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RESEARCH ARTICLE

Effects of ambient $PM_{2.5}$ on pathological injury, inflammation, oxidative stress, metabolic enzyme activity, and expression of c-fos and c-jun in lungs of rats

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Abstract Fine particulate matter ($PM_{2.5}$) exposure is associated with morbidity and mortality induced by respiratory diseases and increases the lung cancer risk. However, the mechanisms therein involved are not yet fully clarified. In this study, the $PM_{2.5}$ suspensions at different dosages (0.375, 1.5, 6.0, and 24.0 mg/kg body weight) were respectively given to rats by the intratracheal instillation. The results showed that $PM_{2.5}$ exposure induced inflammatory cell infiltration and hyperemia in the lung tissues and increased the inflammatory cell numbers in bronchoalveolar lavage fluid. Furthermore, $PM_{2.5}$ significantly elevated the levels of pro-inflammatory mediators including tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-1β, and intercellular adhesion molecule 1 (ICAM-1) and the expression of c-fos and c-jun in rat lungs exposed to higher dose of $PM_{2.5}$. These changes were accompanied by decreases of activities of superoxide dismutase and increases of levels of malondialdehyde, inducible nitric oxide synthase, nitric oxide, cytochrome P450s, and glutathione *S*-transferase. The results implicated that acute exposure to $PM_{2.5}$ induced pathologically pulmonary changes, unchained inflammatory and oxidative stress processes, activated metabolic enzyme activity, and enhanced proto-oncogene expression, which might be one of the possible mechanisms by which $PM_{2.5}$ pollution induces lung injury and may be the important determinants for the susceptibility to respiratory diseases.

Keywords $PM_{2.5}$ · Rat lung injury · Inflammation · Proto-oncogene · Metabolic enzyme · Oxidative stress

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Introduction

Ambient fine particulate matter ($PM_{2.5}$) with an aerodynamic diameter less than 2.5 μm is easily inhaled into the airway and deposited in lung alveoli, where the toxic particles may affect pulmonary structures and functions. Recent studies have revealed that ambient $PM_{2.5}$ exposure increases the morbidity/mortality of pulmonary diseases and the risk of lung cancer in urban areas (Vinikoor-Imler et al. 2011; Li et al. 2013). $PM_{2.5}$ is a complex mixture of organic and inorganic components including metals, salts, polycyclic aromatic hydrocarbons (PAHs), and carbonaceous material (Li et al. 2014; Cao et al. 2014). $PM_{2.5}$ toxicity is related to pollution sources and its composition (Shi et al. 2015), and the water-soluble and organic-soluble extracts of urban airborne $PM_{2.5}$ may induce DNA damage in a human lung epithelial cell line (Gutiérrez-Castillo et al. 2006; Bonetta et al. 2009). Epidemiologic study showed that ambient PAHs increased lung cancer risk of Chinese population (Zhang et al. 2009). PAHs, the important compositions of $PM_{2.5}$, can be metabolically activated by two enzymes, cytochrome P450s (CYP450s), phase I enzymes, and glutathione *S*-transferases (GSTs), phase II enzymes (Ada et al. 2007).

It is noticed that chronic inflammation-induced production of reactive oxygen species (ROS) in the lung may predispose individuals to lung diseases including lung cancer (Azad et al. 2008; Rosanna and Salvatore 2012). Further, inhaled toxic agents stimulate the generation of reactive oxygen/nitrogen species (ROS/RNS), which in turn provoke inflammatory responses resulting in the release of proinflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-1β, leading to chronic inflammation and lung injury. During inflammation, enhanced ROS/RNS production may induce recurring DNA damage, inhibition of apoptosis, and activation of proto-oncogenes by initiating signal transduction pathways. Activation of proto-oncogenes, such as c-fos and c-jun, always occurs at an early stage of tumor development induced by the chemical carcinogens (Spandidos 2007). Increasing evidences have indicated that the production of oxidative stress, DNA breakage, micronucleus formation, 8-hydroxy-deoxyguanosine (8-OHdG) formation, and inflammatory responses are involved in $PM_{2.5}$-mediated lung injury (Oh et al. 2011; Vattanasit et al. 2014).

However, further studies aiming at the elucidation of pathological injury, inflammation, oxidative stress, metabolizing enzyme activity, and expression of c-fos and c-jun in lungs of rats have hitherto not been fully established. In this work, we determined the histopathological changes, the levels of oxidative stress markers (superoxide dismutase, SOD; malondialdehyde, MDA; inducible nitric oxide synthase, iNOS; nitric oxide, NO), activities of CYP450s and GST, and the alterations of inflammatory cell numbers and inflammatory cytokines [TNF-α, IL-6, IL-1β, and intercellular adhesion molecule 1 (ICAM-1)] as well as the mRNA and protein expression of c-fos and c-jun in lungs of rats exposed to $PM_{2.5}$ using real-time quantitative RT-PCR, Western blot, enzyme-linked immunosorbent assay (ELISA), hematoxylin-eosin staining (HE staining), and biochemical analysis methods, respectively.

Materials and methods

Sampling and preparation of particle samples

The sampling site and methods were performed as described in our previous study (Li et al. 2014). During $PM_{2.5}$ concentration monitoring, aerosol samples were collected on quartz fiber filters (QFFs) for 24 h/day using a $PM_{2.5}$ high-volume air sampler (Thermo Anderson, USA). The pump flow rate was about 1.13 m^3/min. The QFFs after sampling were packed in clean aluminum foil and stored at −20 °C until analysis. The QFFs loading $PM_{2.5}$ during the sampling time were, respectively, cut and surged in Milli-Q water with sonication. The $PM_{2.5}$ suspensions of each filter were obtained and frozen dried in vacuum. Prior to use, the dried samples were mixed, weighted, and then diluted with sterilized 0.9 % physiological saline and swirled for 10 min.

