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Methionine oxidation in albumin by fine haze particulate matter: An in vitro and in vivo study

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HIGHLIGHTS

- Effects of protein oxidation by haze $PM_{2.5}$ were investigated.
- Oxidative stress was increased by the haze $PM_{2.5}$.
- Haze episodes to albumin resulted in oxidation of methionine moieties.
- Oxidation of methionine associated with oxidative stress and PAHs in the $PM_{2.5}$.

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ABSTRACT

The potential effects of inhaled fine particulate matter ($PM_{2.5}$), found in haze episodes, on the oxidation of the proteins in the lungs are not well understood. We investigated the effects of $PM_{2.5}$ from haze episodes on protein oxidation. $PM_{2.5}$ was collected from the air pollution in Beijing (BJ), Xian (XA), Xiamen (XM) and Hong Kong (HK) during a period of intensive haze episodes. The chemical characteristics of these samples and their effects on albumin oxidation were investigated. The levels of $PM_{2.5}$ in BJ and XA were 4–6 times higher than in XM and HK. The concentrations of the polycyclic aromatic hydrocarbons (PAHs) components of the $PM_{2.5}$ from BJ and XA were 10 times higher than those found in XM and HK. The haze $PM_{2.5}$ increased oxidative stress. Addition of $PM_{2.5}$ samples collected from haze episodes to albumin *in vitro* resulted in oxidation of methionine moieties; nasal instillation of $PM_{2.5}$ suspensions in mice resulted in oxidation of methionine in the albumin in the bronchoalveolar lavage fluid. The methionine moieties participate in peptide chain crosslinking, and methionine oxidation in the albumin could be attributed to the PAH compounds. Our findings may be helpful in explaining the potential respiratory effects during haze episodes.

Abbreviations: DCF, 2′,7′-dichlorofluorescin; DCFH, 2′,7′-dichlorodihydrofluorescein; DCFH-DA, 2′,7′-dichlorodohydrofluorescein diacetate; BJ, Beijing; BALF, bronchoalveolar lavage fluid; CB, carbon black; DMSO, dimethyl sulfoxide; HK, Hong Kong; $PM_{1.8}$, particulate matter less than 1.8 μm in aerodynamic diameter; $PM_{2.5}$, particulate matter less than 2.5 μm in aerodynamic diameter; PBS, phosphate buffered saline; PAH, spolycyclic aromatic hydrocarbons; ROS, reactive oxygen species; XM, Xiamen; XA, Xian.

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1. Introduction

Haze is defined as a weather phenomenon that leads to atmospheric visibility less than 10 km due to of the mixture of dust, moisture, smoke and vapour in the atmosphere [1]. Combustion of biomass and fossil fuels by power plants, factories, residential homes and vehicles are major contributors to haze. For example, with a population of over 13 million, Beijing (BJ), the capital of the People's Republic of China, is affected by haze episodes every year [2]. Haze can severely degrade visibility, and more importantly, an increasing number of epidemiological studies have shown that haze episodes provide considerably increase the risks for cardiopulmonary diseases [3,4]. Previous studies have shown that haze episodes are important public health issues that may contribute to 24% of hospital admissions for respiratory conditions [5] and a 30% increase in outpatient attendance [6]. Mao et al. (2013) suggested that particulate matter less than 2.5 μm in aerodynamic diameter ($PM_{2.5}$) may be the main cause of cardiopulmonary syndromes during the haze episodes observed in Chinese cities [7]; however, the possible mechanisms underlying the health effects of haze $PM_{2.5}$ are not well understood.

The mechanisms of action underlying the health effects of $PM_{2.5}$ involve oxidative stress and inflammation, which are attributable to the physicochemical characteristics of the particles. Generally, albumin concentrations remain very low in the lung bronchoalveolar lining fluid when compared with the plasma. $PM_{2.5}$-induced inflammation enhances vascular permeability mainly through chemicals released by activated neutrophils, leading to an increased concentration of albumin in the lung environment [8]. One beneficial effect that arises from this apparent damage is that albumin concentrations may be increased in the sites of inflammation, where the protein can exert its multiple antioxidant properties [8–10]. Albumin is a non-glycosylated protein of 66 kDa. It is synthesised by the liver in mammals and is the most abundant protein in the serum, comprising approximately 60% of the total globular protein in the blood plasma. In general, albumin represents the major and predominant antioxidant in the plasma and other places in the body that are exposed to continuous oxidative stress [8]. Native albumin contains 6 methionines as well as 35 cysteine residues, which are involved in the formation of 17 disulphide bonds [9]. Numerous studies have suggested that albumin plays an important role in the regulation of physiological and pharmacological functions and is involved in disease development [8,9]. For example, significant albumin oxidation was observed in the lung tissues of patients with chronic obstructive pulmonary disease (COPD) and current smokers. This oxidation resulted from an antioxidant imbalance [11]. Pulmonary exposure to $PM_{2.5}$ has also been linked to protein oxidation in healthy non-smokers [12] and drivers [13]. This oxidative stress could be attributed to the physicochemistry of particles including the presence of polycyclic aromatic hydrocarbons (PAHs) [14]. However, little is known regarding the pathophysiological significance of this oxidation due to poor characterisation of the structure and functional properties of oxidised albumin. The effects of $PM_{2.5}$ from haze episodes on albumin oxidation also remain unclear.

