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RESEARCH ARTICLE

Effect of PM2.5 environmental pollution on rat lung

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Abstract

Particulate matter smaller than 2.5 μm (PM2.5) is a continuing challenge to pulmonary health. Here, we investigated the mechanisms involved in PM2.5 exposure-induced acute lung injury in rats. We analyzed biochemical and morphological changes following a 2-week "real-world" exposure. And then we found that PM2.5 exposure increased the concentrations of total protein, malondialdehyde, hydrogen peroxide, nitric oxide, and soluble elastin in bronchoalveolar lavage fluid, levels of cytokines in blood, and expression of MMP-9 in airways. Further, alveolar macrophage and neutrophil counts increased following PM2.5 exposure, and edema and lung lesions were observed. Our results suggest that PM2.5 exposure can induce oxidative stress and acute inflammatory responses, which can damage the micro-environment and decrease the repair ability of the lung, resulting in tissue damage.

Keywords PM2.5 · Lung · Injury · Inflammation · Oxidative stress · Extracellular matrix

Introduction

Airborne particulate matter (PM) smaller than 2.5 μm (PM2.5) is generated by various sources and is therefore a heterogeneous mixture of various substances. PM2.5 is a continuing challenge to public health, and epidemiological studies have linked PM2.5 to adverse health effects, such as pulmonary and cardiovascular diseases (Beelen et al. 2015; Pope et al. 2009; Shah et al. 2013; Líbalová et al. 2014). The complex mechanisms underlying PM2.5-induced health effects require further study.

Multiple experimental studies have been conducted to elucidate the effects of PM2.5 on human health and the mechanism of its toxicity (Xu et al. 2016; Kim et al. 2017; Riva et al. 2011; Wang et al. 2017). Because the constituents of PM2.5 are known to vary substantially between regions and sources, affecting toxicity, many studies have analyzed PM2.5 composition in different regions, and investigated the function of signaling pathways involved in redox homeostasis and inflammation (Li et al. 2015; Wang et al. 2013; Kumar et al. 2015). However, our knowledge of the molecular and cellular mechanisms underlying PM2.5-associated systemic diseases remains incomplete.

We used a "real-world" PM2.5 system to perform a mouth-nose exposure of rats to environmentally relevant PM2.5 or neutral saline (NS). To elucidate the molecular basis of ambient PM2.5-associated cytotoxicity, we investigated biochemical markers following exposure. We hypothesized that the PM2.5-induced stress response relies on the production of reactive oxygen species (ROS) and regulates the morphological remodeling. The findings on PM2.5-induced pathological changes such as cytotoxicity, leukocyte infiltration, pro-inflammatory cytokines, oxidative stress, damage to the alveolar epithelium-capillary barrier, and vascular permeability will not only contribute to a better understanding of molecular and cellular mechanisms by which PM2.5 elicits its effects, but will also inform the prevention and treatment of air pollution-induced systemic diseases.

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Materials and methods

Animals

Wistar rats that were obtained from Vital River, CP (Beijing, China), were used at 6~8 weeks of age (180~220 g), and they were randomly divided into PM2.5-exposed group and a control group. All studies were performed according to protocols reviewed and approved by the Ethics Committee of Animal Care and Experimentation of the National Institute for Environmental Studies, China. Wistar rats received the same food and water and were kept at the same ambient conditions of temperature, humidity, and noise. After 2 weeks acclimation, all rats were executed according to study design (Fig. 1).

PM2.5 sampling

PM2.5 samples were prepared as described in our previous studies, and the analysis of PM2.5 composition was performed (Ma et al. 2015). Briefly, using a high-volume sampler particle collector equipped with a Hi-Vol PM2.5 inlet (Tisch Environmental, USA) at a constant flow rate of 1.13m^3/min, PM2.5 samples were collected on nitrocellulose filters (Waterman, USA) in the center of the downtown area of ShenYang, China. We confirmed that heavy metals and polycyclic aromatic hydrocarbons are the compositions of PM2.5.

PM2.5 administration

Using the Mouth-nose Exposed system (Beijing Huironghe Technology Co., Led, China), the experimental group animals were exposed to concentrated PM2.5 at nominal 10 ambient concentrations of 750 μg/m^3 for 4 h per day, 5 days per week for a total of 2 weeks, and the control group animals were treated with similar condition. Twenty-four hours following the last exposure, animals were euthanized and BAL fluid and tissue samples were collected for further analysis.

Lung harvesting, tissue processing, and H&E staining

The lungs were inflated and fixed with 10% neutral formalin overnight at room temperature. Lung tissue was dehydrated with increasing ethanol (EtOH) concentrations and then embedded in paraffin. Five-micrometer-thick paraffin sections were stained with hematoxylin and eosin (H&E). Five-micron sections were placed on slides and stained with H&E in order to observe cell morphology.

Transmission electron microscopy insection

We performed the transmission electron microscopy insection as previous described (Wang et al. 2013). Two apexes of the left lungs were selected and immersed in 2.5% glutaraldehyde

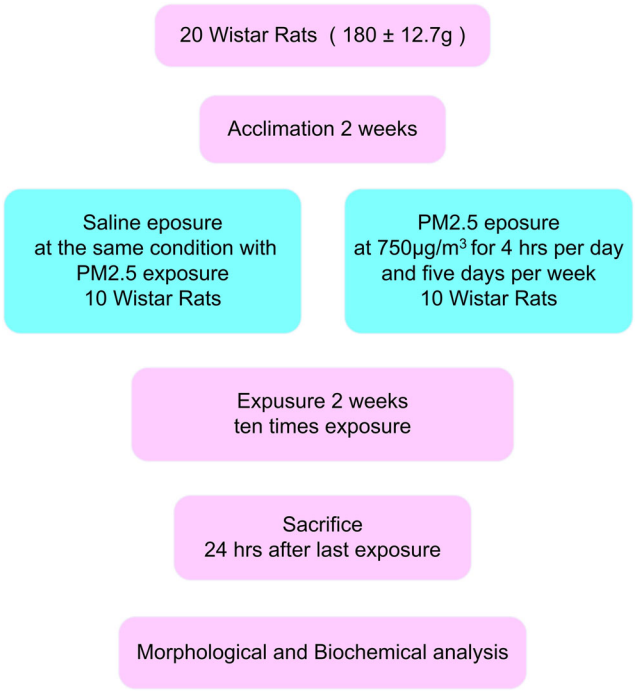

Fig. 1 Study design

at 4 °C. After washing with phosphate buffer solution sufficiently, they were fixed with 1% osmium tetroxide, dehydrated in a graded series of ethanol, embedded in araldite and polymerized for 24 h at 37 °C. Ultrathin sections (50–60 nm) were cut with ultramicrotome (LKB-I, Sweden). Thin sections were mounted directly on copper grids, stained with lead citrate, and observed with H-7650 electron microscopy (HITACHI, Japan).

