Airborne Fine Particulate Matter Induces Oxidative Stress and Inflammation in Human Nasal Epithelial Cells

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Airborne fine particulate matter with an aerodynamic diameter equal to or smaller than 2.5 μm is abbreviated as $PM_{2.5}$, which is one of the main components in air pollution. Exposure to $PM_{2.5}$ is associated with increased risk of many human diseases, including chronic and allergic rhinitis, but the underlying molecular mechanism for its toxicity has not been fully elucidated. We have hypothesized that $PM_{2.5}$ may cause oxidative stress and enhance inflammatory responses in nasal epithelial cells. Accordingly, we used human RPMI 2650 cells, derived from squamous cell carcinoma of the nasal septum, as a model of nasal epithelial cells, and exposed them to $PM_{2.5}$ that was collected at Fudan University (31.3°N, 121.5°E) in Shanghai, China. $PM_{2.5}$ exposure decreased the viability of RPMI 2650 cells, suggesting that $PM_{2.5}$ may impair the barrier function of nasal epithelial cells. Moreover, $PM_{2.5}$ increased the levels of intracellular reactive oxygen species (ROS) and the nuclear translocation of NF-E2-related factor-2 (Nrf2). Importantly, $PM_{2.5}$ also decreased the activities of superoxide dismutase, catalase and glutathione peroxidase. Pretreatment with N-Acetyl-L-cysteine (an anti-oxidant) reduced the degree of the $PM_{2.5}$-induced oxidative stress in RPMI 2650 cells. In addition, $PM_{2.5}$ increased the production of granulocyte-macrophage colony-stimulating factor, tumor necrosis factor-α, interleukin-13 and eotaxin (C-C motif chemokine ligand 11), each of which initiates and/or augments local inflammation. These results suggest that $PM_{2.5}$ may induce oxidative stress and inflammatory responses in human nasal epithelial cells, thereby leading to nasal inflammatory diseases. The present study provides insights into cellular injury induced by $PM_{2.5}$.

Introduction

Nowadays, exposure to airborne particulate matter (PM) is closely related to public health hazards in many developing countries. PM with an aerodynamic diameter equal to or smaller than 2.5 μm is abbreviated as $PM_{2.5}$ that is associated with increased risk of many human diseases. Epidemiological studies have found that $PM_{2.5}$ exposure increases the risk of cardiac (Delfino et al. 2005) and respiratory (Kloog et al. 2012) diseases. The size, morphology and component of $PM_{2.5}$ play key roles in the adverse effects on human health. Generally, smaller PM has higher health risks. $PM_{2.5}$ is a small particulate, which is inhalable and able to penetrate to tissues (Harrison and Yin 2000). Due to numerous sources, the chemical constituents of $PM_{2.5}$ can contain nitrate, sulfate, ammonium, metals and poly aromatic hydrocarbons (PAHs) (Sun et al. 2004). Although specific constituents in charge of their toxicity

have not been fully investigated, metals, PAHs, biological agents and ultrafine particles that adhere to particles are considered to be the constituents that have adverse effects on the respiratory system (Li et al. 2002). Inhaled $PM_{2.5}$ makes contact with human nasal epithelial cells and may impair the function of these cells, thereby causing nasal inflammatory diseases such as chronic and allergic rhinitis. However, its underlying molecular mechanism is largely unknown.

Oxidative stress is a state of imbalance between oxidation and anti-oxidation, and plays an important role in the pathology of many human diseases (Limón-Pacheco and Gonsebatt 2009). One source of oxidative stresses results from the generation of reactive oxygen species (ROS). $PM_{2.5}$ can induce ROS that directly interact with superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), and alter their enzymatic activities (Pamplona and Costantini 2011). Nuclear factor NF-E2-

related factor-2 (Nrf2) plays a key role in keeping cellular redox balance by protecting cells from oxidative stress (Li et al. 2004). The activation of Nrf2 increases the expression of antioxidant enzymes, which in turn removes ROS and decreases cell damage (Satoh et al. 2010). However, whether $PM_{2.5}$ alters the activity of Nrf2 remains elusive.

In addition, airway epithelial cells play a key role in nasal inflammatory diseases by producing and releasing numerous inflammatory cytokines, leading to inflammatory response. Previous studies have suggested that $PM_{2.5}$ has a potential to stimulate cells to produce and release inflammatory cytokines (Ogino et al. 2014). *In vitro* studies have found that diesel exhaust particles (DEP) and PM increase the expression of granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor-α (TNF-α), interleukin 8 (IL-8), IL-6, IL-1β and eotaxin (C-C motif chemokine ligand 11) in airway epithelial cells (Boland et al. 1999; Gioda et al. 2011; Patel et al. 2011; Tang et al. 2012). Since nasal epithelial cells function as the first epithelial barrier in the nasal cavity to protect from inhaled particles, $PM_{2.5}$ may induce inflammatory responses in nasal epithelial cells.

We hypothesize that inhaled $PM_{2.5}$ is involved in the pathogenesis of nasal inflammatory disorders by inducing oxidative stress and inflammatory response in nasal epithelial cells. In this study, we used well-characterized $PM_{2.5}$ collected from the atmosphere and investigated its effects on cell viability, intracellular ROS generation, enzymatic antioxidant activity, nuclear translocation of Nrf2, and expression of inflammatory cytokines in RPMI 2650 human nasal epithelial cells.