Mao et al. (2013) stated that the investigation of the health implications of exposure to haze $PM_{2.5}$ is an urgent issue for the protection of human health [7]. The harmful effects of pulmonary exposure to haze $PM_{2.5}$ may be related to the potential mechanism of the sedimentation of particles in the lung, leading to pulmonary damage and oxidative-inflammatory responses followed by remodelling of the lung. However, the mechanisms underlying the interaction of albumin with haze $PM_{2.5}$ remain unclear. To elucidate the relevant mechanism(s), $PM_{2.5}$ was collected in severely haze-affected cities, BJ and Xian (XA), and less haze-affected cities, Xiamen (XM) and Hong Kong (HK), and the PAH composition and oxidative stress levels were determined. Next, albumin oxidation was examined following interactions with the four haze $PM_{2.5}$ samples using *in vitro* and *in vivo* models.

2. Materials and methods

2.1. $PM_{2.5}$ collection

Two inland cities [BJ (39°59′10.78″ N, 116°23′09.25″ E) and XA (34°13′49.36″ N, 108°52′58.59″ E)] and two coastal cities [XM (24°29′11.20″ N, 118°06′08.04″ E) and HK (22°18′11.49″ N, 114°11′00.17″ E)] in China were selected for high and low levels of $PM_{2.5}$ exposure during haze episodes, respectively (Fig. 1a). The $PM_{2.5}$ samples were collected for between six and eight days during a haze air pollution episode from 26 January 2013 to 1 February 2013. Mini-volume samplers equipped with two $PM_{2.5}$ impactors (Airmetrics, Oregon, USA), operated at flow rates of 5 L/min, were used to collect the $PM_{2.5}$. All samples were collected onto pre-weighed 47-mm Teflon filters for 24 h (from 10:00 a.m. to 10:00 a.m.) every day. All filters were equilibrated for 48 h in 40% ± 5% relative humidity pre- and post-sampling to obtain the particle mass and were then stored at −20 °C until further analyses.

2.2. $PM_{2.5}$ preparation

Methanol $PM_{2.5}$-extracts were prepared as previously described using two-stages of sonication in methanol [15]. The extract was then dried using a pure nitrogen stream. The particles were then resuspended in dimethyl sulphoxide (DMSO) [<0.01% vol] in phosphate buffered saline (PBS) at 0, 50 and 150 μg/ml. Fresh samples were kept at 4 °C and used within one week of preparation. Near-pure, manufactured, chemical-less, carbon-core carbon black (CB) with an average diameter of 65 nm (Monarch 120; Cabot Corporation, UK) was selected as a control particle. CB is an industrial carbon produced by the thermal decomposition of hydrocarbons. The chemical characteristics of CB have been described previously [16].

2.3. PAH determination

The filters were extracted for 16–18 h with 60 ml of a solution of acetone:dichloromethane:n-hexane (1:1:1) in a Soxhlet extractor at 68 °C. The extracted solution was concentrated to 1 ml using a rotary evaporator, followed by a florisil clean-up step. The cleaned extract was dried under a nitrogen stream and resuspended in 1 ml of n-hexane. Deuterated PAH internal standards (acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12} and perylene-d_{12}) were added to the extracts, each at a concentration of 320 ng/g, prior to GC-MSD analysis [Hewlett–Packard (HP) 6890 N gas chromatograph (GC) coupled with an HP-5973 mass selective detector (MSD) and a 30 m × 0.25 mm × 0.25 μm DB-5 capillary column (J&W Scientific Co. Ltd., Folsom, CA)]. The native PAH standards [naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b + k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a, h)anthracene and benzo(g, h, i)pyrelene] were used to generate the standard curve using the concentrations of 0, 2, 5, 10, 20, 50, 100 and 200 ng/g. The limit of detection for each PAH was determined as the concentration of an individual PAH detected in a sample with a signal-to-noise ratio of 3; these values ranged from 0.05 to 0.15 ng/g. The recoveries of individual PAHs were from 74 to 108%. For each batch of samples, a method solvent blank, a spiked blank, sample duplicates and the standard reference material were analysed. The coefficient of variation in the concentrations of PAHs

