PM-induced ROS activity analysis through *in vivo*, *in vitro*, chemical experiments and epidemiological studies have been conducted around the world. In Asia, however, few studies on PM related health effects from the molecular to the epidemiological level have been reported recently (Chen et al., 2017; He et al., 2017; Li et al., 2018; Hamad et al., 2015). In Korea, epidemiological studies of adverse health effects of PM-related to respiratory disease, cardiovascular disease, cerebrovascular disease (Kim et al., 2015) and mortality rate (Heo et al., 2014) have been done, however PM-induced ROS activity in cellular level has not been actively studied.

Due to South Korea's geological characteristics and westerly winds, transport of PM from China is inevitable. The supposition that the chemical constituents and sources of $PM_{2.5}$ of Korea will be different from that of other countries due to the effects of local and long range transport led to analysis of ROS potential of $PM_{2.5}$ in Korea. Thus, the objective of this study is to assess toxicological effects of $PM_{2.5}$ in Korea by relating PM-induced oxidative potential with chemical constituents and possible sources in Seoul, Korea.

2. Experimental methods

2.1. Sample collection and analysis

Ambient $PM_{2.5}$ samples were collected at the rooftop of former Seoul National University Graduate School of Public Health building (37.58°N, 127.00°E, and 17 m above ground) from September 2013 to May 2015. The sampling site was located in the center of the Seoul which is suitable for characterizing various sources and effects of $PM_{2.5}$ in the most populated urban area in Korea. A low-volume air sampler consisting of Cyclone (URG-2000-30EH, URG, USA) and filter pack system (URG-2000-30FG, URG, USA) was used with a flow rate of 16.7 L min^{-1}. Teflon filter (PTFE membrane, Pall Corporation, USA), quartz microfiber filter (Quartz microfiber filter, 1851-047, Whatman™, UK), and zefluor filter (Zefluor™ Membrane, Pall Corporation, USA) with a diameter of 47 mm were loaded in the sampler.

$PM_{2.5}$ mass concentration was obtained by measuring Teflon filter which were stored in desiccator for at least 24 h before and after sampling. Collected samples were weighed with balance (0.01 mg) (HM202, A&D, San Jose, CA, USA) and sent to Clarkson University (Postdam, NY) for elemental analysis of a total of 18 elements (Cl, Al, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Pb, Si, Ti, V, Zn, Br, Ni, and Br) using X-ray Fluorescence (XRF).

Samples collected on zefluor filters were used for ionic chemical analysis. Soluble ions (SO_4^{2-}, NO_3^-, NH_4^+) were analyzed by Ion Chromatography (ICS-1100, Thermo Fisher Scientific). Carbon species, including organic carbon (OC) and elemental carbon (EC) were quantified by Carbon Aerosol Analyzer (Model 3, Sunset Laboratory Inc., Oregon, USA). Quartz filters were punched (1.5 cm × 1.0 cm) and the oven was ramped up to 870 °C by following National Institute of Occupational Safety and Health (NIOSH) 5040, thermal/optical transmittance (TOT) method. Water-soluble organic carbon (WSOC) was measured by total carbon analyzer (TOC-V CPH, Shimadzu, Japan).

2.2. Macrophage ROS analysis

Samples were sent to Wisconsin State Laboratory of Hygiene (Wisconsin, USA) to measure the ROS activity induced by fine PM. To quantify ROS activity, rat alveolar macrophage cells (*NR8383*, American Type Culture Collection, VA, USA) were exposed to extracted $PM_{2.5}$ samples and then fluorescence intensity measured with a flow cytometer (Coulter EPICS XL, Beckman Coulter, Miami, FL). Teflon filters were used for ROS analysis and each filter was cut in half before use. Extraction was prepared by continuous agitation of the sectioned filter in Type I purified water for 16 h. Cell-permeable, 2′7′-dichlorodihydrofluorescein diacetate (DCFH-DA) was used as the ROS probe and got loaded into cells. Since ROS oxidize DCFH-DA and yield highly fluorescent 2′7-dichlorofluorescein (DCF), measurements of DCF generated by the extracted samples represents each sample's ROS potential (Eruslanov and Kusmartsev, 2010). Zymosan was used as a positive control and results of ROS activity shown as units of μg zymosan (Landreman et al., 2008).

2.3. Data analysis

Pearson correlation (r) and stepwise multiple linear regression (MLR) were performed through R 3.4.0, to find correlation between chemical constituents and ROS activity. All 26 chemical species were used and ROS activity was logged. Principal component analysis (PCA) was applied to the selected samples for identification of possible sources. SPSS Statistics 23 (SPSS Statistics, IBM, U.S.A.) was used for PCA. A VARIMAX rotation with Kaiser normalization was applied and factors with eigenvalues > 1.0 were extracted. The number of components to be extracted was decided upon scree plot. PCA was coupled with principal component regression analysis (PCR) to determine the importance of each sources to ROS activity. In this study, principal component scores from PCA and logged ROS activity results were used for PCR.

3. Results

3.1. Chemical constituents

Time series plot of $PM_{2.5}$ speciation data in Seoul, Korea from September 2013 to December 2015 is shown in Fig. 1. Overall, the average mass concentration of $PM_{2.5}$ for this period (41.5 μg/m^3) shows relatively high concentration during spring and winter. The sum of the ionic species NO_3^-, SO_4^{2-}, and NH_4^+ accounted for about 42% of the total mass and was most abundant component of $PM_{2.5}$ mass. Metal species accounted for 23% of total $PM_{2.5}$ mass and OC (6.3 μg/m^3) and EC (1.2 μg/m^3) accounted for 15% and 3% of total mass, respectively. When divided into warm and cold seasons, average $PM_{2.5}$ concentration of cold season (48.1 μg/m^3) was shown to be higher than that of warm season (32.7 μg/m^3). Average concentrations of OC, EC, NH_4^+, NO_3^-, crustal and non-crustal species in the cold season are higher than that of warm season. SO_4^{2-} was the only species to show higher concentrations in the warm than cold season.

52 samples collected during the sampling period were selected to be utilized in the *in vitro* macrophage assay and Table 1 summarizes concentrations of chemical species and ROS activity for three different categories: warm, cold and total. Selected samples were divided into two categories, warm and cold, based on temperature. Samples in the warm season had an average temperature of 22.1°C and were collected between May and October while cold season samples had an average temperature of 5.1°C and were collected between November and April. Among 52 samples, 28 samples were classified as warm season samples and 24 samples were selected as cold season samples. Deming regression was applied to obtain the values of primary organic carbon (POC) and secondary organic carbon (SOC) was obtained by subtracting POC from the total measured OC. A different number of samples (warm = 16, cold = 12) were used to calculate mass concentration of water soluble organic carbon (WSOC). A concentration of crustal dust was calculated by using Eq. (1) and the sum of the rest of the elements was categorized into non-crustal.