Materials and Methods

$PM_{2.5}$ sampling and preparation

Sampling of $PM_{2.5}$ was performed as previously described (Huang et al. 2012). In brief, $PM_{2.5}$ was collected on Whatman 41 filters (Whatman, Maidstone, UK) using $TSP/PM_{10}/PM_{2.5}$-2 samplers (Dickel, Beijing, China) with the flow rate of 77.59 Liter/min. The samplers were on the roof (approximately 20 meters high to the ground) of a building at Fudan University (31.3°N, 121.5°E) in Shanghai, China. This site could be regarded as representative of the megacity Shanghai, standing for the mixture of residential, traffic, construction and industrial sources. Before and after sampling, the filters were stabilized in constant temperature (20 ± 1°C) and humidity (40 ± 1%), and weighed using a Sartorius 2004MP analytical balance (Sartorius, Germany) with a reading precision of 10 μg.

$PM_{2.5}$ was prepared according to the method as described (Imrich et al. 2000). Briefly, the sample filter containing $PM_{2.5}$ was immersed in deionized water and sonicated for 45 min using a KQ-50B water-bath sonicator (Kunshan Ultrasonic Instruments, Jiangsu, China). $PM_{2.5}$ was extracted and stored at −20°C. Aliquots containing 1 mL of $PM_{2.5}$ were placed on filters and air-dried, and the samples and filters were weighed on a Sartorius 2004MP analytical balance (Sartorius) after stabilization at constant temperature (20 ± 1°C) and humidity (40 ± 1%) to determine the concentration of $PM_{2.5}$.

$PM_{2.5}$ physical and chemical characterization

In order to determine the size and morphology of $PM_{2.5}$, scanning electron microscopy S4800 (SEM; Hitachi, Japan) was used to perform a particle analysis. One-fourth of the sample filter was extracted ultrasonically by 20 mL of deionized water. Inductively coupled plasma atomic emission spectroscopy (ICP-OES; SPECTRO, Germany) was used to measure 18 metals (Al, As, Ba, Ce, Co, Cr, Cu, Fe, Ge, K, Mn, Na, Ni, Sb, Se, Sn, V and Zn). Ten inorganic ions (F^-, CH_3COO^-, $HCOO^-$, Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, Na^+, NH_4^+ and K^+) were analyzed by ICS 3000 ion chromatography (Dionex, USA). Detailed procedures of the element and ion analysis were based on the procedures of as procedures previously described (Huang et al. 2013). The PAH analytical procedure was carried out as previously described (Guo et al. 2009). PAHs in $PM_{2.5}$ were measured using a gas chromatography-mass spectrometer (GC-MS; Agilent, USA). The sixteen targeted PAHs analyzed in $PM_{2.5}$ samples were as follows: acenaphthylene, acenaphthene, anthracene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene, chrysene, dibenz[a,h]anthracene, fluoranthene, fluorene, indeno[123-cd]pyrene, naphthalene, phenanthrene and pyrene.

Cell culture and $PM_{2.5}$ exposure

RPMI 2650 cell line derived from squamous cell carcinoma of nasal septum was used as a model of human nasal epithelial cells. The cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in Eagle's minimal essential medium (ATCC) supplemented with 10% fetal bovine serum (FBS; Hyclone, GA, USA), 2 mM of L-glutamine (Gibco, Paisley, UK), 100 U/mL of penicillin (Gibco) and 100 μg/mL of streptomycin (Gibco). Cells were maintained at 37°C with 5% CO_2 and 95% air. All cell exposure experiments were performed at 80-90% of cell confluence. Then, cells were harvested and sub-cultured into six-well plates, 24-well plates or 96-well plates, according to the selection of experiments. Subsequently, cells were exposed to serum-free medium with different concentrations of $PM_{2.5}$ with or without N-Acetyl-L-cysteine (NAC; Sigma, USA) pretreatment. Cell growth status was observed with a phase contrast microscope (Olympus, Japan).

Cell morphology characterization

Cell morphology after $PM_{2.5}$ exposure was evaluated with SEM SU8010 (Hitachi). RPMI 2650 cells were seeded in six-well plates and cultured for 24 hours. After attachment, the cells were exposed to 800 μg/ml of $PM_{2.5}$ for 24 hours and were examined by SEM.

Acute cytotoxicity assay

Acute cytotoxicity of $PM_{2.5}$ was evaluated in RPMI 2650 cells with Cell Counting Kit-8 (CCK-8; Obio Technology, Shanghai, China). RPMI 2650 cells were seeded in 96-well plates (2,000 cells/well) for 24 hours, and treated with $PM_{2.5}$ at final concentrations of 50, 100, 200, 400, 600 or 800 μg/ml for 12, 24 or 48 hours, respectively. The doses and exposure time of $PM_{2.5}$ were based on preliminary experiments, and appropriate doses and exposure time were selected according to the growth curves. Ten μl of the CCK-8 solution was added into each well and incubated at 37°C for two hours. Absorbance at 490 nm was measured with a microplate reader (Tecan US, Inc., Charlotte, NC, USA).

Reactive oxygen species assay

A Reactive Oxygen Species Assay Kit (Beyotime, Hainan,

China) was used to measure intracellular levels of ROS in RPMI 2650 cells. Briefly, 2′,7′-Dichlorofluorescin diacetate (DCFH-DA) was added to the cell culture at a final concentration of 40 μM for 30 minutes. RPMI 2650 cells (2×10^5 cells) were washed with PBS and incubated with zero, 100, 400 and 800 μg/ml of PM$_{2.5}$ for four hours at 37°C, with or without pretreatment with NAC (0.5 mM). Then, cells were lysed by 200 mM of sodium hydroxide (NaOH). Total fluorescence intensity was detected with a fluorescence multi-well plate reader TriStar LB 941 (Berthold, Germany) with excitation and emission wavelengths of 485 nm and 530 nm, respectively. Protein concentration was determined with Bicinchoninic Acid protein assay kit (Beyotime).