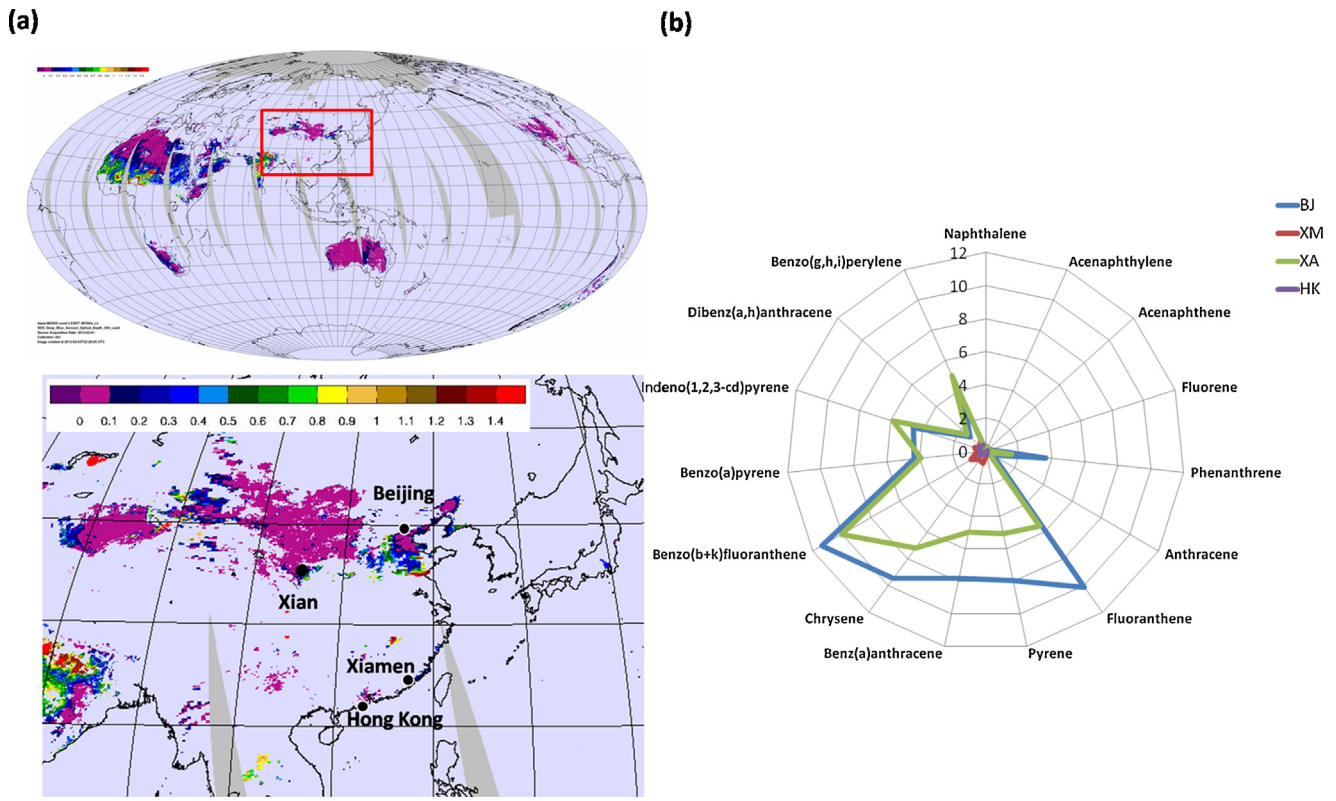

Fig. 1. (a) Geographic location of the sampling cities, Beijing (BJ), Xian (XA), Xiamen (XM) and Hong Kong (HK), from which $PM_{2.5}$ was collected during the haze episodes from 26 January 2013 to 1 February 2013. The colour on the map is the satellite (MODIS) integrated aerosol optical depth (AOP), which showed that BJ and XA deomonstrated higher concentrations of haze particles than XM and HK during the sampling period. (b) The concentrations of 15 PAHs determined from the BJ, XA, XM and HK $PM_{2.5}$ in the haze episodes.

in the duplicate samples was less than 10%. The concentrations of PAHs in the method blank were less than the limit of detection.

2.4. Cell culture

A549 cells were obtained from the American Type Culture Collection and cultured in RPMI containing 10% foetal bovine serum, penicillin and streptomycin. The cells were incubated in air at 37 °C, 95% humidity and 5% CO_2. All chemicals used in this study were reagent grade and were obtained from Sigma–Aldrich (UK), unless otherwise stated.

2.5. A549 cell treatment

For the *in vitro* experiments, the A549 cells were seeded onto surface-treated, 24-well transwells at a density of 1×10^5 cells/ml and incubated for 24 h (3×10^4 cells/well; BD Biosciences, UK). The RPMI medium was removed before adding 300 μl of the $PM_{2.5}$ samples at particle concentrations of 0 (control), 50 and 150 μg/ml. The cells were then incubated at 37 °C for 4 h in a humidified atmosphere with 5% CO_2. Each experiment was conducted in quadruplicate. The concentrations of $PM_{2.5}$ were chosen to produce oxidative effects with >80% cell viability, according to criteria that were described previously [17].

