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Characteristics and cellular effects of ambient particulate matter from Beijing

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ABSTRACT

In vitro tests using human adenocarcinomic alveolar epithelial cell line A549 and small mouse monocyte-macrophage cell line J774A.1 were conducted to test toxicity of six PM (particulate matter) samples from Beijing. The properties of the samples differ significantly. The production of inflammatory cytokine (TNF-α for J774A.1) and chemokine (IL-8 for A549) and the level of intracellular reactive oxygen species (ROS) were used as endpoints. There was a positive correlation between water soluble organic carbon and DTT-based redox activity. Both cell types produced increased levels of inflammatory mediators and had higher level of intracelllar ROS, indicating the presence of PM-induced inflammatory response and oxidative stress, which were dose-dependent and significantly different among the samples. The releases of IL-8 from A549 and TNF-α from J774A.1 were significantly correlated to PM size, Zeta potential, endotoxin, major metals, and polycyclic aromatic hydrocarbons. No correlation between ROS and these properties was identified.

1. Introduction

Airborne particulate matter (PM) is of a global public health concern due to a variety of adverse health effects. This is particularly relevant in the countries during economic transition (Evans et al., 2013). In early 2013, officially monitored maximum daily mean concentrations of $PM_{2.5}$ (PM with aerodynamic diameter less than 2.5 μm) was above 600 μg/m^3 (Wang et al., 2013). Correlation between ambient PM and population morbidity has been demonstrated by numerous epidemiological studies (Kelly and Clancy, 1984; Godlee, 1991; Pope and Dockery, 2006). It was estimated that inhalation exposure to ambient PM can cause millions of premature deaths in China each year (Zhang and Smith, 2007) and northern China including Beijing is one of the most heavily polluted regions in the world.

Toxic effects of ambient PM on human health have been extensively studied in developed countries while data in developing countries are scarce. Among various methods, *in vitro* models using human or animal cell lines, including human lung epithelial cell line A549 and small mouse monocyte-macrophage cell line J774A.1, are often used as a quick screening procedure for assessing the adverse effects of air pollutants including PM, and to perform in-depth mechanistic studies (Alfaro-Moreno et al., 2002; Donaldson and Borm, 2006; Ayres et al., 2008). Inflammation and oxidative stress damage induced by PM depositing on respiratory system are two critical endpoints among various adverse effects (Li et al., 2003; Hetland et al., 2004).

PM is a complex mixture of particles of different origins. Its ambient levels and composition often vary greatly depending on the emission sources and metrological conditions. For example, it has been reported that the contents of mineral elements and PAHs for the PM_{10} (PM with aerodynamic diameter less than 10 μm) collected in Milan during the winter and summer were significantly different and difference was also observed for their cytotoxic and pro-inflammatory effects (Gualtieri et al., 2010).

The association of adverse cellular effects with a number of PM properties, including size, water soluble organic carbon (WSOC), Zeta potential, redox activity, trace and major elements, polycyclic aromatic hydrocarbons (PAHs), and endotoxin has been studied (Prieditis and Adamson, 2002; Gerlofs-Nijland et al., 2009; Cho et al., 2012). A recent study by Wang et al. (2013) has demonstrated a close link between size distributions and injurious cellular effects of PM from Beijing.

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Our long-term goal is to reveal the contribution of individual components of PM to the cellular toxicities and possible synergic effects among them. This knowledge is essential for a better understanding of the health impact of PM exposure. This work was designed as one of the pilot projects to evaluate the feasibility of developing such *in vitro* model(s). We performed detailed characterization of PM_{10} collected in the urban area of Beijing during summer and fall of 2010 and used A549 and J774A.1 cell lines as *in vitro* systems to assess the pro-oxidative and pro-inflammatory effects of these particles and the dependence of such effects on physicochemical properties of the PM.

