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Human bronchial epithelial cell injuries induced by fine particulate matter from sandstorm and non-sandstorm periods: Association with particle constituents

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ABSTRACT

Epidemiological studies have demonstrated the exacerbation of respiratory diseases following sandstorm-derived particulate matter (PM) exposure. The presence of anthropogenic and biological agents on the sandstorm PM and the escalation of PM < 2.5 μm ($PM_{2.5}$) pollution in China have led to serious concerns regarding the health effects of $PM_{2.5}$ during Asian sandstorms. We investigated how changes in $PM_{2.5}$ composition, as the weather transitioned towards a sandstorm, affected human airway epithelial cells. Six $PM_{2.5}$ samples covering two sandstorm events and their respective background and transition periods were collected in Baotou, an industrial city near the Gobi Desert in China. PM samples from all three periods had mild cytotoxicity in human bronchial epithelial cell line BEAS-2B, which was positively correlated with the contents of polycyclic aromatic hydrocarbons and several metals. All PM samples potently increased the release of interleukin-6 (IL-6) and interleukin-8 (IL-8). Endotoxin in all samples contributed significantly to the IL-6 response, with only a minor effect on IL-8. Cr was positively correlated with both IL-6 and IL-8 release, while Si was only associated with the increase of IL-6. Our study suggests that local agricultural and industrial surroundings in addition to the sandstorm play important roles in the respiratory effects of sandstorm-derived PM.

Introduction

An elevated level of ambient fine particulate matter (PM) with aerodynamic diameter < 2.5 μm ($PM_{2.5}$) is a major risk factor for increased cardiopulmonary morbidity and mortality (Brook et al., 2008; Pope and Dockery, 2006). Epidemiological studies conducted in different continents (Chang et al., 2006; Gyan et al., 2005; Hirsch et al., 1974; Kanatani et al., 2010) have demonstrated that exposure to sandstorm particles can increase daily mortality and exacerbate cardiovascular and

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respiratory diseases. Sandstorm-derived particles are responsible for the surges of PM levels in Asia and can have both a short- and long-term impact on air quality at a local, regional, and even global scale (Duce et al., 1980; Fairlie et al., 2007; Wang et al., 2007). Sandstorm $PM_{2.5}$ is a combination of fine sand dust from the desert surface and daily non-sandstorm $PM_{2.5}$ accumulated prior to the sandstorm. Non-sandstorm $PM_{2.5}$ mainly originates from local combustion and industrial emission sources or through secondary aerosol formation. These particles consist primarily of sulfate, nitrate, ammonium, organic and soil crust matter, elemental carbon, and some biological components (Wilson, 1998; Wu et al., 2014); whereas sand dust particles from Asia are mostly composed of metals (*e.g.*, silicon, aluminum, calcium, and iron) (Honda et al., 2014). With sandstorms lasting for days, other chemical and biological agents can also attach to the sand dust particles during transport (Esmaeil et al., 2014).

Asian sandstorm particles, mainly from the Gobi Desert that covers southern Mongolia and parts of northern and northwestern China, can be transported to regions far beyond China (Duce et al., 1980; He et al., 2013). Because the combination of anthropogenic pollutants and biological agents on sandstorm PM may have an additional impact on human health, as $PM_{2.5}$ pollution in China continues to intensify, there are serious concerns about the health effects of Asian sandstorm-derived PM among East Asian countries (He et al., 2013; Honda et al., 2014; Ichinose et al., 2009; Kanatani et al., 2010; Matsukawa et al., 2014; Mori et al., 2003). The adverse respiratory effects of non-sandstorm $PM_{2.5}$ are mainly linked to the particles' chemical composition. Polycyclic aromatic hydrocarbons (PAHs) and metals are considered the primary components responsible for PM-induced inflammation in the lung (Charrier et al., 2014; Delfino, 2002; Li et al., 2003; Gerlofs-Nijland et al., 2009; Wu et al., 2012, 2013). Several studies have identified endotoxin as one of the biological components responsible for the inflammatory response of human airway epithelial cells and the organic contents responsible for PM-induced DNA damage; the latter has been postulated to contribute to the increase of lung cancer in China (Honda et al., 2014; Meng and Zhang, 2007; Osornio-Vargas et al., 2003; Wang et al., 2013; Zhang et al., 2009).

Currently, there is limited information on how the transition from the background weather to a sandstorm will affect $PM_{2.5}$ composition and their cellular effects. Previous studies have focused on the adverse effects and properties of either sandstorm or non-sandstorm PM. This has resulted in a lack of information on the link between the health effects and time-resolved changes in particle composition. Moreover, there is an imperative need in understanding how the local agricultural and industrial surroundings contribute to the health effects of these particles. The objective of this study was to determine how the changes in the biological and chemical composition of $PM_{2.5}$ affected the human airway epithelial cell response as sandstorms approached Baotou; a city surrounded by animal farming, rare earth mining, precious mineral smelting, and steel industries. The data from this study provides detailed information on the changes in the chemical and biological characteristics of ambient $PM_{2.5}$ during a period covering three different weather conditions (*i.e.*, normal local weather, a sandstorm, and a transition period between the two) of two sandstorm episodes, and their effects on human bronchial epithelial cells. Correlation analyses suggest that local agricultural and industrial surrounding may affect the adverse cellular effects of these particles.