Bronchoalveolar lavage fluid collection and cell count

Following removal of the lung's lower right lobe, bronchoalveolar lavage (BAL) fluid was collected by flushing 5 × 5 ml of phosphate buffered saline (PBS) containing 0.1 mM EDTA into the lung via a tracheal cannula. The pooled BAL fluid was centrifuged at 500×*g* at 4 °C for 5 min. Pelleted cells were then resuspended in 2 ml of PBS. Total cell number was counted by hemocytometer and a differential cell count was performed by cytospin staining with Diff-Quik (Siemens, Newark, China).

BAL fluid protein

Increase in BAL fluid protein concentration was taken as a measure of increased permeability of alveolar-capillary barriers. Total protein concentration in the supernatant following BAL fluid centrifugation was determined using the BCA Protein Assay kit (Thermo Fisher Scientific, MA, USA).

Elastolytic activity assay

Extracellular elastin degradation study was performed using soluble elastin by ELISA kit (Elastin Products Company, Inc., Owensville, MO). To determine extracellular elastolytic concentration, BAL fluid was performed according to the manufacturer's instructions. After incubation, soluble elastin was determined using absorbance fold at 490 nm.

Measurement of oxidant stress

Oxidant stress production, including hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and total nitric oxide, in lung tissue was determined using the Hydrogen Peroxide Assay kit, Lipid Peroxidation MDA Assay kit, and Total Nitric Oxide Assay kit (Beyotime, JiangSu, China) according to the manufacturer's instructions.

Cell proliferation assay

Cell viability was assessed with CellTiter 96 AQueous One Solution Reagent (Promega, USA) according to the manufacturer's recommendations. The cells that were collected from the BAL fluid were seeded at a density of 1.0×10^4 per well in triplicate in 96-well plates, and cultured in a CO_2 incubator for 12 h. Alternatively, absorbance was taken on an infinite M200 Pro Reader (TECAN, Switzerland) at a wavelength of 490 nm in PM2.5 exposure group and NS exposure group.

Cell apoptosis analysis

Cell apoptosis assay was performed by the previous described (Chuturgoon et al. 2015). The cells were collected from the bronchoalveolar lavage fluid (BALF). Cells were fixed in 70% ethanol for 24 h and centrifuged once. Then DNA-binding dye propidium iodide (DAPI) (10 μg/ml) was added to the cell suspensions for staining, and the mixed solution was incubated at room temperature for 5 min in the dark. Then the samples were observed using the fluorescence microscope. Additionally, the transfected cells after 48 h were washed for three times by cold PBS and fixed in 70% ethanol. Then, the cell suspensions were stained in 500 μl precooled 1× binding buffer containing 5 μl annexin V/propidium iodide medium and 2.5 μl propidium iodide. Cell apoptosis was analyzed with FACScan flow cytometer (Becton-Dickinson, Sunny-vale, CA, USA). Incubated for another 30 min, the cells were diluted by buffer and then analyzed with a flow cytometry.

Measurement of plasma cytokine levels

Twenty-four hours following the last exposure, wistar rats were euthanized and blood serum was collected for further analysis. The production of cytokine in PM2.5 the exposure group and NS exposure group was measured by rat cytokine antibody array kit (Abcam, Cambridge, UK). The experimental procedures were carried out strictly according to the manufacturer's instructions, and chemiluminescence detection was performed with MF chemi BIS 2020 (DNR imaging systems, Inc., Neve Yamin, Israel).

Lung wet:dry weight ratio

As an index of lung edema, the level of extra-vascular lung water was calculated. The lower lobe of the right lung was excised and the wet weight was recorded (Hu et al. 2016). To obtain the dry weight, the lung was then placed in an incubator at 60 °C for 24 h. The wet:dry ratio was calculated by dividing the wet weight by the dry weight.

Assessment of capillary leakage

To further assess lung permeability, 50 mg/kg of Evans blue dye (EBD; Sigma-Aldrich, St. Louis, MO) dissolved in 200 μl of PBS was injected into the tail veins of mice following ALI induction. The EBD concentration that was expressed as micrograms per gram of lung was calculated as described previously (Reddy et al. 2012).

Immunofluorescent detection

Fluorescence imaging was performed according to a previously established protocol (Liu et al. 2017). Tissue sections were processed for antigen retrieval with permeabilizing solution. Then, the slides were blocked with 1% BSA and incubated overnight with relevant primary antibodies. Followed by washing with PBS-T, the sections were incubated with either Alexa Fluor 488 (for DAPI) or Alexa Fluor 562 conjugated anti–mouse antibodies (for MMP-9). The slides were then examined under a laser scanning confocal microscope (Leica TCS SPE).

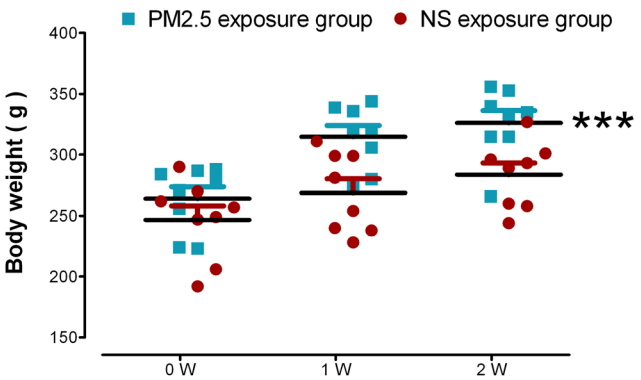

Fig. 2 Average of body weight for both experimental group. Data representative results derived from a minimum of three independent experiments. $P < 0.05$ compared with the NS exposure group