Antioxidant enzyme assays

The intracellular superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities were measured with SOD, CAT and glutathione peroxidase assay kits, according to manufacturer's instructions (Beyotime). Briefly, RPMI 2650 cells were seeded at a density of 1×10^5 cells/ml in six-well plates and were allowed to attach for 24 hours, followed by exposure to 100, 400 or 800 μg/ml of PM$_{2.5}$ for 24 hours at 37°C with or without one hour treatment of NAC (0.5 mM). Cell extracts were used to measure the activities of SOD, CAT and GSH-Px. Total protein concentration was determined with Bicinchoninic Acid protein assay kit (Beyotime), and enzyme activity was standardized to milligram protein.

Real-time quantitative reverse transcription PCR (qRT-PCR)

After exposure of cells to zero, 100, 400 or 800 μg/ml of PM$_{2.5}$ for 24 hours, the mRNA levels of GM-CSF, TNF-α, IL-13, eotaxin, IL-6 and IL-8 were measured by real-time qRT-PCR as previously described (Teng et al. 2015). Briefly, total RNA of RPMI 2650 cells was extracted using Trizol reagent (Invitrogen, Shanghai, China), and reverse transcribed into cDNA using SuperScript III Reverse Transcriptase (Invitrogen). The synthesized cDNA was then quantified by SYBR Green assay on a CFX96 Touch™ Real-Time Detection System (Bio-Rad, Hercules, CA, USA). β-actin was used as an endogenous reference for mRNAs. All primers were obtained from Invitrogen, and their sequences are presented in Table 1. Relative expression was calculated using the comparative cycle threshold method (Livak and Schmittgen 2001).

Enzyme-linked immunosorbent assay (ELISA)

After exposure of cells to zero, 100, 400 or 800 μg/ml of PM$_{2.5}$ for 24 hours, GM-CSF, TNF-α, IL-13, eotaxin, IL-6 and IL-8 levels in the cell culture supernatant were measured using GM-CSF Quantikine ELISA Kit (R&D Systems, Minneapolis, MN, USA), TNF-α Quantikine ELISA Kit (R&D Systems), IL-13 Quantikine ELISA Kit (R&D Systems), CCL11/eotaxin Quantikine ELISA Kit (R&D Systems), IL-6 Quantikine ELISA Kit (R&D Systems) and IL-8 Quantikine ELISA Kit (R&D Systems), respectively, in accordance with manufacturers' instructions.

Western blotting analysis

After exposure of cells to zero, 100, 400 or 800 μg/ml of PM$_{2.5}$ for 24 hours, a nuclear and cytoplasmic protein extraction kit (Beyotime) was used to purify the nuclear proteins and cytoplasmic proteins of RPMI 2650 cells, according to the manufacturer's instruction. Nrf2 and Histone H1 in nuclear lysates, as well as Nrf2 and β-actin in cytosolic lysates, were evaluated by western blot analysis,

Table 1. Primer sequences used for qRT-PCR.

Symbol	Primer (5' to 3')
β-actin	Forward: 5'-GTACGCCAACACAGTGCTGTC-3'
	Reverse: 5'-GCTCAGGAGGAGCAATGATCTTG-3'
GM-CSF	Forward: 5'-AATGTTTGACCTCCAGGAGCC-3'
	Reverse: 5'-TCTGGGTTGCACAGGAAGTTT-3'
TNF-α	Forward: 5'-TGGGATCATTGCCCTGTGAG-3'
	Reverse: 5'-GGTGTCTGAAGGAGGGGGTA-3'
IL13	Forward: 5'-GAGCTGGTCAACATCACCCA-3'
	Reverse: 5'-AGCTGTCAGGTTGATGCTCC-3'
eotaxin	Forward: 5'-CCGACCCCAAGAAGAAGTGG-3'
	Reverse: 5'-AACCCATGCCCTTTGGACTG-3'
IL6	Forward: 5'-TTAGAGTCTCAACCCCCAATAAAT-3'
	Reverse: 5'-ATTTGTGGTTGGGTCAGGGG-3'
IL8	Forward: 5'-GTTGTACTCATGACCAGAAAGACC-3'
	Reverse: 5'-AACCAAGGCACAGTGGAACA-3'

as described (Yu et al. 2013). Anti-Nrf2 primary antibody was purchased from Abcam (Cambridge, UK). Protein bands were visualized using ECL detection reagent (GE Healthcare Life Sciences, Piscataway, NJ, USA). Scanned images were quantified using Quantity One software.

Statistics

Statistical analysis was performed using SPSS software for Windows (version 16.0; SPSS, Inc., Chicago, IL, USA). Results are expressed as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA). A P value < 0.05 was considered statistically significant.