1. Materials and methods

1.1. Reagents and materials

The human bronchial epithelial cell line BEAS-2B was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Bronchial epithelial cell growth medium (BEGM) was obtained from Lonza (Walkersville, MD, USA). Cell culture-grade water, phosphate-buffered saline and polymyxin B (PB) were purchased from Sigma-Aldrich (St Louis, MO, USA). Trypsin–EDTA and a penicillin/streptomycin mixture were obtained from Invitrogen (San Diego, CA, USA). Limulus amebocyte lysate (LAL) assay kit was purchased from Thermo Fisher Scientific (Rockford, IL, USA). Pierce™ Lactate Dehydrogenase Cytotoxicity Assay (LDH assay) kit was purchased from Life Technologies (Grand Island, NY, USA). Enzyme-linked immunosorbent assay (ELISA) kits for human interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), and type I rat tail collagen were obtained from BD Biosciences (San Diego, CA, USA). ELISA kit for human thymic stromal lymphopoietin (TSLP) was purchased from eBioscience (San Diego, CA, USA). The PAH standards and their recovery surrogates (*p*-terphenyl D_{14} and 2-fluorobiphenyl) were obtained from AccuStandard, Inc. (New Haven, CT, USA). Acetonitrile and *n*-hexane of Ultra Resi-Analyzed® standard were purchased from Merck KGaA (Darmstadt, Hesse-Darmstadt, Germany). Acetone and dichloromethane of Suprasolv® standard were purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ, USA). Silica gel (100–200 mesh), neutral aluminum oxide (200–300 mesh), and granular anhydrous sodium sulfate were purchased from Beijing Chemical Reagent Co. (Beijing, China). Anhydrous sodium sulfate was baked at 600°C for 6 hr, and silica gel and neutral aluminum oxide were heated at 450°C for 8 hr to remove impurities. Silica gel and neutral aluminum oxide were further reactivated, then stored in a sealed desiccator and heated at 150°C for at least 16 hr before use. Granular anhydrous sodium sulfate was stored in a sealed glass bottle after cleaning. All glassware was cleaned with an ultrasonic cleaner (KQ-500B; Kunshan Ultrasonic Instruments Co., Jiangsu, China) for 30 min with liquid detergent, rinsed with distilled water, and finally heated at 450°C for 8 hr.

1.2. PM sampling and extraction

The details of the $PM_{2.5}$ sampling have been described previously (Deng et al., 2007). Briefly, samples were collected using a high-volume sampler (Anderson, USA) with a quartz filter from March 6 to May 6, 2004 in Baotou. $PM_{2.5}$ samples were collected daily for 24 hr (noon-to-noon) and six $PM_{2.5}$ samples were used in this study. Based on local meteorological conditions and air-quality monitoring data, the selected

samples were divided into three groups designated as: background $PM_{2.5}$ (BG) on non-sandstorm days, sandstorm $PM_{2.5}$ (SS) during a sandstorm, and transition $PM_{2.5}$ (TR) during the transitional days between BG and SS. The sampling dates (mm/dd) for the six samples were 03/06 (BG#1), 03/31 (TR#1), 04/01 (SS#1), 04/04 (BG#2), 04/21 (TR#2), and 04/29 (SS#2). The samples were stored at −20°C in a tightly sealed container. The particles were extracted from filters by ultrasonication for 5 min using cell-culture-grade water. A stock suspension (2.5 mg/mL) was prepared for each sample and stored at −80°C prior to use. One blank filter was extracted as the filter control, which was used to exclude interference by filter materials in the cellular study.

1.3. Cell culture and cytotoxicity analysis

BEAS-2B cells were cultured in BEGM in collagen-coated cell culture plates at 37°C in a humidified incubator supplemented with 5% CO_2 (Li et al., 2002, 2003). To determine cell viability, BEAS-2B cells were plated at 6000 cells/well in a 96-well cell culture plate containing 100 μL/well of BEGM. Each stimulation group contained three independent wells. The cells were allowed to rest for 24 hr before being exposed to 10, 50, and 100 μg/mL of PM in 100 μL/well of fresh BEGM. Sixteen hours after the addition of PM the cell culture media were collected and analyzed for cytotoxicity using the LDH assay kit.

1.4. Analysis of inflammatory mediators

Cells (1×10^5/well, 12-well plate) were exposed to 50 μg/mL of PM in a final volume of 1 mL of BEGM. The cell culture media were collected after 16 hr of exposure and stored at −80°C for cytokine analysis. The levels of four cytokines including interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), thymic stromal lymphopoietin (TSLP), and one chemokine, interleukin-8 (IL-8), were measured using an ELISA kit. To determine the role of endotoxin in PM-induced IL-6 and IL-8 response, BEAS-2B cells were plated at 0.5×10^5/well in 24-well plates and allowed to rest for 24 hr. PM samples were diluted in BEGM at a final concentration of 50 μg/mL and pre-incubated with PB (174 Units for 1 ng endotoxin) or equal volume of water for 2 hr before being given to the cells at 0.5 mL/well for a 16 hr exposure. Each stimulation group contained three independent wells. Endotoxin was measured using the LAL assay kit, following the manufacturer's instructions.