2.6. Determination of dichlorodihydrofluorescein oxidation

The cell assay used to determine the reactive oxygen species (ROS) levels was described in a previous study [15]. Briefly, ROS production was determined using a 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) probe. Intracellular cleavage of the diacetate groups by esterase enzymes produces the relatively lipid-insoluble and non-fluorescent dichlorodihydrofluorescein (DCFH). The highly fluorescent compound 2′,7′-dichlorofluorescin (DCF) produced by the oxidation reactions of DCFH with ROS was measured using a GeminiXS spectrofluorometer (Molecular Devices, USA) at an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

2.7. PM and BSA interaction

In this study, we investigated the modification of the serum protein after $PM_{2.5}$ exposure. The preparation of samples was described previously [18]. Briefly, a 1 mg/ml solution of recombinant BSA was prepared with sterile PBS and then filter-sterilised. The $PM_{2.5}$ samples were suspended by sonication for 15 min. Aliquots of the PM suspensions were then combined with 5 ml of the BSA solution to yield final particle concentrations of 0, 50 and 150 μg/ml. The $PM_{2.5}$ samples in the BSA solution were vortexed and incubated at 37 °C for 2 h under constant shaking at 500 rpm to ensure thorough mixing. The PM suspensions were used to investigate the protein modifications.

2.8. Animal study

Female BALB/c mice (6 weeks old) were obtained from BiOLASCO (Taipei, Taiwan). The mice were maintained at a constant temperature and relative humidity of 22 °C ± 2 °C and 55% ± 10%, respectively, with a 12 h-12 h light–dark cycle throughout the study. The mice weighed between 16 and 19 g during the experimental period. The animals were housed in plastic cages and were provided Lab Diet 5001 (PMI Nutrition International, USA) and water *ad libitum* during acclimatisation as well as during pre-exposure and post-exposure. The animal experiments were

performed in compliance with the animal and ethics review committee of the Laboratory Animal Centre at the Taipei Medical University, Taiwan (Approval No: LAC-101-0003).

2.9. Pulmonary exposure to $PM_{2.5}$

To assess the effects of PM on the modification of the pulmonary protein, 4 exposure groups were used (6 mice/group): PBS control, BJ $PM_{2.5}$, XA $PM_{2.5}$ and HK $PM_{2.5}$. Due to the limited amount of the XM $PM_{2.5}$ collected, no mice were exposed to the XM $PM_{2.5}$ samples. On day 0, the mice in the exposure groups received an intranasal instillation of 150 μg of $PM_{2.5}$ in PBS under light anaesthesia induced by Ultrane (Abbott Laboratories, UK), whereas those in the control group received the same volume of PBS alone. The dosing was repeated on day 7. On day 14, the animals were euthanised, and bronchoalveolar lavage fluid (BALF) samples were collected. BALF collection was performed as previously described [19]. Briefly, animals were euthanised with a single intraperitoneal injection of 2 ml of sodium pentobarbitone (200 mg/ml). A single 1 ml volume of PBS was used to lavage the lungs. The BALF samples were centrifuged at 1500 × *g* for 5 min at 4 °C, and the supernatant was collected for the determination of serum protein oxidation.

2.10. Determination of BSA and BALF serum protein modification

BSA and BALF samples were diluted with 6.5 mM dithiothreitol at 37 °C for 1 h and then alkylated using 10 mM iodoacetamide in the dark at room temperature for 30 min. The samples were digested with trypsin in 25 mM ammonium bicarbonate at 37 °C for 18 h and then acidified with 0.1% formic acid. The tryptic peptides were analysed with a Q-Exactive MS (Thermo Fisher Scientific, Bremen, Germany) coupled to an UltiMate 3000 RSLC system. The peptide separation was performed using LC with a C18 column (Acclaim PepMap RSLC, 75 μm × 150 mm, 2 μm, Dionex) under the conditions described previously [18]. Full MS scans were performed with an m/z range of 300–2000, and the ten most intense ions from the MS scan were subjected to fragmentation to yield MS/MS spectra. The raw data were processed into peak lists by Proteome Discoverer v1.4 for Mascot database searches (http://www.matrixscience.com). The search parameters specified variable modification for deamidation (NQ), oxidation (M) and methylation (K) and a fixed modification for carbamidomethylation (C). The maximum mass tolerance was set to 10 ppm for the precursor ions and 0.05 Da for the fragment ions. To estimate the degree of oxidation, the signal intensities from the extracted ion chromatograms of the peptides with and without oxidation were compared [18]. The degree of oxidation was calculated based on the peak area [(peak area of oxidised peptide/total peak area of peptides with and without oxidation) × 100%]. The Swiss-Pdb Viewer Version 4.1.0 (Swiss Institute of Bioinformatics, Switzerland) was used to analyse the peptides oxidised in the BSA oxidised by the $PM_{2.5}$ samples [20].

2.11. Statistical analysis

The statistical analyses were performed using GraphPad Version 5 for Windows. The Mann–Whitney *U*-test was used for comparisons between the groups [21]. Pearson's correlation coefficient was used to examine the correlation of the percentage of BSA peptide oxidation to oxidative stress and PAH levels. The significance criterion was set at $p < 0.05$.