Results

Physical and chemical characteristics of PM$_{2.5}$ used in this study

Physical characteristics of PM$_{2.5}$ such as size and morphology were analyzed with SEM. PM with a diameter equal or smaller than 2.5 μm were the most abundant on the porous membrane. Some morphological differences between PM$_{2.5}$ particulates were observed according to their respective origins. These particles possessed diverse forms including spheres, clusters, plate and reticular forms (Fig. 1A). ICP-OES, ICS and GC-MS were used to analyze PM$_{2.5}$ chemical characteristics by analyzing metals, ions and PAHs constituents of PM$_{2.5}$ (Table 2). Analytical results of the 18 elements revealed that Na, K, Al, Zn, Cu and Fe were the most abundant elements in PM$_{2.5}$. Among the 10 ions analyzed, NO_3^-, SO_4^{2-} and NH_4^+ were the domi-
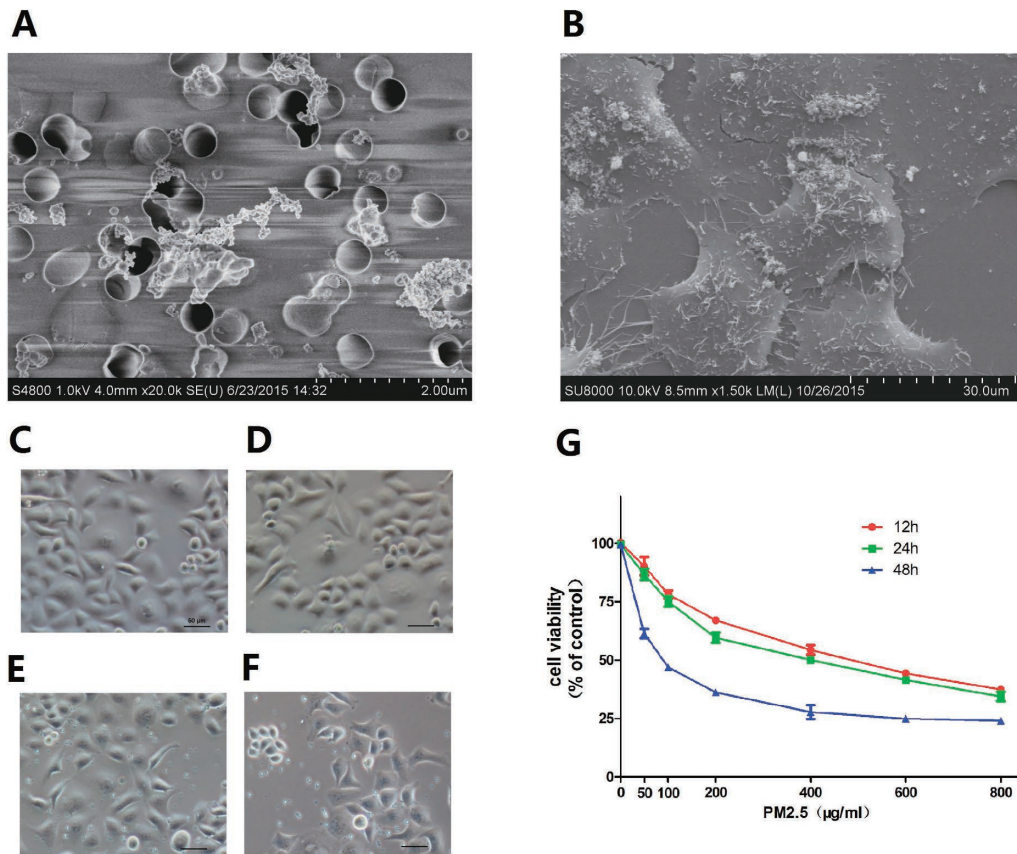
Fig. 1. PM$_{2.5}$ inhibits the viability of RPMI 2650 cells.
(A) SEM observation of PM$_{2.5}$ particles. (B) SEM observation of RPMI 2650 cells incubated with 800 μg/ml of PM$_{2.5}$. Phase contrast microscope observation of RPMI 2650 cells incubated with 0 μg/ml (C), 100 μg/ml (D), 400 μg/ml (E) and 800 μg/ml (F) for 24 hours. (G) Time- and concentration-dependent effect of PM$_{2.5}$ on cell viability of RPMI 2650 cells (n = 5). Cell viability was expressed as a percentage of the untreated group (mean ± SD). (Original magnification: A, ×20,000; Bar 2 μm; B, ×1,500, Bar 30 μm; C-F, ×400, Bar 50 μm).

nant ions in PM$_{2.5}$. The analysis of PAHs revealed that benzo[b]fluoranthene, phenanthrene, fluoranthene, pyrene and chrysene were the dominant PAHs in PM$_{2.5}$.

PM$_{2.5}$ alters morphology and decrease the viability of RPMI 2650 cells

In order to elucidate whether PM$_{2.5}$ is able to affect the morphology of cells, RPMI 2650 cells were challenged with 800 μg/ml of PM$_{2.5}$ for 24 hours and scanned by SEM. PM$_{2.5}$ firmly adhered to the ciliated cell membrane of RPMI 2650 cells, and PM$_{2.5}$ provoked the rearrangement of microvilli (Fig. 1B). In order to explore the cytotoxic effects of PM$_{2.5}$, a phase contrast microscope was used to measure the morphology of RPMI 2650 cells following treatment with different concentrations of PM$_{2.5}$ for 24 hours. Results revealed that with the increase of PM$_{2.5}$ concentration, the number of particles on cells obviously increased, and cell growth was inhibited (Fig. 1C-F). Next, cells were exposed to different concentrations of PM$_{2.5}$ for 12, 24, or 48 hours, and cell proliferation was assessed by CCK-8 assay. Compared with unexposed control cells, PM$_{2.5}$ decreased the cell viability of RPMI 2650 cells in a time- and concen-

tration-dependent manner (Fig. 1G). These results demonstrate that PM$_{2.5}$ was toxic to RPMI 2650 cells.