1.5. PM characterization

The water-soluble organic carbon fraction (WSOC) of the PM was extracted and measured following a previously published method (Wang et al., 2013). PAH concentrations of the PM were measured following an extraction-cleanup-analysis procedure (see "Extraction and Cleanup for PAH Analysis" section in the Supplementary Information for more details). Twenty-seven parent PAHs were analyzed: acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLE), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLU), retene (RET), pyrene (PYR), benzo[c]phenanthrene (BCP), cyclopenta[c,d]pyrene (CcdP), benz[a]anthracene (BAA), chrysene (CHR), benzo[b]fluoranthene (BBF), benzo[k]fluoranthene (BKF), benzo[e]pyrene (BEP), benzo[a]pyrene (BAP), perylene (PER), indeno[1,2,3-cd]pyrene (IcdP), dibenz[a,h]anthracene (DahA), benzo[g,h,i]perylene (BghiP), di-benzo[a,c]pyrene (dBacP), di-benzo[a,l]pyrene (dBalP), dibenzo[a,e]fluoranthen (dBaeF), coronene (COR), di-benzo[a,e]pyrene (dBaeP), di-benzo[a,i]pyrene (dBaiP), and di-benzo[a,h]pyrene (dBahP). Qualitative and quantitative analyses were performed using a gas chromatograph–mass spectrometer (GC–MS: Model 7890, Agilent, Santa Clara, CA, USA) coupled with a mass spectrometer (MS: Model 5975, Agilent) and equipped with a HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm). PAHs were identified based upon the retention time and the qualifying ions of the standards in selected ion monitoring mode with an electronic impaction source. Two procedure blanks and a reagent blank were included. If the PAH concentration of PM sample was lower than its operation blanks, it was set to be zero. The recoveries of the two surrogate standards [2-fluorobiphenyl for low molecular-weight PAHs with 2–3 benzene rings and *p*-terphenyl D_{14} for high molecular-weight PAHs with 4–5 benzene rings] were (97.2 ± 37.0)% and (104 ± 13.0)%, respectively. To determine the metal content, a PM suspension of each sample was digested using a mixture of HNO_3 and $HClO_4$. A blank filter extract was set as the operation background for the six $PM_{2.5}$ samples. The concentrations of 15 major and trace metals were measured using inductively coupled plasma-atomic emission spectrometry (ICP-AES: Optima 3300DV, Perkin-Elmer, Waltham, MA, USA): aluminum (Al), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), Silicon (Si), vanadium (V), and zinc (Zn).

1.6. Data analysis

The results are presented as means ± standard error of the mean (SEM). Student's *t*-test was used to compare the differences between PM-exposed and the control groups (cell-only and filter controls). The $PM_{2.5}$ samples were grouped into Episode I (BG#1, TR#1, and SS#1) and Episode II (BG#2, TR#2, and SS#2). A one-way analysis of variance (ANOVA) was performed to compare the means of the three types of PM samples (BG, TR, and SS) in the same episode, and they were further classified by multiple comparisons using Fisher's least significant difference test (LSD). Statistical analysis was performed using SPSS (v. 16.0). A two-tailed p value < 0.05 was considered statistically significant.

2. Results

2.1. Cytotoxic effect of PM

The impact of the PM on cell viability was determined by the dose–response experiment (Fig. 1). LDH is a stable enzyme that is present in the cytoplasm of most cells. Upon disruption of the cell membrane integrity LDH is released into the extracellular environment; thus, the amount of LDH in the cell culture media has been used as an indicator of toxicity levels presupposing cell death. The overall cell viability

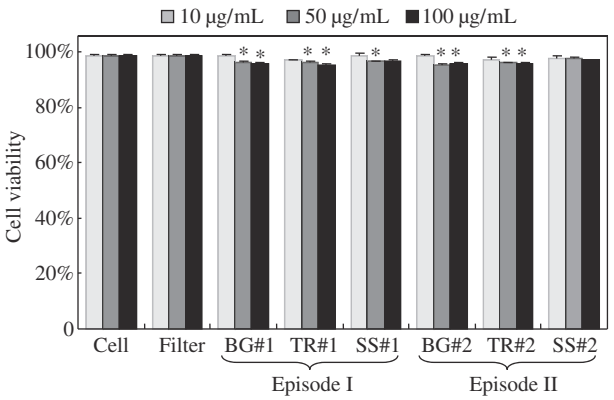

Fig. 1 – Impact of PM on cell viability. Cell: cell-only negative control; filter: blank filter extract; SS: sandstorm $PM_{2.5}$; BG: background $PM_{2.5}$; TR: transition $PM_{2.5}$. *$p < 0.05$ compared with the filter control. PM: particulate matter.

decreased by less than 5% at the concentrations tested (10, 50, and 100 μg/mL), indicating that all the PM samples had quite weak toxicity. The exposure of BEAS-2B cells to BG#1, BG#2, and TR#1, but not SS, led to a dose–dependent decrease in cell viability. With the exception of SS#2 all other PM samples (50 μg/mL) were found to induce a minor, but statistically significant, loss of cell viability (Fig. 1). No significant difference was found among BG, TR, and SS in Episode I, whereas BG tended to exhibit slightly greater toxicity than SS in Episode II.