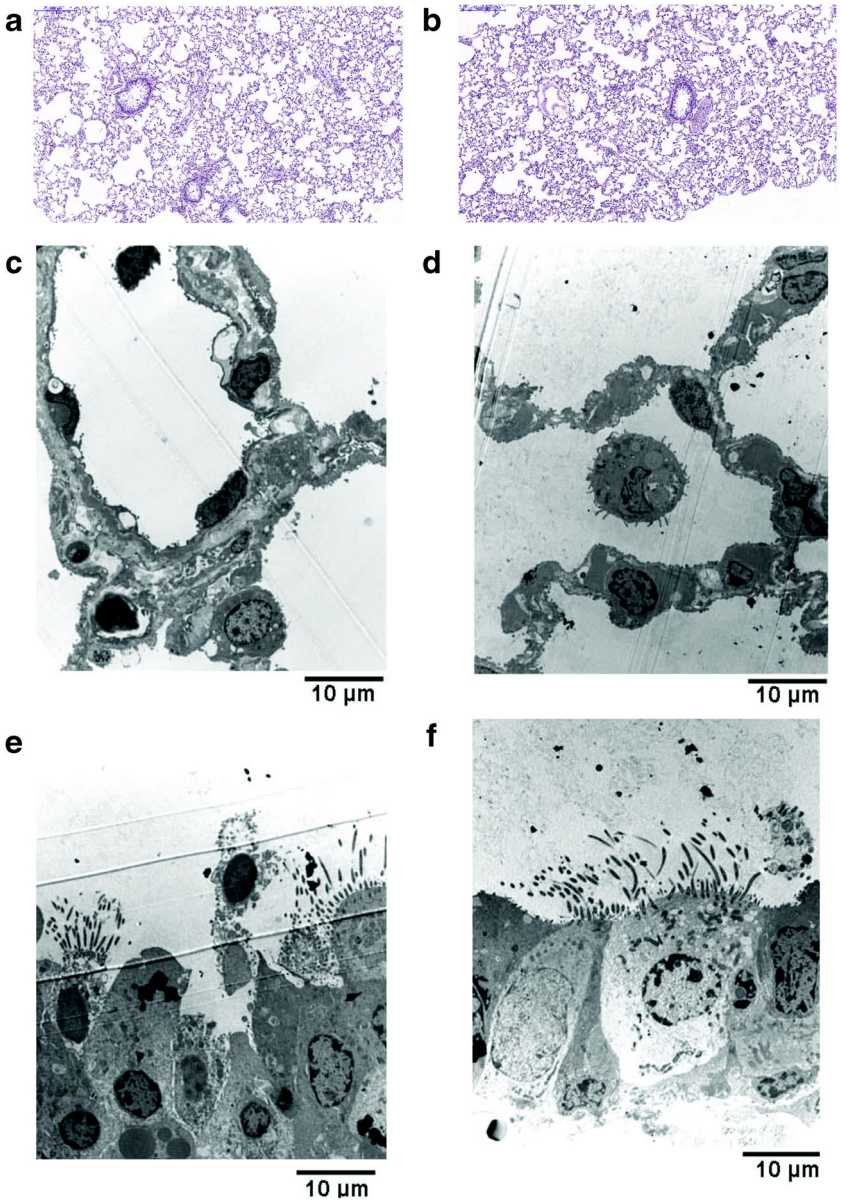

Fig. 3 PM2.5 exposure induced lung injury. **a** PM2.5 exposure group (H&E stain). Line indicates 200 μm; **b** Neutral saline exposure group (H&E stain). Line indicates 200 μm. **c**–**f** Transmission electron microscopy inspection. **c** PM2.5 exposure group, alveolar cell and macrophage. **d** Neutral saline exposure group, alveolar cell and macrophage. **e** PM2.5 exposure group, the epithelials of the respiratory tract. **f** Neutral saline exposure group, the epithelials of the respiratory tract

Statistical analysis

Experimental data were analyzed by using the software GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA). Data are presented as mean ± SD. Differences between groups were analyzed using ANOVA. Statistical comparisons were made using an unpaired two-tailed Student's *t* test for two groups. Differences were considered significant if $P < 0.05$.

Table 1 Total cell, macrophages, and neutrophil number in BAL fluid

Groups	Total cell counts (10^5 ml)	Macrophage counts (10^5 ml)	Neutrophil counts (10^5 ml)
PM2.5 exposure group	11.50 ± 0.4041***	6.86 ± 0.3528 *	4.43 ± 0.2906***
NS exposure group	6.73 ± 0.3283	4.73 ± 0.3480	1.23 ± 0.1764

* $P < 0.05$ PM2.5 exposure compared to NS exposure

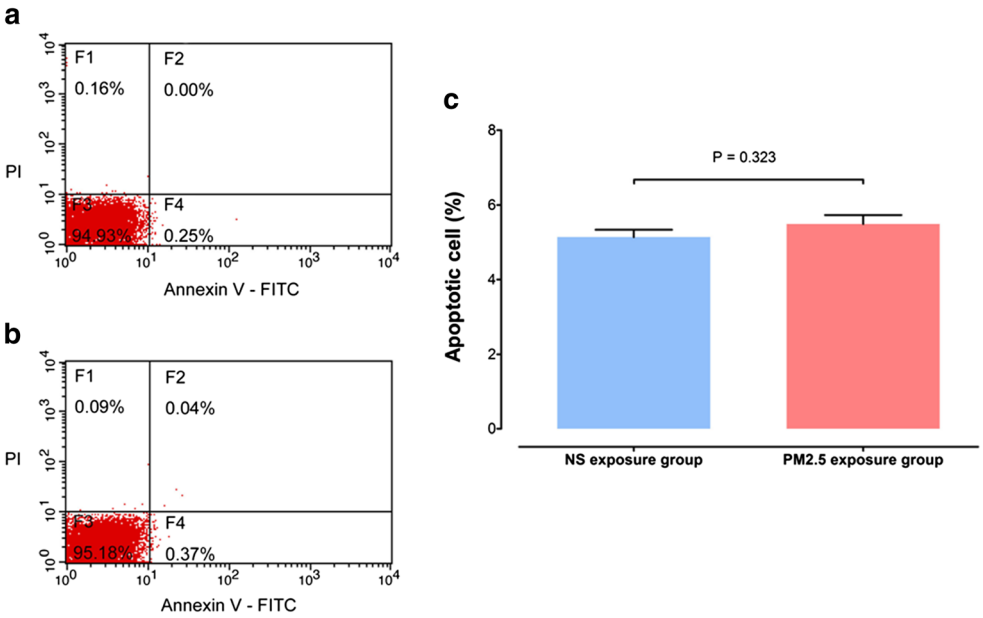

Fig. 4 The apoptosis of BAL cells stimulated by PM2.5 detected by flow cytometry. **a** NS exposure group; **b** PM2.5 exposure group; **c** Histogram results of the apoptotic BAL cells of different groups compared to the NS exposure group. Data representative results derived from a minimum of three independent experiments. $P < 0.05$ compared with the NS exposure group

Results

Body weight change

Body weight changes in rats during the whole exposure period were showed by Fig. 2. Body weights of the PM2.5 exposure group showed a normal increase trend during the 2 weeks, and the results did show significant difference compared to the NS exposure group.

Morphological evaluation of lung lesions

To test the lung lesions followed the PM2.5 exposure, we assessed the histological changes of lung tissues by the H&E staining and the transmission electron microscopy inspection. We can see that pathological changes were significant by H&E staining as shown in Fig. 3a, b. Comparing with the NS exposure group, it was apparent that the lung parenchyma were subjected to edema. These pathological characters were enhanced along with lung lesions. The ultrastructure of rat lung was shown in Fig. 3c, d, e, f, parts of alveolar epithelial cell were injury. On the other hand, the cells in stratum mucosum were injured or died, such as the ciliated cell and the brush cell.

Total cell counting

The total cell number and the numbers of macrophages and neutrophils in BALF were shown in Table 1. We observed the numbers of total cell, macrophages, and neutrophils in BALF under the PM2.5 exposure group were significantly higher than the NS exposure group. And the differential staining indicated that neutrophils accounted for most of the increase.

Detection on cell apoptosis

To evaluate the role of PM2.5 in stimulating cell apoptosis in BALF, we performed the flow cytometry assay. As shown in Fig. 4, the rates of cell apoptosis in the PM2.5 exposure group and NS exposure group were 5.140 ± 0.1973 and 5.490 ± 0.2397, respectively, with no significant difference.