PM$_{2.5}$ induces oxidative stress and Nrf2 nuclear translocation in RPMI 2650 cells

In order to assess whether PM$_{2.5}$ induces oxidative stress, RPMI 2650 cells were exposed to PM$_{2.5}$ at zero, 100, 400 or 800 μg/ml for four hours in the presence or absence of anti-oxidant NAC, and intracellular levels of ROS were determined by 2′,7′-Dichlorofluorescin (DCF) fluorescence intensity assay. The exposure to PM$_{2.5}$ for four hours significantly elevated intracellular ROS levels in a concentration-dependent manner (Fig. 2A). However, NAC significantly reduced ROS generation with 800 μg/ml of PM$_{2.5}$. Moreover, exposure of RPMI 2650 cells to PM$_{2.5}$ for 24 hours significantly and concentration-dependently decreased the activities of SOD (Fig. 2B), CAT (Fig. 2C) and GSH-Px (Fig. 2D); however, the degree of the decreases in these activities was significantly alleviated by NAC pretreatment (Fig. 2B-D). These results demonstrate that PM$_{2.5}$ induces oxidative stress and decrease intracellular antioxidant enzymatic defenses in RPMI 2650 cells.

Table 2. The Metals, ions and PAHs in PM2.5

Metals	Concentration (ng/mg)	Metals	Concentration (ng/mg)
Al	13,108±869.52	K	36,940±1042.74
As	550.33±90.59	Mn	325.33±62.07
Ba	97.33±9.50	Na	57,019±2570.54
Ce	14±3.00	Ni	627±44.19
Co	57.33±9.50	Sb	339±18.03
Cr	213.67±11.24	Se	68.67±11.02
Cu	3,073.67±130.42	Sn	222.33±15.53
Fe	2,472±177.54	V	408.33±9.02
Ge	177.67±18.50	Zn	5,756.67±264.71

Anions	Concentration (µg/mg)	Cations	Concentration (µg/mg)
F^-	0.8±0.17	Na^+	4.02±0.21
CH_3COO^-	4.4±0.43	NH_4^+	199.14±7.26
$HCOO^-$	0.19±0.04	K^+	1.53±0.27
Cl^-	57.16±3.36		
NO_3^-	386.32±10.50		
SO_4^{2-}	209.34±9.46		
PO_4^{3-}	15.54±1.81		

PAHs	Concentration (ng/mg)	PAHs	Concentration (ng/mg)
Acenaphthylene	19.03±1.46	Chrysene	191.68±5.71
Acenaphthene	16.32±0.89	Dibenz[a,h]anthracene	12.14±0.99
Anthracene	7.3±0.59	Fluoranthene	239.77±14.75
Benz[a]anthracene	160.17±4.60	Fluorene	7.22±0.30
Benzo[b]fluoranthene	253.52±7.72	Indeno[123-cd]pyrene	99.84±1.27
Benzo[k]fluoranthene	97.88±1.85	Naphthalene	5.57±0.68
Benzo[a]pyrene	95.43±5.70	Phenanthrene	247.61±11.88
Benzo[ghi]perylene	87.58±2.29	Pyrene	198.48±8.23

Data are means ± SD of three independent experiments.

Nrf2 plays a key role in protecting cells from oxidative stress (Li et al. 2004). We further explored the nuclear translocation of Nrf2 after exposure of RPMI 2650 cells to different concentrations of PM$_{2.5}$ for 24 hours with or without NAC. Treatment of RPMI 2650 cells with PM$_{2.5}$ for 24 hours significantly increased the nuclear protein levels of Nrf2, while at the same time, decreased cytosolic Nrf2 levels in a concentration-dependent manner. In contrast, NAC significantly prevented the nuclear translocation of Nrf2 following 800 µg/ml of PM$_{2.5}$ treatment. Thus, PM$_{2.5}$ induced Nrf2 translocation from the cytoplasm into the nucleus, which was inhibited by NAC.