2.2. Cellular inflammatory response to PM exposure

We analyzed five inflammatory mediators including IL-1, IL-6, IL-8, TNF-α and TSLP in the culture media after the BEAS-2B cells were exposed to 50 μg/mL of PM for 16 hr. Only IL-6 and IL-8 were detectable and their levels showed a dose–dependent increase (Fig. S1). Because Asian sand dust (ASD)-induced release of IL-6 and IL-8 by BEAS-2B cells has been found to be associated with the endotoxin on the particle surface we measured the amount of endotoxin in each PM sample to determine whether the endotoxin might have contributed to the observed inflammatory response (Honda et al., 2014). Fig. 2 shows the amount of endotoxin present in the culture media when the cells were stimulated with 50 μg/mL of PM (Fig. 2A) and the endotoxin content on the particles (Fig. 2B). Endotoxin levels varied with changes in the local meteorological conditions. In general, as the sandstorm approached there was a gradual increase in particle-associated endotoxin, reaching the maximum during the two sandstorm periods (SS#1 and SS#2). Overall, the endotoxin content was in the order of SS > TR > BG.

To determine the contribution of endotoxin to PM-induced IL-6 and IL-8 release, we exposed BEAS-2B cells to 50 μg/mL of PM in the absence and presence of endotoxin inhibitor PB. Fig. 3A shows that there was no significant difference in the IL-6 level between the cell-only and filter control groups as well as between respective controls with and without PB. In the absence of PB, all PM samples potently increased the release of IL-6 compared to the cell-only and filter controls ($p \leq 0.01$). During Episode I, SS#1 significantly increased IL-6 release compared to BG#1, while both TR#2 and SS#2 from Episode II had a stronger effect on IL-6 response than BG#2. With the exception of BG#1, PB effectively suppressed the release of IL-6 (59%–84% inhibition), indicating a significant contribution of endotoxin. The IL-8 response had a similar pattern to that of IL-6. Specifically, all PM samples increased the release of IL-8 from BEAS-2B cells compared to the cell-only and filter controls. The culture media of SS#1-, TR#2-, and SS#2-treated cells also had significantly higher levels of IL-8 compared to that in their respective BG groups. However, on the contrary to its effect on IL-6, only the endotoxin on TR#1 contributed to the IL-8 response as evidenced by its 34% decrease in the presence of PB (Fig. 3B). PB failed to suppress the increase of IL-8 induced by all other PM samples. Overall, SS PM from both episodes consistently had a stronger effect in increasing the release of both IL-6 and IL-8 (Fig. 3).

2.3. WSOC and PAH contents of the PM

A large number of studies have demonstrated that organic compounds play important roles in PM-induced cell injury

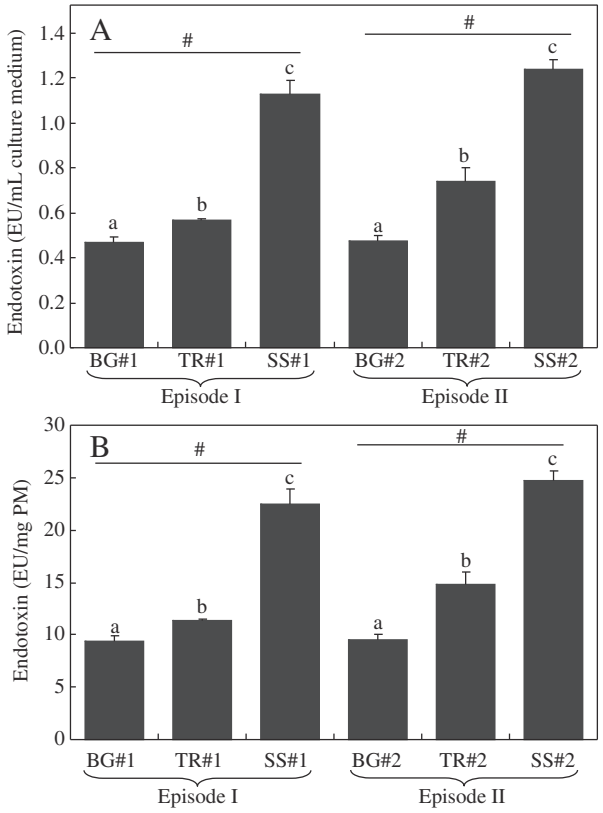

Fig. 2 – The greater endotoxin content on sandstorm PM. (A) Endotoxin concentrations in the cell culture media during exposure; (B) endotoxin content on the PM. #$p < 0.001$ by ANOVA for BG, TR, and SS of the same episode, a, b, and c are significant groups classified by LSD multiple comparison within the same episode; representing statistically significant ranking of c > b > a. PM: particulate matter; ANOVA: analysis of variance; BG: background; TR: transition; SS: sandstorm; LSD: Fisher's least significant difference test.

(Araujo and Nel, 2009; Breysse et al., 2013; Esmaeil et al., 2014; Nel et al., 2006). To evaluate the relationship between the chemical composition and the cellular response we first analyzed particles' WSOC and PAH contents. The WSOC content and the sum of PAHs (ΣPAHs) of the PM fluctuated with the meteorological conditions, and a decreasing trend was observed from the background level, reaching the minimum during the two sandstorm periods (SS#1 and SS#2) (Table 1). Detailed results of the PAH analyses are provided in Table S1. The overall ranking for both WSOC and ΣPAHs contents for both episodes was BG > TR > SS.