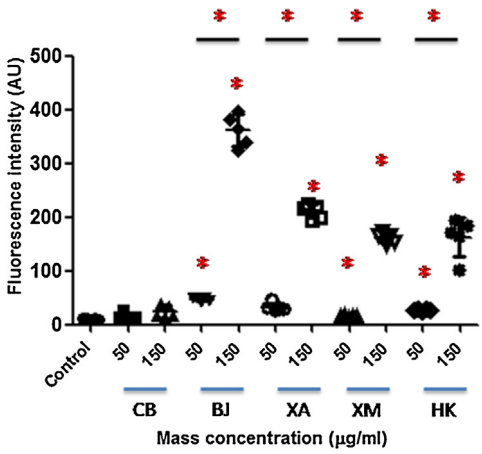

Fig. 2. Levels of oxidative stress induced by the CB, BJ, XA, XM and HK $PM_{2.5}$ at 0, 50 and 150 μg/ml ($n = 6$). The values shown are the means ± SD; * $p < 0.05$.

3. Results

3.1. PM characterisation

The daily average $PM_{2.5}$ mass concentrations in the four cities during the haze episodes were 221 ± 108 μg/m^3 (113–328 μg/m^3) for BJ, 234 ± 27 μg/m^3 (204–253 μg/m^3) for XA, 50 ± 10 μg/m^3 (41–65 μg/m^3) for XM and 40 ± 7 μg/m^3 (35–51 μg/m^3) for HK. The $PM_{2.5}$ concentrations were 5.5-fold higher in BJ, 1.3-fold higher in XM and 5.9-fold higher in XA than in HK. A GC-MS was used to analyse a total of 15 PAHs in the haze $PM_{2.5}$ samples collected from BJ, XA, XM and HK. The concentrations of the bulk PAHs were 64.7 ± 13.4 ng/m^3 for BJ, 51.0 ± 3.3 ng/m^3 for XA, 5.3 ± 0.7 ng/m^3 for XM and 2.8 ± 0.3 ng/m^3 for HK, suggesting that the BJ samples had the highest bulk PAHs when compared to the other cities ($p < 0.05$). The most common PAHs in the BJ samples were phenanthrene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b + k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene and benzo(g, h, i)perylene; the levels of these PAHs ranged from 3.3 ng/m^3 to 11.4 ng/m^3 (Fig. 1b).

3.2. Oxidative stress

To determine the effects of the $PM_{2.5}$ on oxidative stress in the cell-based systems, the A549 cells were exposed to CB and the haze $PM_{2.5}$ collected from BJ, XA, XM and HK (Fig. 2). There were significant increases in the oxidative stress when A549 cells were exposed to any of the $PM_{2.5}$ samples compared to the controls ($p < 0.05$), with the exception of the CB samples and 50 μg/ml XM $PM_{2.5}$. Additionally, a significant dose-dependent response was observed for the BJ, XA, XM and HK $PM_{2.5}$ ($p < 0.05$). Comparisons of the samples at the same mass concentration showed that the levels of oxidative stress induced by the $PM_{2.5}$ were BJ > XA > XM > HK. At the 150 μg/ml concentration, the ROS production was 2.2-fold higher in the BJ samples than in the HK samples.

3.3. BSA oxidation

To investigate the BSA modifications due to the interaction with $PM_{2.5}$, the trypsin-digested peptides from the BSA control and the CB- and the $PM_{2.5}$-treated samples were analysed by LC–MS and the Mascot database. We observed three peptides from the BSA that were oxidised by the $PM_{2.5}$: ETYGDMADCCEK (Met111), MPCTEDYLSLILNR (Met469) and TVMENFVAFVDK (Met571) (Figure S1, Supplementary Information). The estimated degrees of oxidation

Table 1
Estimated percentages of oxidation in the three albumin peptides from BSA and BALF for the control, and after treatment with 150 μg/ml of the CB, BJ, XA, XM and HK $PM_{2.5}$ samples. The estimated oxidation percentage was calculated based on the peak area [(peak area of oxidised peptide/total peak area of peptides with and without oxidation) × 100%].