PM$_{2.5}$ stimulates production of inflammatory cytokines in RPMI 2650 cells

In order to examine the impact of PM$_{2.5}$ on inflammatory cytokines production, RPMI 2650 cells were treated with different concentrations of PM$_{2.5}$ for 24 hours, and the mRNA levels of GM-CSF, TNF-α, IL-13, eotaxin, IL-6 and IL-8 were measured by real-time qRT-PCR. GM-CSF, TNF-α, IL-13, eotaxin, IL-6 and IL-8 mRNA levels significantly increased in RPMI 2650 cells treated with PM$_{2.5}$ for 24 hours when compared with untreated cells (Fig. 3A-F). However, there was no significant difference in mRNA expression levels of IL-13 and eotaxin between cells exposed to 100 µg/ml of PM$_{2.5}$ and control. Consistent with
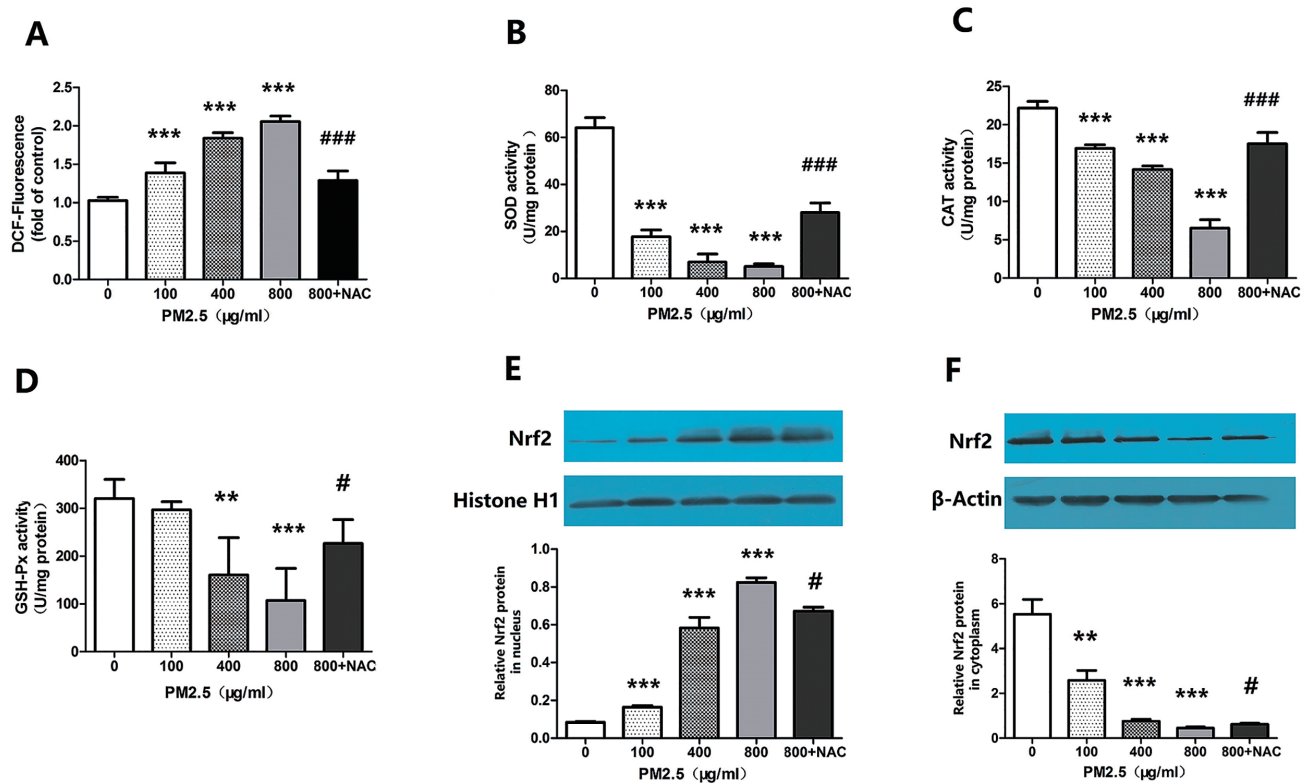
A

B

C

D

E

F

Fig. 2. PM$_{2.5}$ induces oxidative stress and reduces antioxidant enzymatic activities in RPMI 2650 cells.
(A) Intracellular levels of ROS induced by PM$_{2.5}$. (B-D) Activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) in RPMI 2650 cells were decreased by PM$_{2.5}$. (EF) Effect of PM$_{2.5}$ exposure on Nrf2 nuclear translocation is shown. Density of nuclear Nrf2 was relative to the level of Histone H1, and density of cytosolic Nrf2 was relative to the level of β-actin; n = 5.
Data are mean ± SD.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. control; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs. 800 μg/ml of PM$_{2.5}$ without NAC.

the upregulation of mRNA levels, ELISA analysis revealed that treatment of RPMI 2650 cells with PM$_{2.5}$ for 24 hours significantly increased the protein levels of GM-CSF, TNF-α, IL-13, eotaxin, IL-6, and IL-8 in culture supernatants (Fig. 4A-F). These results indicate that PM$_{2.5}$ induced the expression of inflammatory cytokines in RPMI 2650 cells.

Discussion

In this study, we demonstrated that PM$_{2.5}$ altered the morphology and decreased the cell viability of RPMI 2650 cells. Moreover, PM$_{2.5}$ increased ROS levels and reduced intracellular antioxidant enzymatic activity accompanied with Nrf2 translocation from the cytoplasm into the nucleus; all of which were inhibited by NAC. Importantly, PM$_{2.5}$ induced the expression of inflammatory cytokines GM-CSF, TNF-α, IL-13, eotaxin, IL-6 and IL-8. These results revealed that PM$_{2.5}$ induced oxidative stress and inflammatory response in RPMI 2650 cells. Our findings suggest that oxidative stress and inflammatory response may play an important role in the cell growth of RPMI 2650 cells and contribute to the impairment of nasal epithelial barrier dysfunction following PM$_{2.5}$ exposure.