PM2.5 induce the cell proliferation in BALF

To investigate differences in the proliferation of cell in BALF induced by PM2.5-exposed and NS-exposed groups, we performed cell proliferation assays. Compared with the NS exposure group, the PM2.5 exposure group showed higher viability and proliferation (Fig. 5). Our results suggested that PM2.5 exposure induced the cells that had larger populations in BALF.

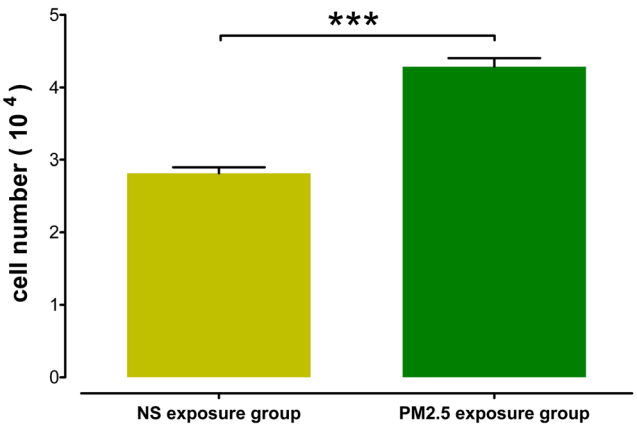

Fig. 5 Effects of PM2.5-exposed-induced cell proliferation in BALF. The cell numbers were detected in 490 nm by cell proliferation assay. Data representative results derived from a minimum of three independent experiments. $P < 0.05$ compared with the NS exposure group

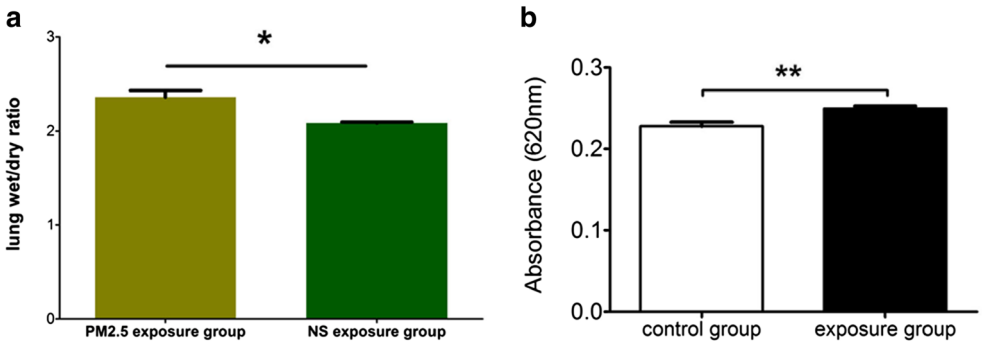

Fig. 6 Measurement on vessel permeability in lung tissue. **a** Ration of lung wet:dry weight. **b** The Evans blue dye was quantitated by spectrophotometry. Data representative results derived from a minimum of three independent experiments

Measurement on vessel permeability in lung tissue

To investigate the effects of PM2.5 exposure on pulmonary edema, we compared the wet:dry weight ratio and capillary permeability between the PM2.5-exposed and NS-exposed groups (Fig. 6). According to the results, we found that the increased vascular permeability following the PM2.5 exposure allowed the plasma proteins and EBD to extravasate from the vasculature into the lung parenchyma. The extravasation of EBD was significantly induced by PM2.5 exposure. The

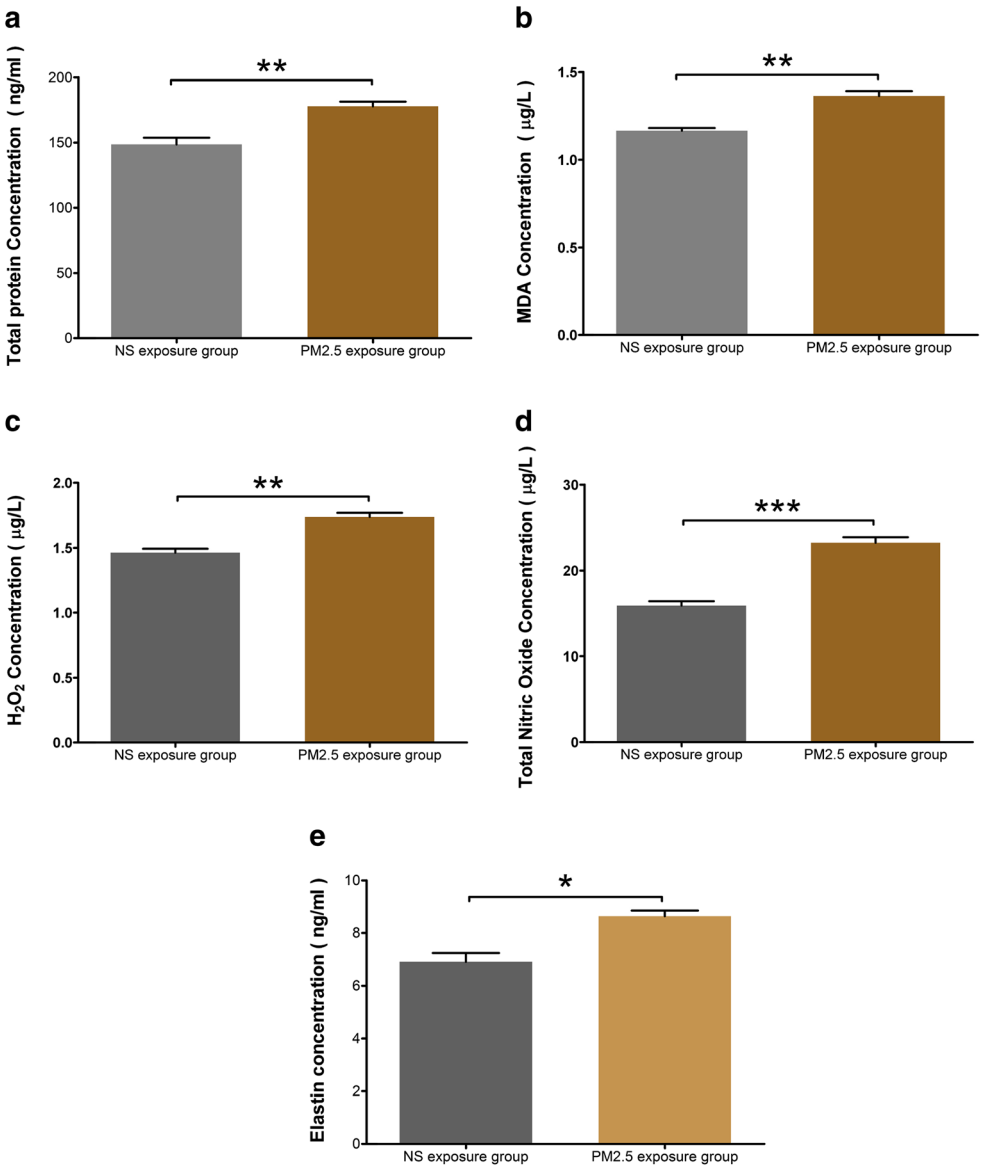

Fig. 7 Biochemical markers in BALF. **a** The total protein concentration in BAL fluid. **b** The levels of MDA in BAL fluid. **c** The levels of H_2O_2 in BAL fluid. **d** The levels of total nitric oxide in BAL fluid. **e** The levels of soluble elastin in BAL fluid. Data representative results derived from a minimum of three independent experiments. $P < 0.05$ compared with the NS exposure group

lung edema that results from capillary permeability is reflected in the wet:dry weight ratio, and PM2.5 exposure increases in this parameter. PM2.5 exposure markedly increased lung wet/dry weight ratio ($P < 0.05$).