2. Material and methods

2.1. Reagents

RPMI 1640 (RPMI) medium, Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), and trypsin-EDTA were purchased from GIBCO, USA. DTT, penicillin, streptomycin sulfate, and dihydroethidium (DHE) were from Sigma–Aldrich, USA. Limulus amebocyte lysate assay kit for endotoxin analysis was from Chinese Horseshoe Crab Reagent Manufactory, China. Dichloromethane, *n*-hexane, methanol, silica gel (100–200 mesh), and granular anhydrous sodium sulfate were from Beijing Reagent, China. PAH standard solution (PPH-10JM) was purchased from Chem Service, USA. Surrogates (2-fluoro-1,1′-biphenyl and *p*-terphenyl-d_{14}) and internal standards (NAP-d_8, ACE-d_{10}, ANT-d_{10}, CHR-d_{12}, and perylene-d_{12}) for PAH measurement were from J&K Chemical, USA. Phosphate buffered saline (PBS, pH 7.2–7.4) was prepared by mixing NaCl (8.5 g), Na_2HPO_4 (1.4 g), and NaH_2PO_4 (0.20 g) in 1.0 L ultrapure water. Glass fiber membranes were heated at 450 °C for 6 h. Silica gel was heated at 450 °C for 4 h, reactivated at 130 °C over 16 h, and kept in a desiccator before use. Anhydrous sodium sulfate was baked at 650 °C for 6 h.

2.2. Sample collection

Twelve PM_{10} samples were collected on Peking University campus, a typical urban setting of Beijing, using a median volume active sampler (Laoying 2031, China) at a flow rate of 1 m^3/min in 2010. PM_{10} in urban area of Beijing are originally from various sources including primary emissions from coal burning, biomass burning, and motor vehicles and secondarily formation. Atmospheric transport from neighboring provinces also contributes to the air pollution in Beijing (Wang et al., 2013). The samples were collected during the periods of July 31 to August 9 (S1), August 31 to September 6 (S2), September 23 to October 1 (S3), October 1 to October 8 (S4), October 15 to October 22 (S5), and October 27 to November 12 (S6). Six of them were selected to cover a wide range of PAH concentrations for this study. Glass fiber filters were used as sampling medium. The particles were extracted from the filters with 20 ml ultrapure water ultrasonically for 3 min (50W, KQ-50, Kun Shan, China). The suspensions were diluted to 2 mg/ml with 100 U/ml penicillin and 100 mg/ml streptomycin sulfate added. The samples were stored at −80 °C prior to use. It should be noted that the possible effects on PM size and composition can not be totally avoided by using this procedure.

2.3. PM sample characterization

Size distribution and Zeta potential were measured using a Zetasizer Nano (ZS90, Malvern, UK) and the measurement was conducted in 400 μg PM/ml in RPMI supplemented with 10% FBS. For WSOC analysis, PM water suspension (400 μg/ml) was shaken (100 rpm) for 16 h at 25°C and filtered through a PTFE filter (0.22 μm). The filtrate was then analyzed for WSOC using a total carbon analyzer (Shimadzu 5000A, Japan). The procedures for extraction, cleanup, and analysis of PAHs were performed as previously described by Wang et al. (2011). Briefly, PAHs in the PM samples were extracted using a microwave accelerated reaction system (CEM, USA). The extracts were purified using silica/alumina columns (10 mm i.d. × 30 cm). PAHs were analyzed using a gas chromatograph (Agilent 6890, USA) coupled with a DB-5MS capillary column (0.25 mm i.d. × 30 m, 0.25 μm film thickness) and a mass spectrometer (Agilent 5973, USA). The mass spectrometer was operated in electron impact ionization mode (70 eV) and the scanning range was from 35 to 500 mass units. PAHs were identified based on the retention time and qualitative ions of standards in selected ion monitoring mode and were quantified by the internal standards added after extraction and cleanup. The 18 parent PAHs quantified include acenaphthene (ACE), acenaphthylene (ACY), fluorene (FLO), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), benzo(a)anthracene (BaA), chrysene (CHR), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(e) pyrene (BeP), benzo(a)pyrene (BaP), diben(a,h)anthracene (DahA), indeno(1,2,3-cd) pyrene (IcdP), and benzo(g,h,i)perylene (BghiP), dibenz[a,l]pyrene (DalP), and coronene (COR). The total concentration of the 18 PAHs is designated as PAH18 in this paper. For metal analysis, PM samples were digested using a mixture of HNO_3 and $HClO_4$ following the method of Lee et al. (2007). The concentrations of As, Cd, Cu, Pb, V, Zn, Ca, Fe, and Mg were determined using an inductively coupled plasma-atomic emission spectrometry (Optima 3300DV, Perkin Elmer, USA) (Lee et al., 2007). The intrinsic oxidant potential of PM samples were determined by the DTT assay using a cell-free system as described by Cho et al. (2005). This assay quantitatively measures reactive oxygen species (ROS) formation by PM-associated redox cycling organic chemicals such as quinones (Li et al., 2003; Cho et al., 2005). Endotoxin levels was determined by Limulus amebocyte lysate assay kits following manufacturer's instructions (Chinese Horseshoe Crab Reagent Manufactory, China).