2.4. Particles' metal content

To determine whether metals contribute to the PM-induced BEAS-2B cellular responses, the contents of 15 PM-associated metals were analyzed. The selection of these metals was based on the previous reports (Ichinose et al., 2005; Meng and Zhang, 2007; Mori et al., 2003). The levels of these metals varied among the samples (Table 2). During both sandstorm episodes the SS samples contained smaller amounts of Ca, Na, K, Mg, Mn, and Zn, yet higher levels of Al, Cr, Fe, Si, and V than BG. There was no statistically significant difference in the levels of other metals between SS and BG.

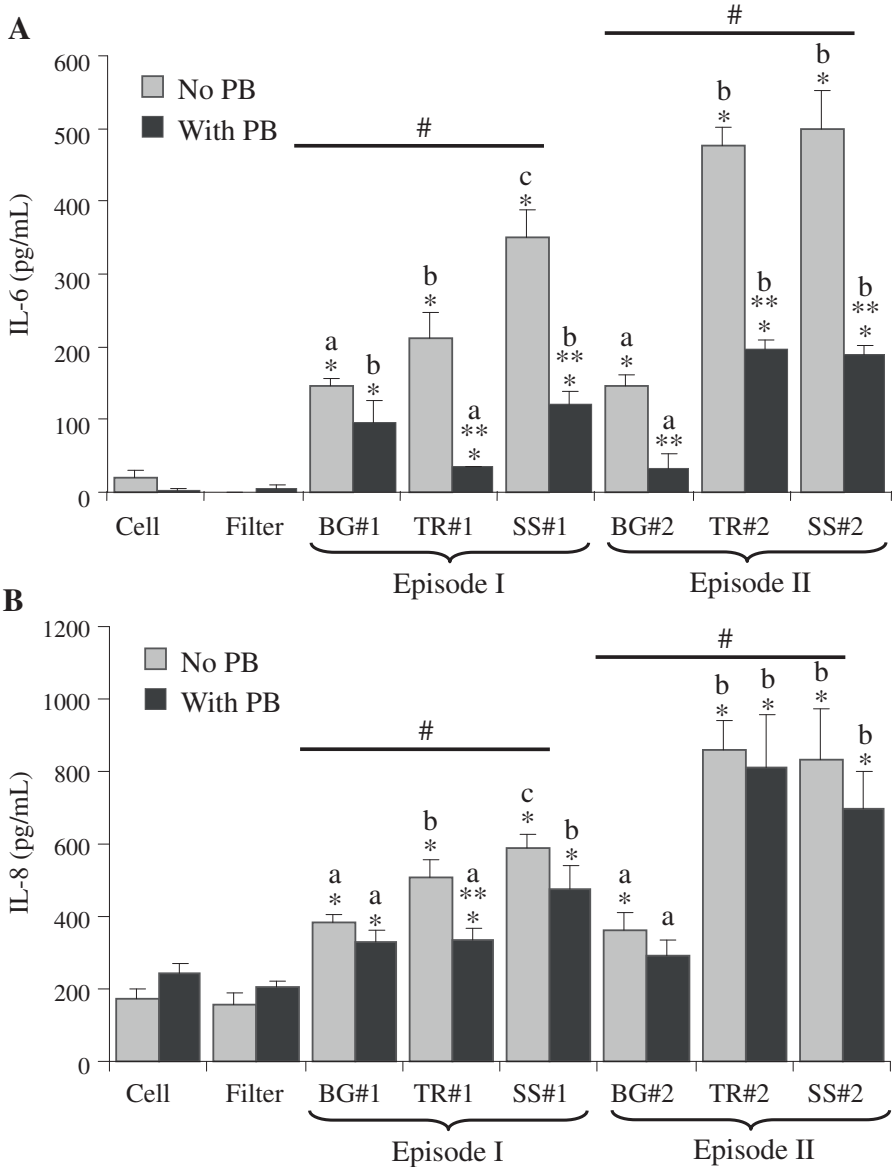

Fig. 3 – The inhibitory effect of polymyxin B on PM-induced release of IL-6 (A) and IL-8 (B). *$p < 0.05$ compared with respective filter control; **$p < 0.05$ compared with respective group without PB; #$p < 0.01$ for BG, TR, and SS of the same episode; a, b, and c are significant groups classified by LSD multiple comparison within the same episode; representing statistically significant ranking of c > b > a. PM: particulate matter; IL-6: interleukin-6; IL-8: interleukin-8; PB: polymyxin B; BG: background; TR: transition; SS: sandstorm; LSD: Fisher's least significant difference test.

2.5. Correlations between cellular responses and PM constituents

Pearson correlation coefficients were analyzed in order to identify the association between PM constituents and cellular inflammatory response (Table 3). Table 3 shows that the cell viability was negatively correlated with ΣPAHs and the contents of K, Mg, Mn and Zn. While Cr was positively correlated with both IL-6 and IL-8 release, Si was only positively correlated with IL-6. There was no significant correlation between the remaining metals and these two inflammatory mediators. Similarly, there was no association between the contents of ΣPAHs and WSOC and the inflammatory response induced by the non-endotoxin fraction (Table 3).