Animal and treatment protocols

Healthy adult and clean grade male Wistar rats, weighing 180–200 g, were purchased from the Animal Center of Hebei Medical University. Animals were housed in metallic cages under standard conditions (24±2 °C and 50±5 % humidity) with a 12-h light–dark cycle. Rats were divided randomly into five equal groups of five animals each: (1) the control group (saline, pH 4.5), (2) 0.375 mg/kg body weight $PM_{2.5}$ group, (3) 1.5 mg/kg body weight $PM_{2.5}$ group, (4) 6.0 mg/kg body weight $PM_{2.5}$ group, and (5) 24.0 mg/kg body weight $PM_{2.5}$ group. The main doses of $PM_{2.5}$ were based on our study (Li et al. 2015). The $PM_{2.5}$ suspensions were respectively given to rats once by the intratracheal instillation at the first, third, fifth, seventh, and ninth day. Correspondingly, the control rats were treated with physiological saline.

Inflammatory cell counts and HE staining

The rats were narcotized by sodium pentobarbital (80 mg/kg, i.p.) 24 h after the last intratracheal instillation. Then, the right lung was ligated, and the left lung was immediately lavaged with phosphate-buffered saline (PBS pH 7.4) solution. Such process was repeated three times with 5 ml PBS as total volume. The counts of total cells in bronchoalveolar lavage fluids (BALF) were determined with a hemacytometer, and inflammatory cells were counted with Wright-Giemsa staining. After the collection of BALF, a piece of the right lungs was cut and fixed in 4 % paraformaldehyde in PBS and paraffin-embedded for the HE staining analysis, and the right lung was quickly frozen in liquid nitrogen and stored at −80 °C until analysis.

All animal procedures were approved by the Shanxi University Animal Investigational Committee and performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the Ministry of Health People's Republic of China.

Real-time quantitative RT-PCR

The frozen lung tissue samples were thawed, and mRNA was extracted using the Transzol reagent (Transgen, Beijing, China). Then, first-strand cDNA was synthesized using AMV RT First Strand cDNA Synthesis Kit (Transgen, Beijing, China) according to the manufacturer's protocols. cDNA product was stored at −80 °C until use.

Expression levels were assessed by real-time PCR in an iCycler iQ Real-Time PCR Detection System (Bio-Rad, Richmond, CA, USA) with the Quantitect SYBR Green I PCR kit (Qiagen, Valencia, CA, USA). The PCR amplification of TNF-α, IL-6, IL-1β, ICAM-1, c-fos and c-jun was

performed as described previously (Li et al. 2015). The relative quantification of the expression of the target genes was measured using the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA as an internal control. The copy numbers of target gene/actin mRNA ratio were measured in all samples. The GenBank accession numbers and the primer sequences of the tested genes and actin together with the PCR product-amplified fragments are listed in Table 1.

Western blotting

Total proteins for ICAM-1, c-fos, c-jun, and actin from frozen lung tissues were respectively extracted with protein extraction kit (Beyotime, Shanghai, China) according to the manufacturer's instructions. Protein concentrations were determined by BCA Protein Assay Kit (Beyotime, Shanghai, China). Samples were mixed with loading buffer and boiled for 5 min. Western blot analysis was performed as described previously (Li et al. 2015). The rabbit polyclonal primary antibodies for rat c-fos, c-jun, ICAM-1, and actin (Santa Cruz, CA, USA) were incubated overnight at 4 °C, whereas the infrared-labeled anti-rabbit secondary antibody (LI-COR Biosciences, USA) at a concentration of 1:5,000 were added to membranes and incubated for 1.5 h at room temperature. The membranes were scanned and the band densities were quantified using the Odyssey Infrared Imaging System (Li-COR Biosciences, USA).

Cytokine and CYP450 immunological assay

The levels of TNF-α, IL-6, and IL-1β in BALF were measured using rat ELISA kit from the R&D Company, USA, and the level of CYP450s was detected using rat ELISA kit from the Beijing Fangcheng Biochemistry, China, according to the manufacturer's instructions.

Measurement of SOD, MDA, iNOS, NO, and GST

Lung proteins were extracted with protein extraction kit (Beyotime, Shanghai, China) according to the manufacturer's instructions. The levels of SOD, MDA, iNOS, NO, and GST in lung tissues were measured using the corresponding kits from the Nanjing Jiancheng Biochemistry, China, according to the manufacturer's protocols.

Statistical analysis

Statistical analyses were performed by the use of one-way analysis of variance (ANOVA) using the SPSS 19.0 package of programs for Windows. Post hoc tests were conducted to determine the difference between groups, followed by Fisher's least significant difference (LSD) test. A level of $P<0.05$ was accepted as statistically significant.