Peptide sequence	Calculated MW	Observed *m/z* (2+)	Oxidation					
			Control	CB	BJ	XA	XM	HK
BSA								
ETYGDMADCCEK	1477.5	739.7	14.3%	20.4%	50.0%	33.0%	23.3%	19.1%
ETYGD**Mox**ADCCEK	1493.5	747.7						
MPCTEDYLSLILNR	1723.8	862.9	13.9%	13.2%	50.2%	42.3%	39.6%	34.0%
MoxPCTEDYLSLILNR	1739.8	870.9						
TVMENFVAFVDK	1398.7	700.3	10.3%	8.4%	33.4%	26.9%	18.0%	17.2%
TV**Mox**ENFVAFVDK	1414.7	708.3						
BALF								
ENPTTFMGHYLHEVAR	1900.8	639.9	N.D.	–	70.6%	54.8%	–	50.0%
ENPTTF**Mox**GHYLHEVAR	1916.8	634.6						
AHCLSEVEHDTMPADLPAIAADFVEDQEVCK	3496.5	1171.8	N.D.	–	2.9%	2.9%	–	1.3%
AHCLSEVEHDT**Mox**PADLPAIAADFVEDQEVCK	3512.5	1166.5						
TVMDDFAQFLDTCCK	1849.7	933.8	N.D.	–	28.6%	23.1%	–	14.6%
TV**Mox**DDFAQFLDTCCK	1865.7	925.8						

MW: molecular weight; N.D.: not detected.

of the three peptides for the BSA control, CB-treated and the $PM_{2.5}$-treated samples are shown in Table 1. There were no significant differences in oxidation between the BSA control and the CB pellet samples for the three peptides, but CB caused 1.4-fold higher ETYGDMADCCEK oxidation than the control. The BJ sample induced the highest oxidation of the three BSA peptides, ranging between 33.4% and 50.2%, whereas the HK samples caused the lowest oxidation of the three BSA peptides, ranging between 17.2% and 34.0%. The XA and XM samples also induced significant oxidation of the three peptides; 26.9–42.3% and 18.0–39.6%, respectively.

3.4. BALF albumin oxidation

To further investigate the modifications of albumin due to the interaction with $PM_{2.5}$ in the lung environment, BALF samples from $PM_{2.5}$-exposed mice were collected and analysed using LC–MS and the Mascot database. Three peptides of BALF albumin were oxidised by $PM_{2.5}$ (Fig. S2, Supplementary Information): ENPTTFMGHYLHEVAR (Met159), AHCLSEVEHDTMPADLPAIAADFVEDQEVCK (Met322) and TVMDDFAQFLDTCCK (Met572). There was no oxidation observed in the BALF control (Table 1). Significant oxidation of the three peptides from BALF albumin was observed in the BJ samples (2.9–70.6%), XA samples (2.9–54.8%) and HK samples (1.3–50.0%).

3.5. Associations of BSA peptide oxidation with oxidative stress and PAHs in the $PM_{2.5}$

The correlations between the oxidation of the three peptides form BSA with oxidative stress in the cell model were evaluated using Pearson's correlation test. The oxidation of the three-peptide was positively correlated with oxidative stress (Fig. 3a), resulting in R^2 values of 0.81 for ETYGDMADCCEK ($p < 0.05$), 0.90 for MPCTEDYLSLILNR ($p < 0.05$) and 0.94 for TVMENFVAFVDK ($p < 0.05$). The potential associations of the three-peptide oxidation of BSA with the PAHs (Fig. 3b) were further investigated. We observed a trend for an association of the PAHs with the oxidation of the three peptides from BSA. Thus, the oxidation of the three peptides from BSA could be positively associated with PAH contents of the $PM_{2.5}$ samples.

4. Discussion and conclusions

Air pollution is a major problem in areas affected by rapid population growth and economic development, and long-term exposure to $PM_{2.5}$ has been suggested to be a risk factor for cardiopulmonary diseases. However, the mechanisms underlying the health effects of exposure to high levels of $PM_{2.5}$, such as those encountered during haze episodes, remain unclear. The major findings of the present study are that exposure to the $PM_{2.5}$ collected from the severe haze-affected cities caused higher

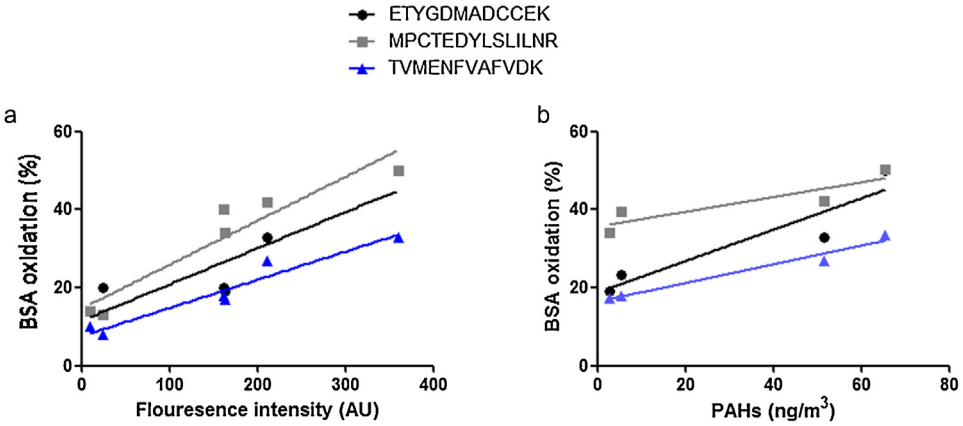

Fig. 3. Correlations between oxidation of the three peptides in BSA with (a) oxidative stress and (b) bulk PAHs in $PM_{2.5}$. The correlations were calculated using Pearson's correlation coefficients.