3. Discussion

One of the major adverse health effects of sandstorm-derived PM is to elicit or exacerbate respiratory diseases, including airway inflammation (Esmaeil et al., 2014; Ghio et al., 2014). Our study used ambient particulate samples covering two sandstorm episodes to compare the adverse effects of sandstorm and non-sandstorm $PM_{2.5}$ in a human bronchial epithelial cell model. Our results showed that all PM samples had very mild cytotoxicity, but they were potent in inducing an inflammatory response in BEAS-2B cells, as evidenced by increased release of IL-6 and IL-8. Moreover, both SS samples had a significantly stronger pro-inflammatory effect than their respective BG (Fig. 3). Using endotoxin inhibitor and Pearson correlation coefficient analysis we have found that the PM constituents including endotoxin, ΣPAHs, and several metals (Cr, K, Mg, Mn, Si, and Zn) contributed differently to the pro-inflammatory effect of our PM samples.

The endotoxin on ASD has been suggested to play a role in aggravating airway inflammation (Ren et al., 2014; Yamada et al., 2012). In this study the PM samples from the sandstorm periods had greater amounts of endotoxin than the BG and TR samples. A major activity in our study region is intensive animal farming. Particles derived from animal farms have been found to contain more endotoxin than those from urban areas (Traversi et al., 2011). Thus, the strong winds during the sandstorms could transport a greater amount of endotoxin from the animal farms to the downwind urban area where the $PM_{2.5}$ samples were collected. In the present study the endotoxin was responsible for as much as 59%–84% of IL-6 release induced by all PM samples. However, it only had a limited contribution (34%) to TR#1-induced IL-8 response (Fig. 3).

Two metals (Cr and Si) were positively, but differently, correlated with the IL-6 and IL-8 response (Table 3). Si was only associated with the IL-6 response, whereas Cr was linked to the increase of both IL-6 and IL-8. That SiO_2 could up-regulate the expression of several cytokines and chemokines, including IL-6 and IL-8, in BEAS-2B cells has been reported (Ovrevik et al., 2009). Because Si is an indicator of the SiO_2 content on sandstorm-derived particles, the positive correlation between Si and the IL-6 response corroborates our assumption that SiO_2 may be a significant contributor to the greater pro-inflammatory effect of PM during sandstorms. Exposure to Cr, a heavy metal, has been reported to induce an inflammatory response in BEAS-2B cells, including increased production of IL-6 and IL-8 and the activation of MAPK and NFκB pathways (O'Hara et al., 2007; Pascal and Tessier, 2004; Pratheeshkumar et al., 2014). Cr contamination primarily comes from the combustion processes and the metal industries including electroplating, alloy and steel welding, and metalliferous smelting (http://www.atsdr.cdc.gov/csem/chromium/docs/chromium.pdf) (Gao et al., 2015). China is a major producer of rare earth with two-thirds of its output located in Baotou (http://www.theguardian.com/environment/2012/aug/07/china-rare-earth-village-pollution). Thus, the presence of China's largest rare earth mining, precious mineral smelting, and steel industries in this city may provide an explanation to local Cr pollution and its contribution to both the IL-6 and IL-8 response. It is also possible that the stronger effect of Cr on IL-8 outweighed that of silica; this may be one of the explanations to the lack of association between Si and IL-8 in the present study.

The sandstorm PM samples had less WSOC and PAHs than those collected during the background and transition periods, while the metal contents varied among all the samples. The differences in the contents of Fe and Al, which have been shown to be the major metals in sandstorm-derived PM (Mori et al., 2003), were consistent with the findings of a previous study on ASD (Ichinose et al., 2005). The level of silicon, which is a major mineralogical component of ASD and is derived mainly from feldspar and quartz (Esmaeil et al., 2014), was considerably higher in SS indicating the large SiO_2 input from desert sand (Table 2). The cytotoxic effect of our SS samples was weaker than that previously observed in an ASD study (Fig. 1) (Honda et al., 2014). The difference in PM toxicity between the previous study and ours is likely due to the local surroundings (*i.e.* Japan *vs.* China) as well as the meteorological conditions when the particles were collected. The loss of cell viability, albeit mild, was positively correlated with the particles' contents of PAH, K, Mg, Mn, and Zn (Table 3). Detection of LDH in the cell culture media is a sensitive assay to assess cytotoxicity because the enzyme is released upon cytoplasmic membrane damage; this may explain why we

Table 1 – Concentrations of water soluble organic carbon and sum of the polycyclic aromatic hydrocarbons.

Components	Episode I			Episode II		
	BG#1	TR#1	SS#1	BG#2	TR#2	SS#2
WSOC (mg carbon/mg PM)	0.108	0.074	0.025	0.112	0.091	0.037
ΣPAHs (ng/mg PM)	1376	1357	226	1312	869	119

WSOC: water soluble organic carbon; ΣPAHs: sum of polycyclic aromatic hydrocarbons; SS: sandstorm $PM_{2.5}$; BG: background $PM_{2.5}$; TR: transition $PM_{2.5}$.

Table 2 – Metal contents of PM samples (unit: ng metal/mg PM).