Results

Histopathological observation

Representative H&E staining images were shown in Fig. 1a–d. No histopathological abnormalities were observed in control (Fig. 1a) and 0.375 mg/kg body weight (b.w.) $PM_{2.5}$ group animals (data not shown). In 1.5 mg/kg b.w. $PM_{2.5}$ group, the inflammatory cells existed in the lungs (Fig. 1b). In 6.0 mg/kg b.w. $PM_{2.5}$ group, the inflammatory cell infiltration, thickened alveolar walls, diminished alveolar spaces, and hyperemia were found in the lungs (Fig. 1c). Exposure to 24.0 mg/kg

Table 1 Primer sequences and the PCR product amplified fragments used in real-time RT-PCR

Genes	Accession no.	Sequence	
TNF-α	NM_012675	Forward primer	5′-ACAGAAAGCATGATCCGAGA-3′
Products	148 bp	Reverse primer	5′-TCAGTAGAGAGAAGAGCGTGG-3′
IL-6	NM_012589	Forward primer	5′-TCCTACCCCAACTTCCAATGCTC-3′
Products	79 bp	Reverse primer	5′-TTGGATGGTCTTGGTCCTTAGCC-3′
IL-1β	NM_031512	Forward primer	5′-GCCTCAAGGGGAAGAATCTATACC-3′
Products	172 bp	Reverse primer	5′-GGGAACTGTGCAGACTCAAACT-3′
ICAM-1	NM_012967	Forward primer	5′-GAACTGGACCTGGCAAGAG-3′
Products	150 bp	Reverse primer	5′-GTACCATTCATCTCCCGTTTG-3′
c-fos	XM_022197	Forward primer	5′-GCCTTCACCCTGCCTCTTC-3′
Products	79 bp	Reverse primer	5′-GCTCCATGTTGCTAATGTTCTTGA-3′
c-jun	NM_021835	Forward primer	5′-GCTGAGTGTCTGTATGCTGGG-3′
Products	115 bp	Reverse primer	5′-GGACTTGTGGGTTGCTGGG-3′
GAPDH	NM_017008	Forward primer	5′-ATGTATCCGTTGTGGATCTGAC-3′
Products	78 bp	Reverse primer	5′-CCTGCTTCACCACCTTCTTG-3′

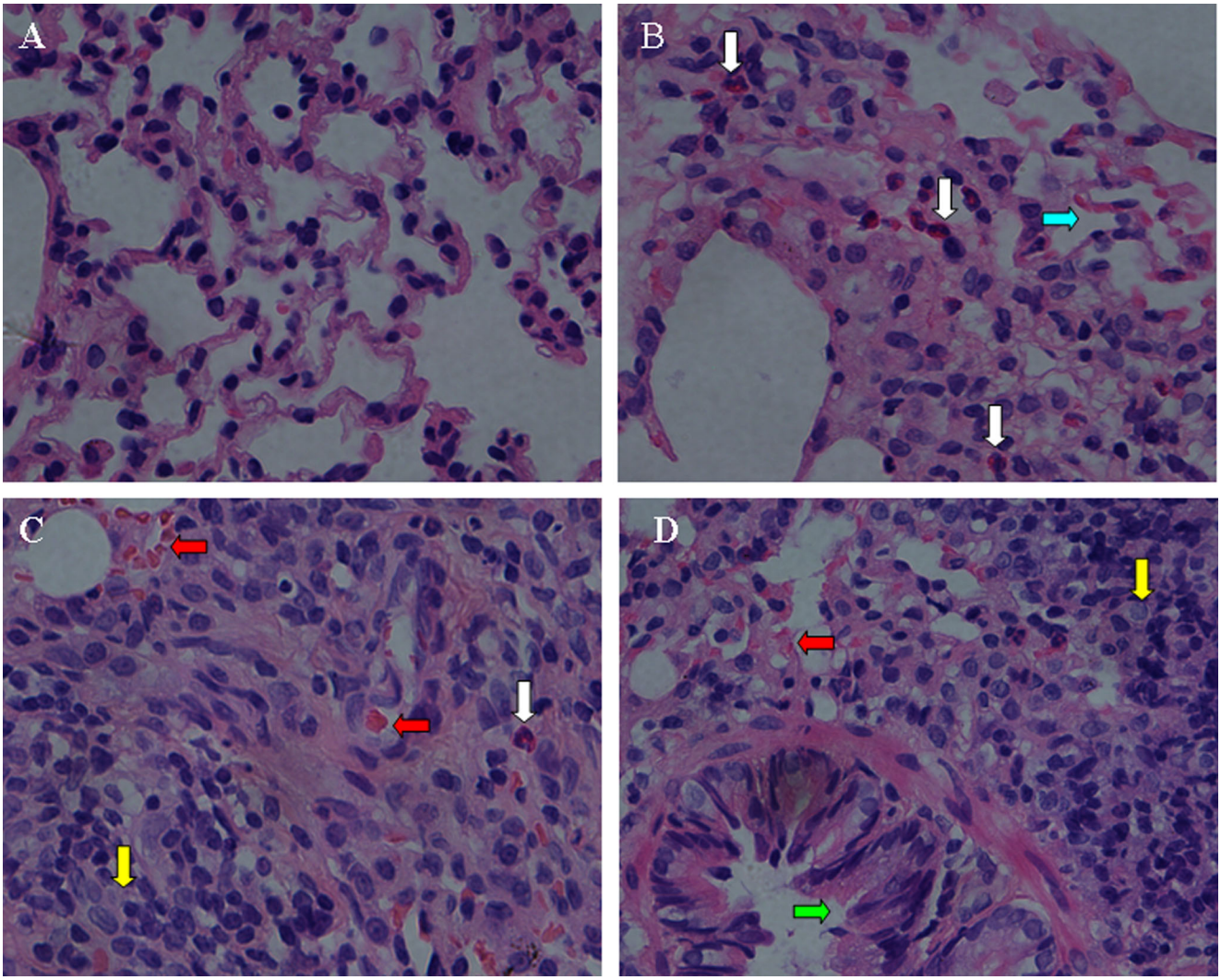

Fig. 1 HE staining results in the lungs of rats from control (**a**), 1.5 mg/kg b.w. (**b**), 6.0 mg/kg b.w. (**c**), and 24.0 mg/kg b.w. (**d**) group, ×400 magnification. The *white*, *red*, *blue*, *yellow*, and *green arrows* indicate sites of inflammatory cell infiltration, hyperemia, alveolar space diminishment, tissue density changes, and bronchial epithelial hyperplasia, respectively. The control group was instilled with same amount of physiological saline

b.w. $PM_{2.5}$ induced the more pathological changes compared to that of other treatment groups, accompanied by the bronchial epithelial hyperplasia (Fig. 1d). It was suggested that $PM_{2.5}$ induced rat lung pathological damage and inflammatory responses.