Biochemical assessments in BALF

To confirm the change of local micro-environment followed the PM2.5 exposure, we collected the BALF and performed the biochemical assessment with concentration of the total protein, MDA, H2O2, the total nitric oxide, and soluble elastin. As shown in Fig. 7, these biomarkers were significantly different between the NS and PM2.5 exposure group, and suggested that PM2.5 exposure directly injures lung tissues.

The expression level of cytokine in blood

To further investigate if PM2.5 exposure impacted inflammatory progression in the side of cytokine, we performed the simultaneous detection of 36 rat cytokines concentrations after PM2.5 exposure and NS exposure in same conditions and procedures. Following the chemiluminescence detection and Image J software analysis, we confirmed that eight cytokines concentration significantly increased after PM2.5 exposure on wistar rats (Fig. 8 and Table S1), including CNTF (cholinergic neurotrophic factor), IL-6 (interleukin-6), IL-10 (interleukin-10), MCP-1 (monocyte chemotactic protein 1), MMP-8 (metalloproteinases-8), RAGE (receptor for advanced glycation endproducts), and Thymus Chemokine-1 and TIMP-1 (tissue inhibitor of metalloproteinase-1).

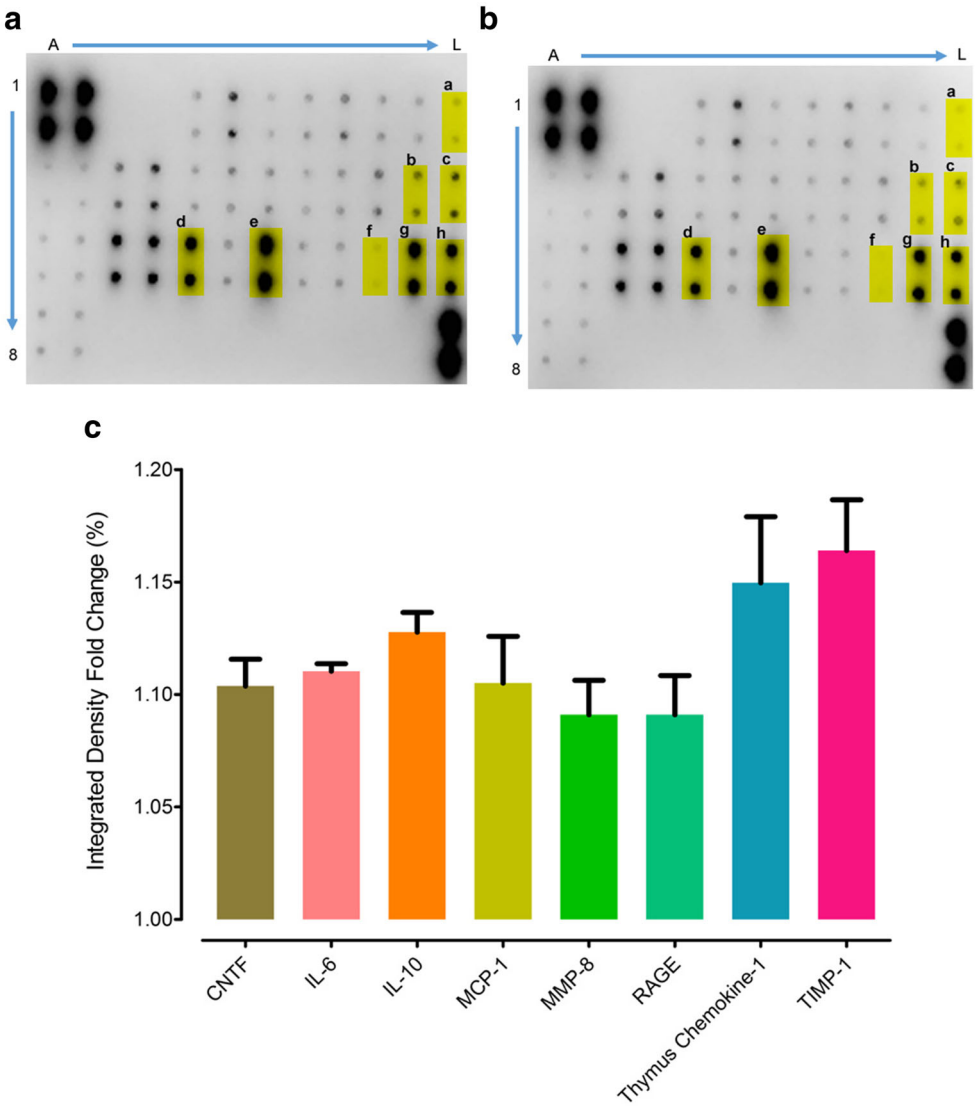

Fig. 8 Plasma cytokine levels. **a** NS group; **b** PM2.5 group; c Graphs show the expression of eight cytokines in blood. Data representative results derived from a minimum of three independent experiments

Evaluation on the remodeling in rat lungs

In the analysis of associated changes in the pulmonary expression of the MMP-9, we performed the confocal assay following the PM2.5 exposure. As shown in Fig. 9, we found that overexpression of MMP-9 in the PM2.5 exposed group, the primary lung metalloproteinase involved in protein degradation, and the levels of MMP-9 were lighter in the NS exposure group.