Metal	Episode I			Episode II		
	BG#1	TR#1	SS#1	BG#2	TR#2	SS#2
Al *	128.84 ± 10[a]	134.88 ± 3.9[a]	166.48 ± 0.5[b]	114.84 ± 1.5[a]	123.61 ± 0.9[a]	152.91 ± 7.0[b]
Ca *	288.91 ± 20[b]	263.23 ± 4.9[b]	209.58 ± 1.6[a]	240.27 ± 6.5[a]	286.03 ± 1.6[b]	227.39 ± 10.4[a]
Cd *	0.03 ± 0.0[b]	0.12 ± 0.0[c]	0.01 ± 0.0[a]	0.14 ± 0.0[b]	0.13 ± 0.0[b]	0.01 ± 0.0[a]
Co	0.07 ± 0.01	0.06 ± 0.0	0.06 ± 0.0	0.05 ± 0.0	0.05 ± 0.0	0.06 ± 0.0
Cr *	0.18 ± 0.02[a]	0.23 ± 0.0[b]	0.25 ± 0.0[b]	0.19 ± 0.0[a]	0.31 ± 0.0[c]	0.25 ± 0.01[b]
Cu *	0.35 ± 0.03[a]	0.61 ± 0.0[b]	0.30 ± 0.0[a]	0.74 ± 0.0[b]	0.6 ± 0.0[b]	0.54 ± 0.1[a]
Fe *	104.33 ± 5.5[a]	116.62 ± 3.7[b]	137.36 ± 1.0[c]	69.68 ± 0.6[a]	112.56 ± 0.2[b]	131.38 ± 8.1[c]
K *	112.71 ± 10[c]	95.72 ± 2.8[b]	65.49 ± 1.7[c]	123.87 ± 6.5[c]	98.91 ± 1.5[b]	54.23 ± 0.4[a]
Mg *	70.07 ± 5.0[b]	73.19 ± 1.5[b]	65.96 ± 0.3[a]	72.90 ± 1.6[b]	75.74 ± 0.8[b]	59.78 ± 3.0[a]
Mn *	15.59 ± 1.0[c]	13.07 ± 0.4[b]	7.86 ± 0.2[a]	12.8 ± 0.1[b]	13.23 ± 0.1[c]	3.80 ± 0.1[a]
Na *	116.29 ± 8.9[c]	74.22 ± 1.3[b]	46.49 ± 1.4[a]	85.26 ± 7.3[b]	97.53 ± 1.2[b]	21.28 ± 2.5[a]
Ni *	0.17 ± 0.01[a]	0.27 ± 0.0[b]	0.18 ± 0.02[a]	0.36 ± 0.0[b]	0.3 ± 0.1[b]	0.18 ± 0.0[a]
Si *	139.41 ± 27[a]	177.65 ± 9.6[a]	222.24 ± 12.9[b]	161.74 ± 1.0[a]	203.21 ± 1.4[b]	228.79 ± 1.4[c]
V *	0.25 ± 0.02[a]	0.24 ± 0.0[a]	0.29 ± 0.0[b]	0.19 ± 0.0[a]	0.22 ± 0.0[b]	0.27 ± 0.0[c]
Zn *	22.67 ± 1.8[c]	17.5 ± 0.4[b]	7.81 ± 0.2[a]	33.37 ± 0.6[b]	29.73 ± 0.1[b]	1.97 ± 0.1[a]

a, b, and c are significant groups classified by LSD multiple comparison for the content of each metal within the same episode representing a statistically significant ranking of c > b > a.
PM: particulate matter; BG: background; TR: transition; SS: sandstorm; LSD: Fisher's least significant difference test.
* $p < 0.05$ for BG, TR, and SS within the same episode.

were able to identify the positive correlations between the small changes in cell viability and different PM components. PAHs have been shown to induce cell death by activating aryl hydrocarbon receptor (Solhaug et al., 2004). In addition, PM with high levels of PAHs and OC has been demonstrated to induce mitochondria damage (Li et al., 2003). It is highly likely that the higher PAH contents in the BG samples are intrinsic to the City of Baotou because of its coal combustion used in the metallurgical, non-ferrous metallurgical, electric power, and coal chemical industries, as well as the heavy traffic burden associated with these industrial activities. A study conducted in the same city has found that background $PM_{2.5}$ was more potent than the sandstorm $PM_{2.5}$ in inducing cell death in rat alveolar macrophages and this effect was more pronounced when the cells were exposed to the solvent-extractable organics (Meng and Zhang, 2007). The positive correlation between the PAH content and the cytotoxicity in the present study was in line with this previous report. The loss of cell viability might also be related to Mn and Zn being transition metals, which have been found to play a role in the cytotoxic effect of ambient PM (Gerlofs-Nijland et al., 2009; Wu et al., 2014). The negative correlation between cell viability and K and Mg cannot be explained at this time.

We used WSOC as an indicator of water-soluble organic matter (Hersey et al., 2011), which has been identified as one

Table 3 – Pearson correlation coefficients between cellular response and PM composition.