Inflammatory cell counts in BALF

The numbers of total cells, alveolar macrophages (AMs), and neutrophils (NEUs) in BALF exposed to $PM_{2.5}$ at the doses of 1.5, 6.0, and 24.0 mg/kg b.w. were significantly increased compared with the control ($P<0.01$), whereas the number changes of total cells and inflammatory cells in 0.375 mg/kg b.w. $PM_{2.5}$ group was not significant relative to the control (Fig. 2a). The counts of lymphocytes (LYMs) were significantly increased only in the 24.0-mg/kg b.w. group ($P<0.01$), versus the control. Furthermore, the percentages of AM and NEU cell numbers in the total cell numbers exposed to $PM_{2.5}$ at the doses of 1.5, 6.0, and 24.0 mg/kg b.w. were markedly elevated compared with the control ($P<0.01$), whereas the percentages of LYM cell numbers in the total cell numbers in the all treatment group were significantly changed relative to the control.

Effects of $PM_{2.5}$ on expression of inflammatory markers in lungs of rats

Figure 3 displays the gene expression of four proinflammatory cytokines (TNF-α, IL-1β, IL-6, and ICAM-1) in the lungs of different group rats. The mRNA and protein levels of TNF-α, IL-1β, IL-6, and ICAM-1 showed an obvious increase in response to the higher dose exposure to $PM_{2.5}$

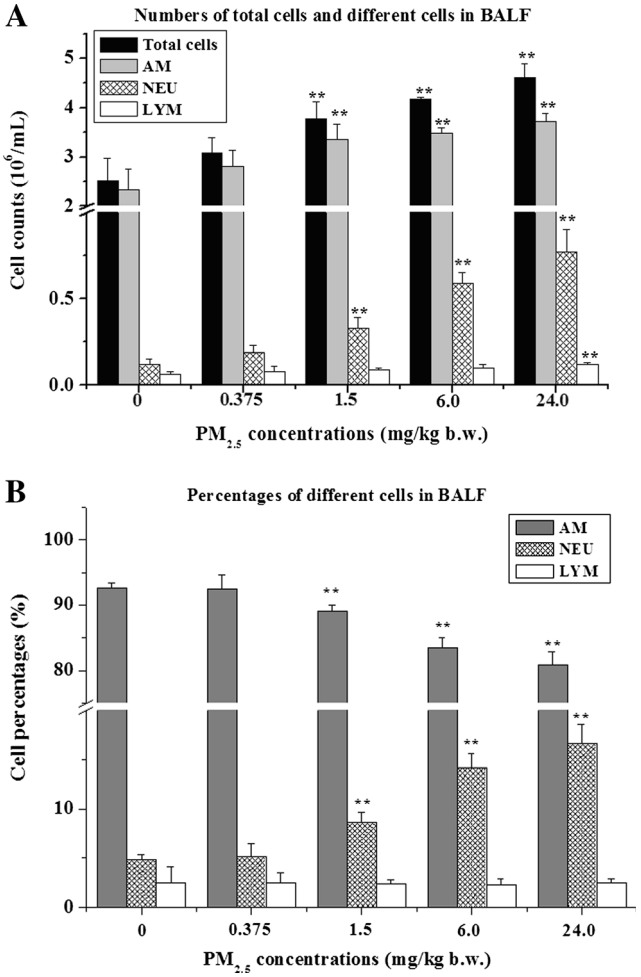

Fig. 2 The numbers of total cells and different inflammatory cells (**a**) and the percentages of different inflammatory cells in total cells (**b**) in BALF of rats treated with different doses of $PM_{2.5}$

(6.0 and 24.0 mg/kg b.w) compared to the control ($P<0.05$ or $P<0.01$). Also, TNF-α and IL-6 mRNA expression as well as TNF-α protein levels were significantly increased at the 1.5-mg/kg b.w. $PM_{2.5}$ group compared with the control. No significant changes of the TNF-α, IL-1β, IL-6, and ICAM-1 were observed in the rats exposed to $PM_{2.5}$ at the concentration of 0.375 mg/kg b.w. compared to that in the control.

Effects of $PM_{2.5}$ on expression of c-fos and c-jun in lungs of rats

In Fig. 4, $PM_{2.5}$ at the concentrations of 1.5, 6.0, and 24.0 mg/kg b.w. significantly induced mRNA expression of c-fos and c-jun ($P<0.05$ or $P<0.01$), while $PM_{2.5}$ at the concentrations of 6.0 and 24.0 mg/kg b.w. significantly increased protein expression of c-fos and c-jun ($P<0.05$; at 6.0 mg/kg b.w., 1.60-fold of control for c-fos, 1.83-fold of control for c-jun; 24.0 mg/kg b.w., 2.13-fold of control