oxidative stress levels. The haze $PM_{2.5}$ resulted in significant oxidation of methionine residues of BSA *in vitro* and BALF albumin *in vivo*, especially in the peptides ETYGDMADCCEK (Met111), MPCTEDYLSLILNR (Met469) and TVMENFVAFVDK (Met571) for BSA and ENPTTFMGHYLHEVAR (Met159), AHCLSEVEHDTMPADLPAIAADFVEDQEVCK (Met322) and TVMDDFAQFLDTCCK (Met572) for BALF albumin. The methionine oxidation was associated with $PM_{2.5}$-driven oxidative stress; this observation could have resulted from the presence of PAHs in the $PM_{2.5}$. Our study provides an explanation the health effects at the macromolecular level.

To understand the biologically relevant differences in the $PM_{2.5}$ during haze episodes in China, we collected $PM_{2.5}$ from two severely haze-affected inland cities, BJ and XM, and from the two less haze-affected coastal cities, XM and HK. During the haze episodes, the daily average $PM_{2.5}$ mass concentrations in BJ and XA were 8.8-fold and 9.4-fold higher than the WHO $PM_{2.5}$ daily average (25 $\mu g/m^3$) [22], respectively, whereas the daily average $PM_{2.5}$ mass concentrations in XM and HK were 2.0-fold and 1.6-fold higher than the WHO $PM_{2.5}$ daily average, respectively. The comparison shows that there could be a crucial health impact on pulmonary function due to exposure to the haze $PM_{2.5}$. A previous haze study reported that the $PM_{2.5}$ mass concentrations during the haze episodes in BJ in 2004 ranged between 206 and 242 $\mu g/m^3$ [1]. Shao and coworkers (2013) showed that the haze $PM_{1.8}$ (less than 1.8 μm in aerodynamic diameter) collected in BJ between 2010 and 2011 ranged between 118 and 379 $\mu g/m^3$ [23]. These previous observations are in line with our findings and suggest that $PM_{2.5}$ was the major fraction of particles in haze episodes [23]. Exposure to such high $PM_{2.5}$ concentrations during the haze episodes could play an important role in inducing acute lung injury resulting from the particle physicochemical characteristics, particularly the associated PAHs [24]. However, few studies have profiled the PAHs in haze $PM_{2.5}$. We found the predominant PAHs in the BJ samples (which had the highest concentration of PAHs) were phenanthrene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b + k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene and benzo(g, h, i)perylene, suggesting that four- and five-ring PAHs were the major PAH compounds in the haze $PM_{2.5}$. The total PAH concentrations in BJ and XA $PM_{2.5}$ were 64.7 and 51.0 ng/m^3, respectively. Consistent with these observations, Tan and colleagues (2011) found 59.8 ng/m^3 of PAHs in $PM_{2.5}$ during haze episodes [25]. The abundant PAHs determined by Tan et al. (2011) were similar to our study, which suggested the haze $PM_{2.5}$ was dominated by the five-ring PAHs. Our previous study has indicated that four- to five-ring PAHs in vehicle-emitted $PM_{2.5}$ have higher toxic potencies [26]; however, the bioreactivity of haze $PM_{2.5}$ remains unclear.