Organic and inorganic components of PM$_{2.5}$ are

involved in toxicological effects (Deng et al. 2013). It was reported that metal content in particles is the main composition that induce inflammatory response, and trace metals were the most toxic substances in ambient PM (Ghio and Devlin 2001). In the present study, both natural sourced elements (Na, K, Al, etc.) and anthropogenic inorganic elements (Zn, Cu, Fe, Ni, Mn, etc.) were detected with high levels of Na, K, Al, Zn, Cu and Fe, indicating that PM$_{2.5}$ was from both natural and anthropogenic sources. The analysis of ions revealed that NO$_3^-$, SO$_4^{2-}$ and NH$_4^+$ were dominant ions in PM$_{2.5}$, which are the representative of secondary aerosols. Furthermore, PAHs analysis revealed that a high proportion of benzo[b]fluoranthene, chrysene, fluoranthene, phenanthrene and pyrene were found in PM$_{2.5}$ samples. These analytical results of PM$_{2.5}$ compositions indicate that PM$_{2.5}$ samples are a complex mixture of chemicals, including natural sourced elements, anthropogenic inorganic elements, secondary aerosols and PAHs. This study focused on the toxic effect of atmospheric PM$_{2.5}$ on human nasal epithelial cells. PM$_{2.5}$ was collected in the atmosphere and used in this study. PAHs and heavy metals are the main components of the fractions, although these components in PM$_{2.5}$ accounted for a small proportion.
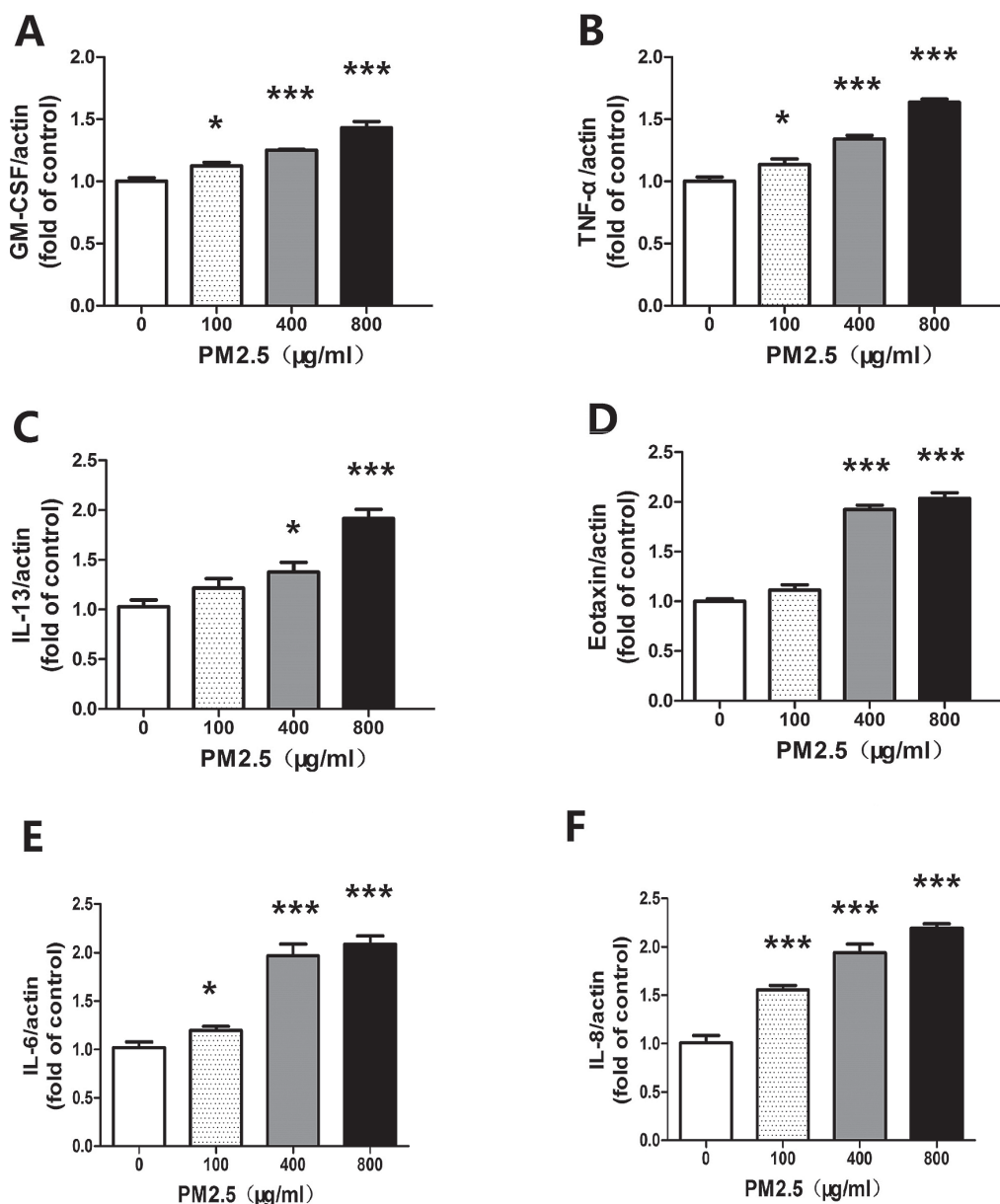
Fig. 3. PM$_{2.5}$ increases inflammatory cytokine mRNA expression in RPMI 2650 cells.
The mRNA expression levels of GM-CSF (A), TNF-α (B), IL-13 (C), eotaxin (D), IL-6 (E), and IL-8 (F) were measured by qRT-PCR analysis. The expression levels of mRNA were normalized to β-actin and given as fold induction compared to the mRNA level in control cells. All data are presented as the mean ± SD (n = 5).
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. control.

Future studies are needed to assess the impact of these components on human health.

Cell viability assays have been used to explore the cellular effects of toxicants (Lee et al. 2011). We found that the cell viability of RPMI 2650 cells exposed to PM$_{2.5}$ decreased in a time- and concentration-dependent manner. Further studies found that PM$_{2.5}$ triggered an increase of intracellular ROS and decrease in activities of SOD, CAT and GSH-Px in RPMI2650 cells. Meanwhile, PM$_{2.5}$ induced the nuclear translocation of Nrf2 in RPMI2650 cells. Moreover, pretreatment with NAC significantly reduced the PM$_{2.5}$-induced generation of ROS, suppression

of SOD, CAT and GSH-Px activities, and nuclear translocation of Nrf2 in RPMI2650 cells. Nrf2-mediated defense mechanisms protect cells from oxidative stress. These results suggest that PM$_{2.5}$-induced oxidative stress may play a crucial role in cell growth, which may contribute to the nasal epithelial barrier dysfunction induced by PM$_{2.5}$. Nevertheless, the underlying molecular mechanisms of PM$_{2.5}$ and NAC on ROS generation, enzyme activities and nuclear translocation of Nrf2 have not been fully elucidated, and need future studies.