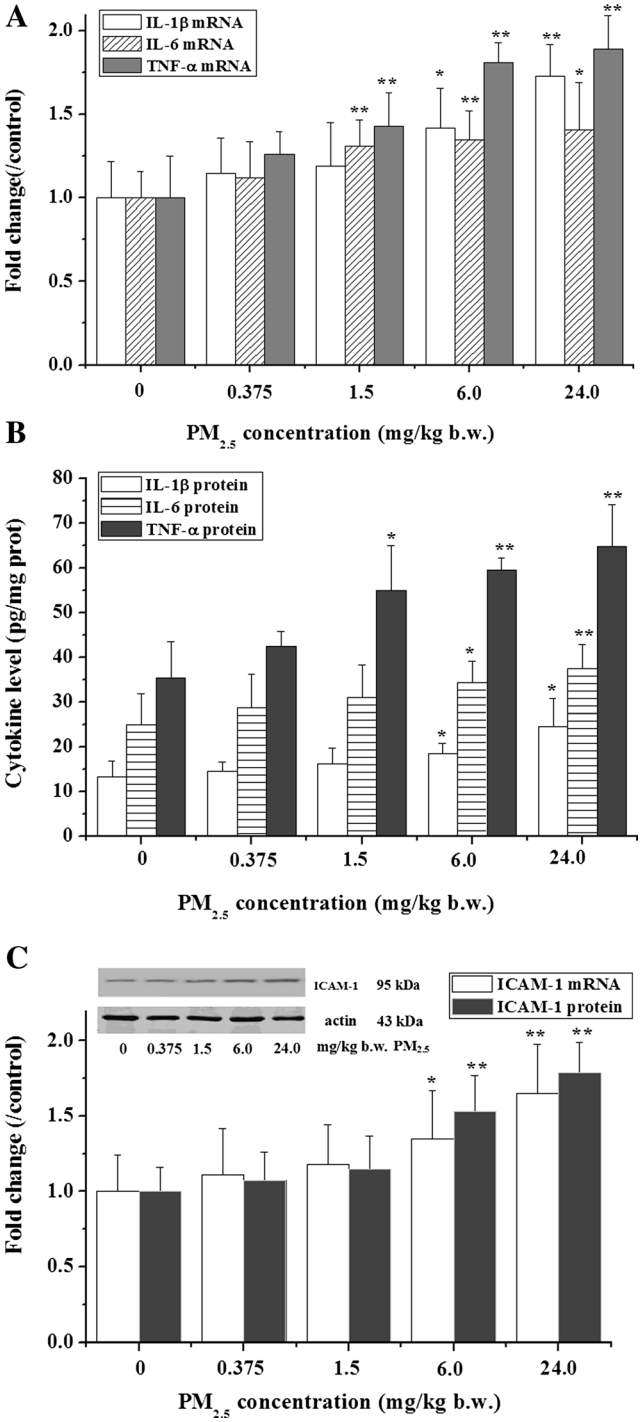

Fig. 3 Expression of mRNA of TNF-α, IL-1β, and IL-6 in rat lungs treated with different $PM_{2.5}$ concentrations (**a**); TNF-α, IL-1β, and IL-6 protein levels in rat lungs treated with different $PM_{2.5}$ concentrations (**b**); and ICAM-1 mRNA and protein expression in rat lungs treated with different $PM_{2.5}$ concentrations. For **a** and **b**, mean expression of mRNA in each treated group is shown as an increase compared to mean expression in control group which has been ascribed an arbitrary value of 1. The values are mean±SD from five individual samples. Using one-way ANOVA, comparing with control group, significant difference is indicated by $*P<0.05$ and $**P<0.01$

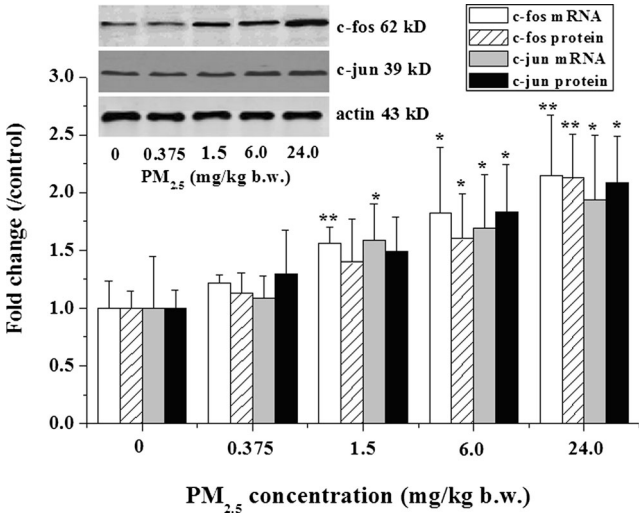

Fig. 4 Expression of mRNA and protein of c-fos and c-jun in lungs of rats treated with different concentrations of $PM_{2.5}$. Mean expression in each treated group is shown as increase compared to mean expression in control group which has been ascribed an arbitrary value of 1. The values are means±SD from five individual samples. Using one-way ANOVA, comparing with control group, significant difference is indicated by *P<0.05 and **P<0.01

for c-fos, 2.09-fold of control for c-jun) in the rat lungs compared with the control.

Effects of $PM_{2.5}$ on CYP450 and GST activity in rat lungs

As shown in Fig. 5, $PM_{2.5}$ at the doses of 1.5, 6.0 and 24.0 mg/kg b.w. significantly increased the levels of CYP450 and GST in the lungs of rats compared with the control group (P<0.05 or P<0.01). The increases of CYP450 and GST activity in the presence of $PM_{2.5}$ (0.375 mg/kg b.w.) were not statistically significant versus the control.

Effects of $PM_{2.5}$ on markers of oxidative stress in rat lungs

As shown in Fig. 6, $PM_{2.5}$ at the doses of 1.5, 6.0, and 24.0 mg/kg b.w. significantly increased the levels of iNOS, NO, and MDA in the lungs of rats compared with the control group (P<0.05 or P<0.01). The higher dose of $PM_{2.5}$ (6.0 and 24.0 mg/kg b.w.) markedly inhibited the SOD activities relative to the control (P<0.05). The level changes of SOD, iNOS, NO, and MDA in the presence of $PM_{2.5}$ (0.375 mg/kg b.w.) were not statistically significant compared with the control group.