Oxidative stress is recognised to be an important mechanism for particle toxicity. Oxidative stress was induced after exposure to the haze $PM_{2.5}$. This finding is consistent with a previous report [27], in which an alteration in glutathione in A549 cells was observed after exposure to haze $PM_{2.5}$. Notably, at equal concentrations (150 $\mu g/ml$), the oxidative potency of the BJ $PM_{2.5}$ was 2.2-fold higher than that of the HK samples. It is well known that protein can be oxidised, including carbonyl oxidation, by inhaled $PM_{2.5}$ due to its physicochemistry [28]. Particles with more ROS on their surfaces may be able to interact with the protein molecules, leading to modifications of the protein structure. However, we know little concerning the effects of interactions of $PM_{2.5}$ with protein, particularly $PM_{2.5}$ from haze episodes. We observed methionine adducts on three peptides induced by the haze $PM_{2.5}$; the results showed that the ETYGDMADCCEK (Met111), MPCTEDYLSLILNR (Met469) and TVMENFVAFVDK (Met571) peptides of BSA were significantly oxidised by haze $PM_{2.5}$. The haze $PM_{2.5}$ collected from BJ, especially at 150 $\mu g/ml$, caused more than 33.4% oxidation of the three peptides. This represented more than 1.5-fold higher oxidative potency than that of the HK $PM_{2.5}$. These results are consistent with the observation that the three methionine-containing peptides of the BALF albumin from the mice, ENPTTFMGHYLHEVAR (Met159), AHCLSEVEHDTMPADLPAIAADFVEDQEVCK (Met322) and TVMDDFAQFLDTCCK (Met572), were oxidised after exposure to the haze $PM_{2.5}$. Consistent with these results, Guedes et al. (2009) investigated the oxidation in BSA and demonstrated that metals catalysed the oxidation of MPCTEDYLSLILNR and TVMENFVAFVDK [29]. The BJ $PM_{2.5}$ caused more than 1.4-fold higher peptide oxidation than the HK $PM_{2.5}$. The high oxidative potency of the haze $PM_{2.5}$ was correlated with the peptide oxidation. Albumin is an important antioxidant that provides ligand-binding properties for endogenous and exogenous compounds. The two sulphur-containing residues in this serum protein, methionine and cysteine, account for 40–80% of the total antioxidant activity of the protein, which in turn is responsible for more than 70% of the ROS trapping activity [30]. Methionine and cysteine residues are particularly sensitive to ROS, being converted to disulphide and methionine sulphoxide residues, respectively [31]. The ligand sites could provide a platform to interact with ROS [8,10]. Another aspect of albumin is its capacity to bind homocysteine, a sulphur-containing amino acid resulting from the catabolism of methionine residues [9]. In this study, we identified three methionine sites that were sensitive to the haze $PM_{2.5}$ in BSA and BALF albumin. Methionine residues in proteins interact with ROS to form methionine sulphoxide, thus scavenging the ROS [8]. The methionine sulphoxide reductases can catalyse a thioredoxin-dependent reduction of methionine sulphoxide back to methionine [8,32]. Thus, the modification of protein methionines by the haze $PM_{2.5}$ could be reversed in biological systems, but there is a possibility that the methionine sulphone produced by the haze $PM_{2.5}$ is irreversible. The oxidation of methionine in peptides is often associated with a loss of biological activity. The sulphone was more active than the sulphoxide, although methionine was the most active, indicating that the anti-inflammatory activity is not correlated with the oxidation state of sulphur. We observed that three methionine residues in the protein, BSA and BALF albumin, were oxidised by the haze $PM_{2.5}$. Notably, methionine sulphoxide-containing peptides were not enriched before analysis in the current method. It may be expected that the peptides from the *in vitro*/*in vivo* oxidation were not recognized during the analytical processes [33]. The corresponding peptides for the three methionines in BSA and BALF oxidised by the haze $PM_{2.5}$ are the most abundant, but additional oxidized derivatives are possible. We suggest that the three methionine sites that are sensitive to haze $PM_{2.5}$ exposure could be related to inflammatory mechanisms [34]; however, further confirmatory studies are required.

PAHs represent a complex mixture of chemicals, some of which have been recognised as cytotoxic and carcinogenic in humans [35]. Tan et al. (2011) reported that the $PM_{2.5}$ from the haze episodes contained consisted of significant amounts of PAHs [25]. Inhaled PAHs are converted to their hydroxyl derivatives by cytochrome P450, epoxide hydrolase and dihydrodiol dehydrogenase [36], leading to increases in ROS [37]. Taken together, these observations suggested that the haze $PM_{2.5}$-associated PAHs could play an important role in the regulation of protein oxidation. Indeed, epidemiological studies have showed an association between albumin adducts and PAH exposure in worker populations [38,39]. Our study provides further information that methionine could be sensitive to the PAH-driven ROS in the haze $PM_{2.5}$. Our findings are supported by a previous study that found that albumin was significantly oxidised by naphthalene exposure [40], that the oxidation of PAHs was associated with their redox states, and that methionine acted to scavenge the ROS production [41]. Additionally, benzo(a)pyrene can be converted by enzymatic metabolism to the final mutagen benzo(a)pyrene diol epoxide, which binds covalently to albumin

to produce protein adducts [42,43]. The mechanisms underlying the interactions between the three peptides and PAHs need further investigation.

Increasing evidence shows that the $PM_{2.5}$ in haze episodes can cause deleterious human health effects; however, a few references are available regarding the mechanisms underlying the interaction of particles with biomolecules. This was the basis for describing biological activity in response to particulate air pollution. In this study, we have characterised the $PM_{2.5}$ and PAH contents of samples collected from four cities in China during the haze episodes. Furthermore, we determined the interactions between albumins and haze $PM_{2.5}$ using *in vitro* and *in vivo* approaches. This study reveals that exposure to the haze $PM_{2.5}$ caused methionine oxidation in the albumins, which could be attributed to the PAH compounds. The oxidation changes of albumin could lead to diverse functional consequences, such as inhibition of enzymatic and binding activities, increased susceptibility to aggregation and proteolysis and increased or decreased uptake by cells. Our findings provide a broader understanding of protein interactions with the haze $PM_{2.5}$ *in vitro* and *in vivo* and provide knowledge of the fate of the particles at the molecular level.

Authors' contributions

All authors have contributed substantially to the concept and design of the study, drafting of the article, and critically revising the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript for publication.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2014.04.029.

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