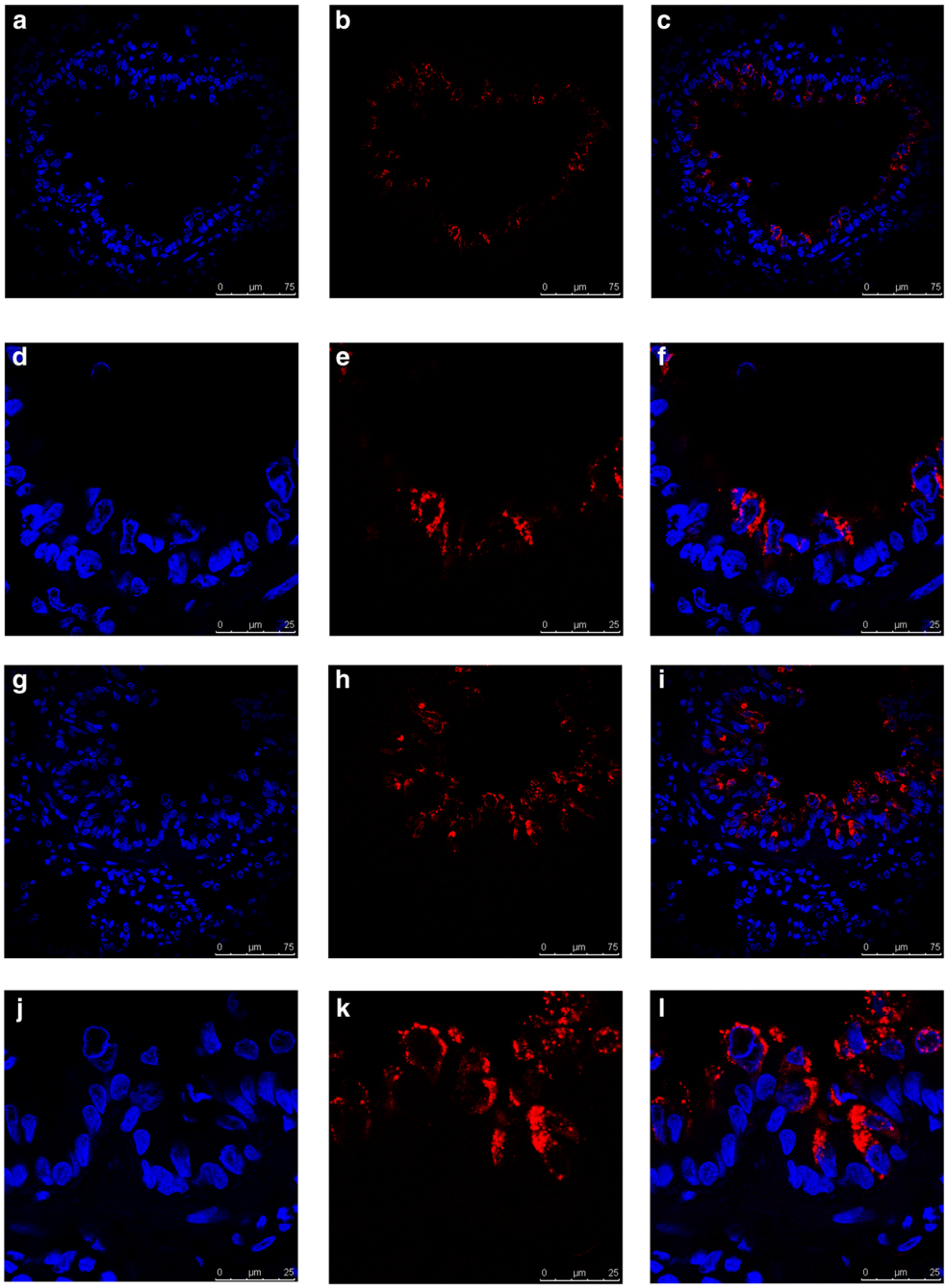

Fig. 9 Representative immunostained lung section profiles of the above treatment groups depicting the levels of MMP-9 in the airway (red), as analyzed by (confocal) assay. Nuclei were stained with DAPI (blue). **a**–**f** Neutral saline exposure group. **g**–**l** PM2.5 exposure group

Discussion

PM2.5 is a heterogeneous mixture of substances, and its larger surface area allows a more significant conveyance of metals and other adsorbed components, increasing its pulmonary toxicity. Acute lung injury (ALI) is a major cause of acute respiratory failure. ALI key events include extensive neutrophil infiltration, release of inflammatory mediators, capillary permeability, and edema (Zhang et al. 2010). In this study, we

investigated the mechanisms on PM2.5-induced ALI. Our key finding was that PM2.5 elicited lung inflammation and injury.

Inflammation is a process of tissue reaction to harmful stimuli, and is an important feature of lung diseases such as asthma and chronic obstructive pulmonary disease. Air pollution can be considered an important trigger of lung inflammation. We found that the inflammatory stimulus of PM2.5 exposure caused an increase in total protein expression in BAL fluid. Airflow obstruction exerts a greater burden of mucus in the small airway, and an increase in mucus may impair clearance functions. Following PM2.5 exposure, the respiratory system may be more susceptible to the effects of deposited PM2.5, thereby exacerbating the pathogenesis of airflow obstruction diseases (Grommes and Soehnlein 2011; Goodman et al. 2003; Tasaka et al. 2008; Hodge et al. 2003). We also found that PM2.5 exposure upregulated the production of inflammatory and pro-inflammatory factors in blood, such as CNTF, IL-6, IL-10, MCP-1, RAGE, MMP-8, TIMP-1, and thymus chemokine 1. These factors could lead directly to inflammatory responses in lung tissues, resulting in alveolar epithelial damage (Murakami and Ohigashi 2007). These biomarkers may correlate with exacerbation of lung disease following PM2.5 exposure.

Our results suggested that PM2.5 exposure in the lung elicits macrophage activation and leakage of inflammatory cells, especially neutrophils. Alveolar macrophages (AMs) isolated from BAL lavage fluid are the classic inflammatory cell type (Barnes, 2004; Butt et al. 2016), and contribute to lung inflammation and injury. In this study, macrophages generated increased ROS, which may mediate inflammatory effects and contribute to tissue injury (Valyi-Nagy et al. 2000). Phagocytes may consume oxygen while engulfing PM2.5, triggering the release of inflammatory factors followed by extracellular production of free radicals. Damaged phagocytes may release oxygen free radicals, protease, and inflammatory factors and activate the inflammatory response, further promoting the release of inflammatory factors (Rücker and Allen 2014). Following macrophage activation, neutrophils accumulate in the lung, and may contribute to oxidant-mediated epithelial injury (Jeyaseelan et al. 2004; Mokra and Kosutova 2015).

We also confirmed that PM2.5 exposure was correlated with the severity of ALI. We found that inflammation and oxidative stress are related, and both are involved in the pathological process of ALI (Su et al. 2014). Increasing evidence has shown that oxidative stress affects the severity of ALI (Tasaka et al. 2008; Shannahan et al. 2012; Ray et al. 2012). In the present study, we confirmed that levels of MDA, H_2O_2, and NO were higher in the PM2.5 exposure group. While MDA and H_2O_2 are upregulated in the respiratory system following PM2.5 exposure, leading to redox imbalance and oxidative stress, NO formation induces inflammation development (Lubos et al. 2008). Increased ROS generation has been associated with enhanced levels of oxidative stress and lipid peroxidation (Porter et al. 2006; Fubini and Hubbard 2003).