Discussion

Epidemiological studies show that $PM_{2.5}$ exposure positively correlates with increased incidence of respiratory diseases, such as pneumonia, asthma, chronic obstructive pulmonary disease (COPD), and lung cancer (Vinikoor-Imler et al. 2011; Li et al. 2013; Tsai et al. 2014). Short-term exposure to $PM_{2.5}$ has been repeatedly associated with evidence of acute airway inflammation (i.e., fractional exhaled nitric oxide increase) and decreased forced expiratory volume in 1 s (FEV1) in human studies (Strak et al. 2012). In one study, Turner et al. (2011) reported that each 10-mg/cm^3 elevation in $PM_{2.5}$ concentration was associated with a 15–27 % increase in lung cancer mortality. Notably, urban airborne $PM_{2.5}$ contains many kinds of harmful organic pollutants. Among them, $PM_{2.5}$-bound PAHs were reported to pose a potential lung cancer risk. For example, Xia et al. (2013) reported the median values of incremental lifetime cancer risk (ILCR) induced by whole year inhalation exposure to both gas and particulate phase PAHs for all population groups in

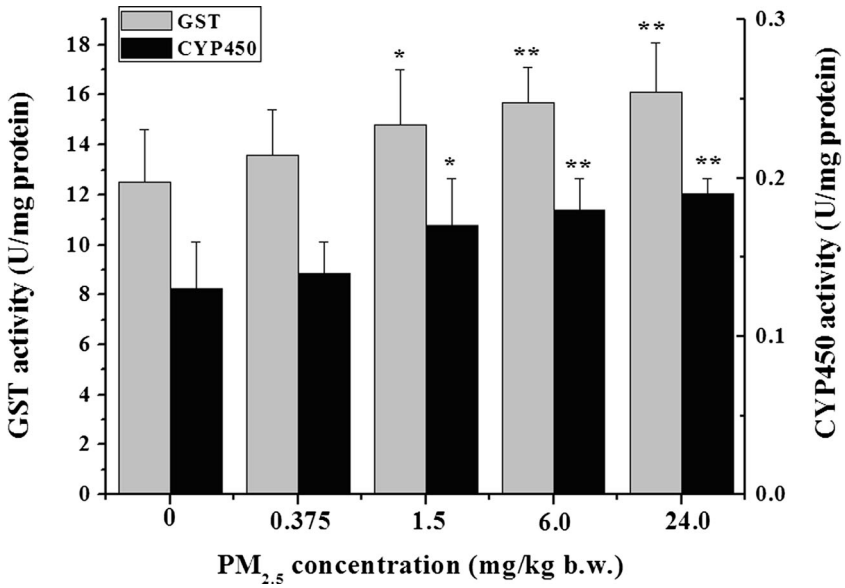

Fig. 5 Activities of CYP450s and GSTs in rat lungs treated with different $PM_{2.5}$ concentrations. The control group was instilled with same amount of physiological saline. The values are mean±SD from five individual samples. Using one-way ANOVA, comparing with control group, significant difference is indicated by *P<0.05 and **P<0.01

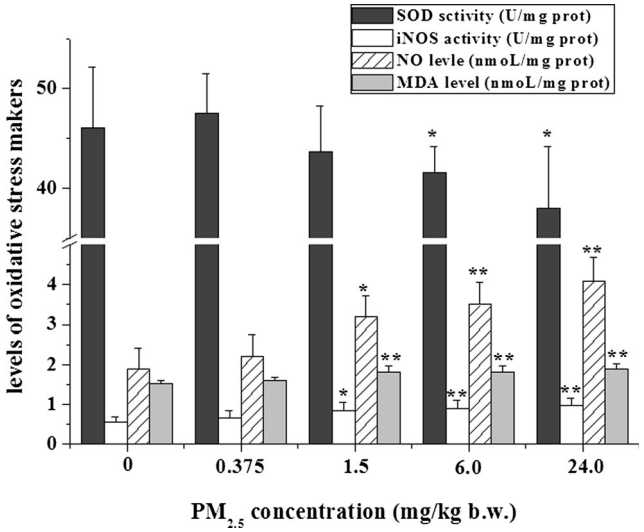

Fig. 6 Activities of SOD and iNOS as well as levels of MDA and NO in rat lungs treated with different $PM_{2.5}$ concentrations. The control group was instilled with the same amount of physiological saline, and the other special control group (vehicle group) was treated with same amount of suspension from extracts of "blank" filter. The values are mean±SD from five individual samples. Using one-way ANOVA, comparing with control group, significant difference is indicated by $*P<0.05$ and $**P<0.01$

Taiyuan, China were basically larger than the risk limit of 10^{-6}, and our study showed that the individual carcinogenicity index of $PM_{2.5}$-bound PAHs exceeded the unit risk of 10^{-5} (Li et al. 2014). However, so far, the detailed mechanisms of $PM_{2.5}$ on lung injury, inflammatory responses, and potential carcinogenic properties remain unclear. Hence, in this study, we investigated the histopathological alterations, inflammatory responses, oxidative stress, changes of metabolic enzyme activity, and proto-oncogene expression in the lungs of rats exposed to $PM_{2.5}$ collected from Taiyuan, China.