PM constituents	LDH viability [a]		Interleukin-6		Interleukin-8	
	r	*p*	*r*	*p*	*r*	*p*
WSOC	−0.80	0.055	−0.61	0.198	−0.51	0.301
ΣPAHs	−0.82	0.046	−0.80	0.055	−0.69	0.131
Al	0.78	0.067	0.46	0.359	0.34	0.505
Ca	−0.39	0.445	−0.22	0.668	−0.08	0.881
Cd	−0.77	0.075	−0.27	0.607	−0.15	0.783
Co	0.13	0.800	−0.37	0.467	−0.34	0.505
Cr	0.32	0.539	0.84	0.037	0.88	0.022
Cu	−0.45	0.370	−0.07	0.900	0.02	0.966
Fe	0.84	0.035	0.65	0.163	0.60	0.204
K	−0.93	0.008	−0.74	0.091	−0.66	0.154
Mg	−0.86	0.030	−0.42	0.412	−0.30	0.565
Mn	−0.82	0.047	−0.67	0.142	−0.57	0.237
Na	−0.75	0.086	−0.57	0.235	−0.48	0.337
Ni	−0.77	0.074	−0.23	0.668	−0.15	0.780
Si	0.72	0.106	0.88	0.021	0.81	0.050
V	0.80	0.057	0.38	0.459	0.29	0.580
Zn	−0.91	0.011	−0.48	0.338	−0.39	0.444

[a] Determined by lactate dehydrogenase (LDH) cytotoxicity assay; WSOC: water soluble organic carbon; ΣPAHs: sum of polycyclic aromatic hydrocarbons; PM: particulate matter.

of the fractions responsible for the adverse effect of PM due to its ability to penetrate respiratory cells (Saffari et al., 2014a, 2014b). A number of studies have demonstrated that OC and PAHs can induce an inflammatory response in human airway epithelial cells (Li et al., 2002; Ovrevik et al., 2010; Totlandsdal et al., 2012, 2015). However, our data suggests that the organic fraction may not be the major inducer of IL-6 and IL-8 release in this study (Table 3). Similarly, a negative correlation between the inflammatory effect and the levels of PAHs, WSOC, and metals in various size fractions of PM has also been reported (Wang et al., 2013). A possible explanation for this may be that the strong inflammatory effect of PM-associated endotoxin, silica, and Cr might have overshadowed the impact of the organic fraction on BEAS-2B cells.

The different response patterns between IL-6 and IL-8 (Fig. 3 and Table 3) may be related to the different kinetics of their gene expression as well as selective adsorption onto the particles (DeForge and Remick, 1991; Seagrave, 2008). A detailed kinetics study by DeForge and Remick has shown marked difference between IL-6 and IL-8. It was demonstrated that IL-6 gene expression reached a peak 2–4 hr after the addition of LPS and the peak of IL-6 protein was at 4–6 hr, respectively, which was followed by its protein stabilization. The expression of IL-8, on the other hand, had a significantly different pattern. The mRNA and protein of IL-8 were induced in two waves. After its first plateau at 6–12 hr it rose again with a continuous increase until the end of the 24-hr study. The different kinetics of IL-6 and IL-8 expression have been suggested to reflect their functional differences with IL-6 being the early mediator to amplify and maintain IL-8 expression (DeForge and Remick, 1991). Based on these findings, the different IL-6 and IL-8 patterns observed in our study are likely the results of different kinetics in the expression and release of these two inflammatory mediators in response to our PM samples. A number of PM including diesel exhaust particles (DEP) and ambient PM have been found to adsorb cytokines and chemokines including IL-8 (Seagrave, 2008). Using different concentrations of DEP, Seagrave has demonstrated that due to particle adsorption the loss of IL-8 from DEP suspension after centrifugation could be as high as 90%, whereas the level of IL-6 was not affected (Seagrave, 2008). In our case, because the cell culture media were centrifuged before IL-6 and IL-8 levels in the clear supernatant were analyzed it is possible that some IL-8 had been removed due to their adsorption by PM, which might have also contributed, albeit to a much lesser extent, to the different patterns of IL-6 and IL-8 responses.

Collectively, our results suggest that PAHs and several metals might be the main components responsible for the PM-induced minor, but statistically significant, loss of cell viability, whereas endotoxin, SiO_2, and Cr were the major contributors to the inflammatory response observed in this study. The inclusion of two TR samples, which were the mixtures of their near-term SS and BG, enabled confirmation of the trend in the differences between SS and BG. Cellular responses induced by TR were intermediate between those of SS and BG providing information regarding the variation in $PM_{2.5}$ composition and its subsequent cellular effects as the weather changed. We were not able to identify an individual factor that played a dominant role in inducing the cell death and the inflammatory response. Therefore, it is highly likely that the adverse cellular effects of these particles (*i.e.* BG, TR, and SS) involve multiple regulatory pathways that were activated by the interactions among different PM components. One limitation of this study was the use of quartz-fiber filters for PM collection. It is possible that some filter fibers may have broken down during the extraction and were present in the particle suspension. To exclude any potential interference from the fibers we included a blank filter extract as a control in all experiments. Our results showed that the filter fibers did not interfere with any of the endpoints we investigated.

4. Conclusions

In summary, our work showed that while the overall cytotoxicity of all PM samples was mild, SS had a significantly greater pro-inflammatory effect on human bronchial epithelial cells. In addition to the sandstorm *per se*, the local agricultural and industrial surroundings had a significant impact on the respiratory effects of sandstorm-associated PM. The results of this study provide insight into the relationship between sandstorm $PM_{2.5}$ composition and its adverse respiratory effects, especially in areas such as China where sandstorms are common meteorological events.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2015.12.015.

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