During inflammation, pathological remodeling occurs as MMPs and neutrophil elastase degrade collagen and elastin (Bihlet et al. 2017). In the present study, we confirmed that MMP-9 expression levels increased substantially following PM2.5 stimulation, in line with findings reported by Gupta et al. (2016). Moreover, MMP-9 has been reported to promote the migration of inflammatory cells into the airways through extracellular matrix (ECM) destruction (Okada et al. 1997). Elastin is a structural protein abundant in lung tissue, and is degraded during lung inflammation and destruction (Saetta et al. 2001; Kristensen et al. 2015; Sand et al. 2015). We confirmed that following PM2.5 exposure, the expression of elastase, which degrades elastin, was upregulated. In ALI, neutrophils produce the serine protease elastase, which degrades the majority of ECM proteins, including the otherwise stable elastin fibers (Kawabata et al. 2002; He et al. 2010).

Accumulating evidence has shown that an increase in capillary permeability and edema is also involved in the pathogenesis of ALI (Zhang et al. 2010). Furthermore, airway microvascular leak, an important feature of airway inflammatory diseases, promotes the associated inflammatory factors from post-capillary influx into the surrounding tissue (Jones et al. 2016). As shown in our findings, elevation of lung W/D ratio and EBD level could account for an increased pulmonary permeability and lung edema following PM2.5 exposure. Importantly, PM2.5-induced inflammation may promote lung vascular permeability and lung injury.

Conclusions

In summary, our findings are likely to be relevant to understanding the mechanisms underlying the relationship between PM2.5 exposure and induction of ALI, as well as exacerbation of existing asthma. Inhalation of particles led to decreased lung function and an inflammatory response characterized by inflammatory cell influx and cytokine release. Further investigation of the molecular mechanisms of ALI is underway in our laboratory, as is the study of the relationship between chronic respiratory disease and development of lung cancer.

Funding information This study was financially supported by the National Natural Science Foundation of China (30872083) and Shenyang Nonprofit Science and Technology Project.

Compliance with ethical standards

All studies were performed according to protocols reviewed and approved by the Ethics Committee of Animal Care and Experimentation of the National Institute for Environmental Studies, China.

Conflict of interest The authors declare that they have no conflicts of interest.

Abbreviations PM2.5, particulate matter with an aerodynamic diameter less than 2.5 μm; NS, neutral saline; MDA, malondialdehyde; H_2O_2, hydrogen peroxide; BALF, bronchoalveolar Lavage (BAL) fluid; AMs, alveolar macrophages; H&E, hematoxylin and eosin; ROS, reactive oxygen species; EDTA, ethylene diamine tetraacetic acid; PBS, phosphate buffer sulfate; BCA, bicinchoninic acid; ELISA, enzyme-linked immunosorbent assay; DAPI, DNA-binding dye propidium iodide; EBD, Evans blue dye; ALI, acute lung injury; ANOVA, analysis of variance; CNTF, cholinergic neurotrophic factor; IL, interleukin; MCP-1, monocyte chemotactic protein 1; MMP, metalloproteinases; RAGE, receptor for advanced glycation endproducts; TIMP-1, tissue inhibitor of metalloproteinase-1; DNA, deoxyribonucleic acid; TEM, transmission electron microscopy

References

Barnes PJ (2004) Alveolar macrophages as orchestrators of COPD. COPD 1:59–70

Beelen R, Hoek G, Raaschou-Nielsen O, Stafoggia M, Andersen ZJ, Weinmayr G, Hoffmann B, Wolf K, Samoli E, Fischer PH, Nieuwenhuijsen MJ, Xun WW, Katsouyanni K, Dimakopoulou K, Marcon A, Vartiainen E, Lanki T, Yli-Tuomi T, Oftedal B, Schwarze PE, Nafstad P, De Faire U, Pedersen NL, Östenson CG, Fratiglioni L, Penell J, Korek M, Pershagen G, Eriksen KT, Overvad K, Sørensen M, Eeftens M, Peeters PH, Meliefste K, Wang M, Bueno-de-Mesquita HB, Sugiri D, Krämer U, Heinrich J, de Hoogh K, Key T, Peters A, Hampel R, Concin H, Nagel G, Jaensch A, Ineichen A, Tsai MY, Schaffner E, Probst-Hensch NM, Schindler C, Ragettli MS, Vilier A, Clavel-Chapelon F, Declercq C, Ricceri F, Sacerdote C, Galassi C, Migliore E, Ranzi A, Cesaroni G, Badaloni C, Forastiere F, Katsoulis M, Trichopoulou A, Keuken M, Jedynska A, Kooter IM, Kukkonen J, Sokhi RS, Vineis P, Brunekreef B (2015) Natural-cause mortality and long-term exposure to particle components: an analysis of 19 European cohorts within the multi-center ESCAPE project. Environ Health Perspect 123:525–533

Bihlet AR, Karsdal MA, Sand JM, Leeming DJ, Roberts M, White W, Bowler R (2017) Biomarkers of extracellular matrix turnover are associated with emphysema and eosinophilic-bronchitis in COPD. Respir Res 18:22

Butt Y, Kurdowska A, Allen TC (2016) Acute lung injury: a clinical and molecular review. Arch Pathol Lab Med 140:345–350

Chuturgoon AA, Phulukdaree A, Moodley D (2015) Fumonisin B_1 inhibits apoptosis in HepG2 cells by inducing Birc-8/ILP-2. Toxicol Lett 235:67–74

Fubini B, Hubbard A (2003) Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation by silica in inflammation and fibrosis. Free Radic Biol Med 34:1507–1516

Goodman RB, Pugin J, Lee JS, Matthay MA (2003) Cytokine-mediated inflammation in acute lung injury. Cytokine Growth Factor Rev 14: 523–535

Grommes J, Soehnlein O (2011) Contribution of neutrophils to acute lung injury. Mol Med 17:293–307

Gupta I, Ganguly S, Rozanas CR, Stuehr DJ, Panda K (2016) Ascorbate attenuates pulmonary emphysema by inhibiting tobacco smoke and Rtp801-triggered lung protein modification and proteolysis. Proc Natl Acad Sci U S A 113:E4208–E4217

He J, Turino GM, Lin YY (2010) Characterization of peptide fragments from lung elastin degradation in chronic obstructive pulmonary disease. Exp Lung Res 36:548–557

Hodge SJ, Hodge GL, Reynolds PN, Scicchitano R, Holmes M (2003) Increased production of TGF-beta and apoptosis of T lymphocytes isolated from peripheral blood in COPD. Am J Physiol Lung Cell Mol Physiol 285:L492–L499

Hu S, Li J, Xu X, Liu A, He H, Xu J, Chen Q, Liu S, Liu L, Qiu H, Yang Y (2016) The hepatocyte growth factor-expressing character is required for mesenchymal stem cells to protect the lung injured by lipopolysaccharide in vivo. Stem Cell Res Ther 7:66

Jeyaseelan S, Chu HW, Young SK, Worthen GS (2004) Transcriptional profiling of lipopolysaccharide-induced acute lung injury. Infect Immun 72:7247–7256