The lung is a prime target for a wide variety of air-borne particles. The histopathological alterations and inflammatory responses in the lungs exposed to $PM_{2.5}$ were observed first. To our knowledge, inflammation is a key host defense response to cellular and tissue injury. However, excessive or persistent inflammation can contribute to the pathogenesis of disease. The previous studies found that macrophages phagocytosed silica particles, produced many inflammatory mediators and chemokines, and enhanced the permeability of the alveolar capillary barrier, which can make for NEUs to enter the alveolar space from blood vessel (Liu et al. 2003). NEUs, further, may enlarge inflammatory cascades through releasing more pro-inflammatory factors such as TNF-α, IL-1β, IL-6, and ICAM-1, recruiting AMs, NEUs, and LYMs into alveolar space and increasing the total numbers of the inflammatory cells and the numbers of AMs, NEUs, and LYMs in BALF (Dimatto et al. 1996). Besides, the inflammatory cells including AMs, NEUs, and LYMs played an important role in the early phase inflammatory reaction, and the counts of AMs, NEUs, and LYMs were elevated in BALF of rats exposed to silica dust in a dose-dependent manner (Last et al. 1986; Qu et al. 2012). The increases of the inflammatory cells and the levels of pro-inflammatory cytokines, eventually, may aggravate inflammation and cause lung damage. The literatures showed that $PM_{2.5}$ exposure (8 mg/rat) elevated the total cell and NEU levels in the BALF compared with the control (Luo et al. 2014), and $PM_{2.5}$ instillation (0.2, 0.8 and 3.2 mg/rat) caused dose-trend increase in TNF-α and IL-6 in the BALF compared to the control (Wang et al. 2015). From the HE experimental results in Fig. 1, the inflammatory cell infiltration and hyperemia in lungs of rats exposed to higher $PM_{2.5}$ doses. Also, the numbers of total cells, AMs, NEU, and LYMs as well as the percentages of AMs and NEUs in the total cells were markedly increased in BALF of rats in the presence of 1.5, 6.0, and 24.0 mg/kg b.w. $PM_{2.5}$ compared to the control. The results showed the same findings as the above reports and suggested that the stimulation of $PM_{2.5}$ induced inflammatory reaction of the lung and increased the numbers of AMs, NEUs, and LYMs, and these may lead to acute lung injury. Since the change of percentage of LYMs in the total cells was not significant relative to the control, it is proposed that LYMs might make a little contribution to pulmonary inflammatory damage under current conditions of $PM_{2.5}$ exposure compared with AMs and NEUs. To investigate whether the lung injury induced by $PM_{2.5}$ was in relation with the increased inflammatory activity, we measured expression of the pro-inflammatory cytokines, such as TNF-α, IL-6, IL-1β, and ICAM-1, which are known to be elevated in BALF of lung disease patients (Yong et al. 1997; Singh et al. 2015). Dagher et al. (2005) reported that exposure to $PM_{2.5}$ induced significant increases (in a concentration- and time-dependent manner) in protein secretion and/or gene expression of inflammatory cytokines including TNF-α, IL-6, and IL-1β in cultured human epithelial lung cells (L132). Brucker et al. (2013) pointed out that TNF-α, IL-6, and IL-1β levels in serum of the taxi drivers exposed to $PM_{2.5}$ with average mass concentrations of 12.4±6.9 μg/m^3. Moreover, ICAM-1 is a major adhesion molecule to induce the inflammatory cell infiltrate to bronchial epithelial cells in the lung and participates in establishing and maintaining inflammation (Wegner et al. 1990). Wang et al. (2015) reported that $PM_{2.5}$ increased mRNA expression of ICAM-1 in lungs and the levels TNF-α and IL-6 in BALF of rats, leading to inflammatory changes and pathological characters in rat lungs. Combining with our data of the inflammatory cells and pro-inflammatory cytokine measurement (see Figs. 1, 2, and 3), it was implied that $PM_{2.5}$ elevated inflammatory cell numbers in BALF and increased the levels of TNF-α, IL-6, IL-1β, and ICAM-1 in the lungs of rat, inducing inflammatory cell infiltration and inflammatory reactions.

In the current study, the $PM_{2.5}$ samples were collected during the heating season from a representative of the coal