Nasal epithelial cells make up the epithelial barrier in the nasal cavity, and secrete various inflammatory media-
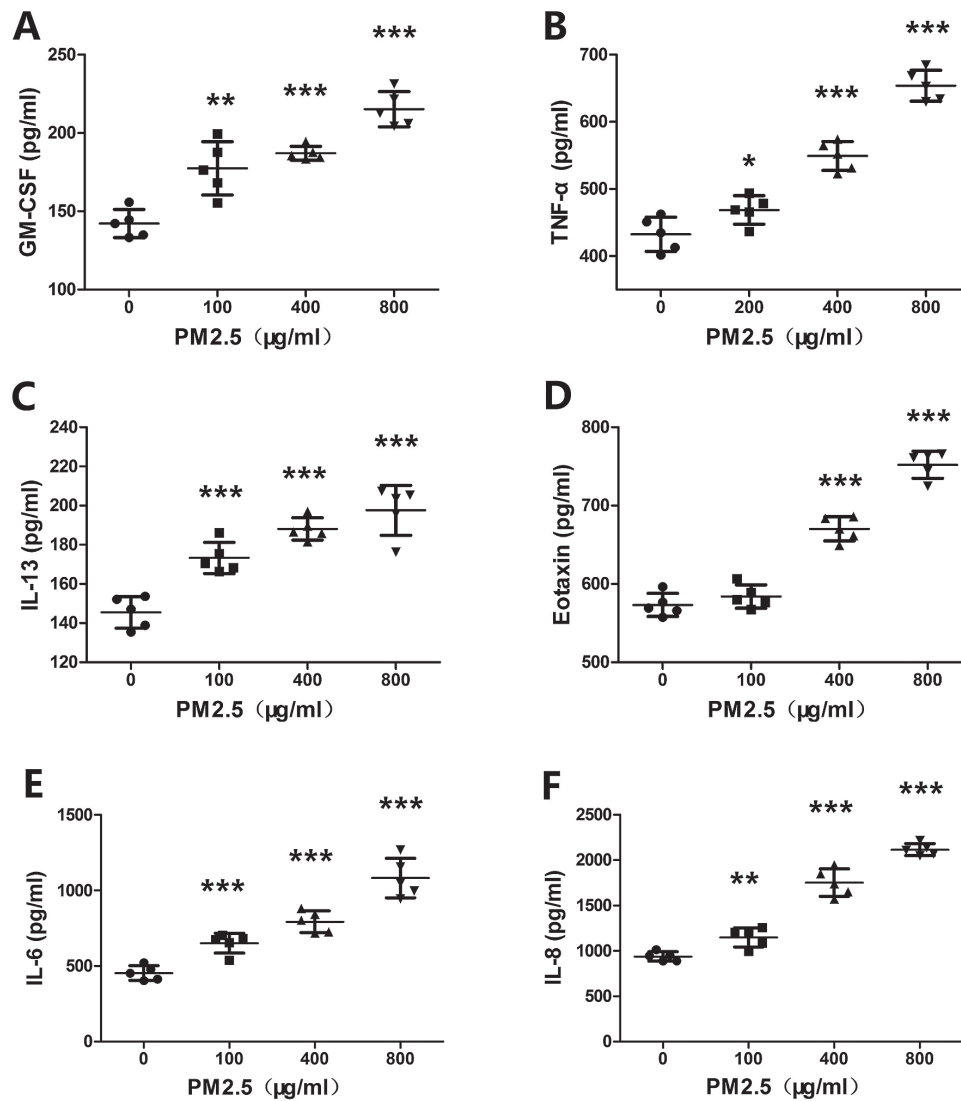
Fig. 4. PM$_{2.5}$ stimulates the protein expression of inflammatory cytokines in RPMI 2650 cells.
The protein levels of GM-CSF (A), TNF-α (B), IL-13 (C), eotaxin (D), IL-6 (E), and IL-8 (F) in the cell supernatant were determined with ELISA. All data are presented as the mean ± SD (n = 5).
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. control.

tors in nasal inflammatory diseases. Inflammatory cytokines GM-CSF and eotaxin can be synthesized and released by airway epithelial cells in local inflammation, and infiltrating inflammatory cells to local tissues (Matsuwaki et al. 2012). IL-13 is considered to be a crucial mediator of the pathogenesis of inflammation (Ingram and Kraft 2012; Corren 2013). This study revealed that PM$_{2.5}$ increased the expression of GM-CSF, TNF-α, IL-13, eotaxin, IL-6 and IL-8. These findings suggest that PM$_{2.5}$ may initiate and augment local inflammation, and lead to disease exacerbation by stimulating the production of GM-CSF, TNF-α, IL-13, eotaxin, IL-6 and IL-8.

In summary, we have shown that exposure to PM$_{2.5}$ causes oxidative stress and inflammatory response, which in turn may inhibit the cell growth and impair the nasal epithelial barrier function. Our results provide insights into the PM$_{2.5}$-mediated cellular injury.

Conflict of Interest

The authors declare no conflict of interest.

Reference

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