Jones VC, Birrell MA, Maher SA, Griffiths M, Grace M, O'Donnell VB, Clark SR, Belvisi MG (2016) Role of EP2 and EP4 receptors in airway microvascular leak induced by prostaglandin E2. Br J Pharmacol 173:992–1004

Kawabata K, Hagio T, Matsuoka S (2002) The role of neutrophil elastase in acute lung injury. Eur J Pharmacol 451:1–10

Kim HJ, Choi MG, Park MK, Seo YR (2017) Predictive and prognostic biomarkers of respiratory diseases due to particulate matter exposure. J Cancer Prev 22:6–15

Kristensen JH, Karsdal MA, Sand JM, Willumsen N, Diefenbach C, Svensson B, Hägglund P, Oersnes-Leeming DJ (2015) Serological assessment of neutrophil elastase activity on elastin during ECM remodeling. BMC Pulm Med 15:53

Kumar RK, Shadie AM, Bucknall MP, Rutlidge H, Garthwaite L, Herbert C, Halliburton B, Parsons KS, Wark PA (2015) Differential injurious effects of ambient and traffic-derived particulate matter on airway epithelial cells. Respirology 20:73–79

Li R, Kou X, Xie L, Cheng F, Geng H (2015) Effects of ambient PM2.5 on pathological injury, inflammation, oxidative stress, metabolic enzyme activity, and expression of c-fos and c-jun in lungs of rats. Environ Sci Pollut Res Int 22:20167–20176

Líbalová H, Krčková S, Uhlířová K, Milcová A, Schmuczerová J, Ciganek M, Kléma J, Machala M, Šrám RJ, Topinka J (2014) Genotoxicity but not the AhR-mediated activity of PAHs is inhibited by other components of complex mixtures of ambient air pollutants. Toxicol Lett 225:350–357

Liu Y, Zhuang J, Zhang X, Yue C, Zhu N, Yang L, Wang Y, Chen T, Wang Y, Zhang LW (2017) Autophagy associated cytotoxicity and cellular uptake mechanisms of bismuth nanoparticles in human kidney cells. Toxicol Lett 275:39–48

Lubos E, Handy DE, Loscalzo J (2008) Role of oxidative stress and nitric oxide in atherothrombosis. Front Biosci 13:5323–5344

Ma M, Li S, Jin H, Zhang Y, Xu J, Chen D, Kuimin C, Yuan Z, Xiao C (2015) Characteristics and oxidative stress on rats and traffic policemen of ambient fine particulate matter from Shenyang. Sci Total Environ 526:110–115

Mokra D, Kosutova P (2015) Biomarkers in acute lung injury. Respir Physiol Neurobiol 209:52–58

Murakami A, Ohigashi H (2007) Targeting NOX, INOS and COX-2 in inflammatory cells: chemoprevention using food phytochemicals. Int J Cancer 121:2357–2363

Okada S, Kita H, George TJ, Gleich GJ, Leiferman KM (1997) Migration of eosinophils through basement membrane components in vitro: role of matrixmetalloproteinase-9. Am J Respir Cell Mol Biol 17: 519–528

Pope CA 3rd, Ezzati M, Dockery DW (2009) Fine-particulate air pollution and life expectancy in the United States. N Engl J Med 360: 376–386

Porter DW, Millecchia LL, Willard P, Robinson VA, Ramsey D, McLaurin J, Khan A, Brumbaugh K, Beighley CM, Teass A, Castranova V (2006) Nitric oxide and reactive oxygen species production causes progressive damage in rats after cessation of silica inhalation. Toxicol Sci 90:188–197

Ray PD, Huang BW, Tsuji Y (2012) Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal 24:981–990

Reddy AT, Lakshmi SP, Reddy RC (2012) The nitrated fatty acid 10-nitro-oleate diminishes severity of lps-induced acute lung injury in mice. PPAR Res 2012:617063

Riva DR, Magalhães CB, Lopes AA, Lanças T, Mauad T, Malm O, Valença SS, Saldiva PH, Faffe DS, Zin WA (2011) Low dose of fine particulate matter (PM2.5) can induce acute oxidative stress, inflammation and pulmonary impairment in healthy mice. Inhal Toxicol 23:257–267

Rückerl D, Allen JE (2014) Macrophage proliferation, provenance, and plasticity in macroparasite infection. Immunol Rev 262:113–133

Saetta M, Turato G, Maestrelli P, Mapp CE, Fabbri LM (2001) Cellular and structural bases of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 163:1304–1309

Sand JM, Knox AJ, Lange P, Sun S, Kristensen JH, Leeming DJ, Karsdal MA, Bolton CE, Johnson SR (2015) Accelerated extracellular matrix turnover during exacerbations of COPD. Respir Res 16:69

Shah AS, Langrish JP, Nair H, McAllister DA, Hunter AL, Donaldson K, Newby DE, Mills NL (2013) Global association of air pollution and heart failure: a systematic review and meta-analysis. Lancet 382: 1039–1048

Shannahan JH, Schladweiler MC, Thomas RF, Ward WO, Ghio AJ, Gavett SH, Kodavanti UP (2012) Vascular and thrombogenic effects of pulmonary exposure to Libby amphibole. J Toxicol Environ Health A 75:213–231

Su ZQ, Mo ZZ, Liao JB, Feng XX, Liang YZ, Zhang X, Liu YH, Chen XY, Chen ZW, Su ZR, Lai XP (2014) Usnic acid protects LPS-induced acute lung injury in mice through attenuating inflammatory responses and oxidative stress. Int Immunopharmacol 22:371–378

Tasaka S, Amaya F, Hashimoto S, Ishizaka A (2008) Roles of oxidants and redox signaling in the pathogenesis of acute respiratory distress syndrome. Antioxid Redox Signal 10:739–753

Valyi-Nagy T, Olson SJ, Valyi-Nagy K, Montine TJ, Dermody TS (2000) Herpes simplex virus type 1 latency in the murine nervous system is associated with oxidative damage to neurons. Virology 278:309–321

Wang G, Zhao J, Jiang R, Song W (2013) Rat lung response to ozone and fine particulate matter (PM2.5) exposures. Environ Toxicol 30:343–356

Wang X, Chen M, Zhong M, Hu Z, Qiu L, Rajagopalan S, Fossett NG, Chen LC, Ying Z (2017) Exposure to concentrated ambient pm2.5 shortens lifespan and induces inflammation-associated signaling and oxidative stress in drosophila. Toxicol Sci 156:199–207

Xu MX, Zhu YF, Chang HF, Liang Y (2016) Nanoceria restrains PM2.5-induced metabolic disorder and hypothalamus inflammation by inhibition of astrocytes activation related NF-κB pathway in Nrf2 deficient mice. Free Radic Biol Med 99:259–272

Zhang X, Huang H, Yang T, Ye Y, Shan J, Yin Z, Luo L (2010) Chlorogenic acid protects mice against lipopolysaccharide-induced acute lung injury. Injury 41:746–752