combustion polluted city, Taiyuan, and the daily mean mass concentration of $PM_{2.5}$ had reached high pollution levels (161 μg/m^3; Li et al. 2014). Besides, the benzo(*a*)pyrene equivalent (BaPeq) concentration of total PAHs in $PM_{2.5}$ was 28.6 ng/m^3, higher than the risk limit (10 ng/m^3), and the individual carcinogenic index of total PAHs was 3.14×10^{-5}, exceeding the recommendation (10^{-5}), suggesting the urban PAHs in $PM_{2.5}$ pose a potential lung cancer risk in Taiyuan. Hence, we specifically focused on the effects of $PM_{2.5}$ on proto-oncogene expression and metabolic enzyme activity in rat lungs. Some studies indicated that $PM_{2.5}$ had a positive relationship with lung cancer incidence and mortality (Vinikoor-Imler et al. 2011; Li et al. 2013). Occupational exposure to PAHs was associated with a risk of lung cancer (Armstrong et al. 2004), while $PM_{2.5}$-bound PAHs have a potential cancer risk (Xia et al. 2013). As we know, overexpression of proto-oncogene c-fos and c-jun plays an important role in proliferation, and they are possible end points of exposure to reactive metabolites in lung (Janssen et al. 1997). They may encode the activator protein (AP)-1 transcription complex, while the activation of AP-1 may mediate inflammation and contribute to the highly malignant transformation in lung cancer (Ichiki et al. 2000; Zenz et al. 2008). As for the present study, the c-fos and c-jun mRNA and protein levels were increased in lungs of rats exposed to $PM_{2.5}$ with higher concentrations (1.5, 6.0, and 24.0 mg/kg b.w., see Fig. 4), which implied that $PM_{2.5}$, especially including PAHs in $PM_{2.5}$, might promote cell proliferation, increasing the potential risk of lung cancer. On the other hand, in the metabolic activation of PAHs, they are initially catalyzed by CYP450s in the phase I metabolic reactions (Shimada and Fujii-Kuriyama 2004), and then the products from phase I reaction can be detoxified by phase II enzymes such as GSTs (Ketterer and Mulder 1990). CYP450 enzymes are central to the metabolic activation of PAHs to epoxide intermediates, which are converted with the aid of epoxide hydrolase to the ultimate carcinogenic metabolites, diol-epoxides (Shimada and Fujii-Kuriyama 2004), suggesting that activation of CYP450s may accumulate more harmful metabolites in the presence of PAHs. GSTs may take the metabolites from phase I and further metabolizes them by catalyzing the conjugation of the tripeptide glutathione with the xenobiotics such as PAHs in the phase II of the biotransformation process promoting its elimination from the organism (Nakamura et al. 2003). To some extent, they may counteract the potential toxicity of PAHs by its role in PAH detoxification (Kabler et al. 2009). A high level of activity of GSTs might be an adaptation or regulation to detoxify the excess of harmful metabolites from phase I reaction. In the present study, $PM_{2.5}$ at the doses of 1.5, 6.0, and 24.0 mg/kg b.w. particles markedly increased the activities of CYP450s and GSTs in rat lung (see Fig. 5), thus modifying the lung toxication/detoxication potential and promoting the toxicity induced by $PM_{2.5}$ or $PM_{2.5}$-bound PAHs. Abbas et al. (2009) demonstrated that PAH-coated onto $PM_{2.5}$ induced the gene expression of CYP1A1, CYP2E1, GST-pi1, and/or GST-mu3 in cultured human alveolar macrophage cells, while Chan et al. (2013) reported ultrafine premixed flame particle induced expression of certain isozymes in the cytochrome P-450 superfamily, such as CYP1A1 and CYP1B1, in rat lungs, which indirectly support our results.

We also paid attention to the relationships between lung injury and oxidative stress. ROS include a wide variety of molecules and free radicals derived from molecular oxygen, such as superoxide ($O_2^{\cdot-}$), hydroxyl radical ($^{\cdot}OH$), hydrogen peroxide (H_2O_2), etc. RNS mainly include NO and peroxynitrite (Snyder and Bredt 1999), which is a very powerful oxidant (Virág et al. 2003). Oxidative stress is an expression used to describe various deleterious processes resulting from an imbalance between the formation and elimination of ROS and/or RNS by antioxidant defenses. Overproduction ROS or RNS can damage to cellular components and biomolecules, including DNA, enzymes, and lipids, inducing oxidative/nitrosative stress and cell dysfunction, which have been suggested to be involved in the etiology of several chronic diseases including cancer and aging (Poljšak and Fink 2014). ROS and RNS can result from exposure to environmental pollutants, such as $PM_{2.5}$ and PAHs (Sagai et al. 1993; Dellinger et al. 2001; Briedé et al. 2004; Chauhan et al. 2004; Gehling et al. 2014). Under stress conditions, activities of antioxidative enzymes such as SOD may be inhibited, whereas excessive ROS are easy to attack the cell membrane and form MDA, which is a typical product of lipid peroxidation (LPO). Also, excessive NO regulated by iNOS may form a more toxic peroxynitrite anion (Snyder and Bredt 1999), causing oxidative damage and cytotoxicity (Virág et al. 2003). The SOD activity decrease and MDA, iNOS, and NO level increase in this study (see Fig. 6) suggest that $PM_{2.5}$ may trigger oxidative stress via the accumulation of ROS and RNS, which may be suggested as an important mechanism of $PM_{2.5}$-mediated lung injury. Except SOD, GST plays a key role in conjugating GSH to the products of endogenous LPO and inactivating organic hydroperoxides, thus protecting the cell from the severe effects of oxidative stress as an important enzyme for quenching and detoxifying ROS (Sharma et al. 2004). A high level of GSTs induced by $PM_{2.5}$ in this study implied the antioxidative ability of the organism. Luo et al. (2014) revealed that $PM_{2.5}$ exposure (8 mg/rat) increased MDA levels and reduced SOD and glutathione peroxidase activity in BALF of Wistar rats, consistent with the results obtained in this study. What is more, on the other hand, the generation of ROS/RNS in turn may provoke inflammatory responses and induce the release of proinflammatory cytokines and chemokines, leading to inflammation and lung injury. During inflammation, enhanced ROS/RNS production may induce recurring DNA damage, inhibition of apoptosis, and activation of proto-oncogenes by initiating signal transduction pathways (Azad et al. 2008).

Accordingly, the role of ROS/RNS in lung inflammation and carcinogenesis should be highlighted.

Summary

The results in this study demonstrated that (1) the lung inflammatory injury was induced by the exposure to $PM_{2.5}$, accompanied by inflammatory cell infiltration and increase of inflammatory cell numbers and pro-inflammatory cytokine levels; (2) the mRNA and protein expression of c-fos and c-jun were raised in the lungs of rats exposed to $PM_{2.5}$ along with the activation of CYP450s and GSTs; and (3) $PM_{2.5}$-caused pathological injury had relations with oxidative stress via inhibiting SOD activity and elevating activity of iNOS and levels of MDA and NO. These data suggest that $PM_{2.5}$-induced inflammatory responses and proto-oncogene high expression in lungs of rats are mediated, in part, by both ROS- and NO-mediated pathways and activation of the metabolic enzymes, which may be one of the important mechanisms by which $PM_{2.5}$ pollution aggravates respiratory disease.

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