2.4. Quality control

For PAH and metal analysis, reagent and procedure blanks were measured together with each batch of samples and subtracted from the results. A control group using extract from glass fiber filter without PM was included to test the possible effect of the filter. At least two replicates were measured except for metal analysis. The detection limits were 0.53–1.32 ng/g for PAHs. Procedure recovery rates for PAHs determined by standard PAH spiking (four duplicates) ranged from 69 to 115%. 2-fluoro-1,1′-biphenyl and *p*-terphenyl-d_{14} were added before extraction as surrogates. The surrogate recoveries were 80–103%. The recovery rates for metals in the reference material (NIST 1648a) were 106, 100, 101, 95, 108, 104, 96, and 87% for Cd, Cu, Pb, V, Zn, Ca, Fe, and Mg, respectively.

2.5. Cell culture and stimulation

A549 and J774A.1 were purchased from Cell Resource Center, IBMS, CAMS/PUMC, China and maintained in a 37 °C humidified incubator supplemented with 5% CO_2. A549 cells were maintained in RPMI medium supplemented with 10% FBS and 1% penicillin/streptomycin. J774A.1 cells were grown in DMEM containing 10% FBS and 1% penicillin/streptomycin. To determine ROS production and cell viability, 3×10^5/well cells were plated in 12-well plates in a total volume of 0.6 ml/well, respectively. For IL-8 and TNF-α analysis, both A549 and J774A.1 were seeded at 5×10^4 cells/well in 96-well plates in a total volume of 0.1 ml/well. Cell densities were chosen based on the results of preliminary experiments to achieve significant dose–response relationship for the two endpoints individually. Cells in all experiments were allowed to rest for 24 h before exposure to PM. Prior to cellular stimulation stock PM suspensions were vigorously vortexed for 30 s followed by a 1-min sonication. PM suspension was then diluted in complete medium to obtain the final working concentrations of 50, 100, 200, or 400 μg/ml. Cellular exposure was achieved by replacing the culture medium in each well with fresh PM-containing medium. The final volume for exposure was 0.6 and 0.1 ml/well in 12- and 96-well plates, respectively. The control groups received equal volume of vehicle. The outer peripheral wells of the 96-well plates were filled with sterile PBS to reduce medium evaporation from the exposure wells. For each cytokines measurement, media from 18 wells were combined to form a composite sample to improve reproducibility.

2.6. Determination of cell viability

After 45-h exposure, A549 and J774A.1 cells were trypsinized and re-suspended in the culture media and stained with 10 μg/ml of propidium iodide for 10 min. Cell viability was determined by flow cytometry (FACSCalibur, Becton Dickinson, USA) as previously described (Hetland et al., 2004).

2.7. Analysis of IL-8 and TNF-α

Cell culture supernatant were collected after 48 h (A549 and J774A.1) of exposure, centrifuged at 3000 rpm in a table top centrifuge to remove cell debris and stored at −80 °C until analysis. The levels of IL-8 and TNF-α in the cell culture supernatant were measured using commercially available ELISA kits following manufacture's instructions (Wuhan Boster, China). For 48-h exposure with serum added, effect of adsorption of serum proteins on PM on the results can not be ruled out. A serum-free method developed by Osornio-Vargas et al. (2003) can be considered in future study.

2.8. Assessment of intracellular ROS

The level of intracellular ROS was analyzed by flow cytometry using DHE staining (Benov et al., 1998). Twenty one hour after the addition of PM the cells were incubated with 20 μM DHE for 10 min at 37 °C and rinsed twice with PBS. The cells were then trypsinized and suspended in their respective culture medium. DHE fluorescence intensity was analyzed on FACSCalibur (Becton Dickinson, USA) with wavelengths of 488 and 630 nm for excitation and emission, respectively. Sumit v.5.0 was used for data analysis.

2.9. Data analysis

The measurements were conducted in duplicate for Zeta potential, WSOC, and endotoxin, triplicate for viability and ROS, quadruplicate for IL-8, quintuplicate for TNF-α, and sextuplicate for DTT. The results are presented as means ± standard deviations. One-way analysis of variance and bivariate correlation analysis were performed using SPSS v. 13.0 (SPSS Inc., USA) at a significance level of 0.05.