Metabolomics analysis reveals that benzo[a]pyrene, a component of PM$_{2.5}$, promotes pulmonary injury by modifying lipid metabolism in a phospholipase A2-dependent manner in vivo and in vitro

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ABSTRACT

Particulate matter with an aerodynamic diameter less than 2.5 $\mu$m (PM$_{2.5}$) is one of the major environmental pollutants in China. In this study, we carried out a metabolomics profile study on PM$_{2.5}$-induced inflammation. PM$_{2.5}$ from Beijing, China, was collected and given to rats through intra-tracheal instillation in vivo. Acute pulmonary injury were observed by pulmonary function assessment and H.E. staining. The lipid metabolic profile was also altered with increased phospholipid and sphingolipid metabolites in broncho-alveolar lavage fluid (BALF) after PM$_{2.5}$ instillation. Organic component analysis revealed that benzo[a]pyrene (BaP) is one of the most abundant and toxic components in the PM$_{2.5}$ collected on the fiber filter. In vitro, BaP was used to treat A549 cells, an alveolar type II cell line. BaP (4 $\mu$m, 24 h) induced inflammation in the cells. Metabolomics analysis revealed that BaP (4 $\mu$m, 6 h) treatment altered the cellular lipid metabolic profile with increased phospholipid metabolites and reduced sphingolipid metabolites and free fatty acids (FFAs). The proportion of $\omega$-3 polyunsaturated fatty acid (PUFA) was also decreased. Mechanically, BaP (4 $\mu$m) increased the phospholipase A2 (PLA2) activity at 4 h as well as the mRNA level of Pla2g2a at 12 h. The pro-inflammatory effect of BaP was reversed by the cytosolic PLA2 (cPLA2) inhibitor and chelator of intracellular Ca$^{2+}$. This study revealed that BaP, as a component of PM$_{2.5}$, induces pulmonary injury by activating PLA2 and elevating lysophosphatidylcholine (LPC) in a Ca$^{2+}$-dependent manner in the alveolar type II cells.

1. Introduction

With rapid industrialization, haze-fog-associated air pollution, characterized by a high level of particulate matter (PM), has become one of the most severe environmental issues in China [1]. The detrimental effect of PM is mainly determined by its size and composition. Due to its small diameter and large surface area, PM with an aerodynamic diameter less than 2.5 $\mu$m (PM$_{2.5}$) carries various toxic components and pathogens into the body and causes harm [2]. Many studies have indicated that there is a close association between mortality and PM$_{2.5}$ level, especially respiratory and cardiovascular mortality [3–6]. As the site for gas exchange and a barrier from the external environment, the respiratory system is exposed directly to high levels of PM$_{2.5}$. PM$_{2.5}$ has been proven to increase the incidence of respiratory diseases, including pneumonia, asthma, chronic obstructive pulmonary disease (COPD) and lung cancer [7,8]. However, the mechanisms of PM$_{2.5}$-induced pulmonary injury is not fully understood.

The lung is characterized by the presence of a complex pattern of lipids, including phospholipids, cholesterol and sphingolipids, which play an important role in multiple physiologic and pathophysiologic processes. Phospholipids are the major composition of pulmonary surfactant, reducing the surface tension of the alveolus [9]. Lysophospholipids and oxidative phospholipids in the lung are increased in pulmonary inflammation and participate in the pathogenesis of pneumonia, asthma and lung fibrosis [10–12]. Cholesterol promotes the spreading, mobility and adsorption of pulmonary surfactant, but excess cholesterol impairs the surface tension reducing function [13,14]. Additionally, an increased number of cholesterol-overloaded macrophages...
has been observed in pneumonia, lung fibrosis and lung cancer, promoting the onset of these diseases [10]. Ceramide (Cer), a sphingolipid metabolite, induces the apoptosis of alveolar epithelial cells and the formation of the alveolus, while sphingosine-1-phosphate (S1P) maintains the survival of cells, which are important for the structural and functional development of the lung [15]. The imbalance of Cer and S1P leads to alveolar enlargement or fibrosis and is involved in the onset of COPD, asthma, lung fibrosis and lung cancer [16]. Although a phospholipid metabolism profile of pulmonary tissue after PM2.5 exposure has been carried out [17], the global lipid metabolism change induced by PM2.5 is largely unknown.

In this study, PM2.5 was given to rats through intra-tracheal instillation in vivo, and metabolomics were utilized to delineate the lipid metabolic profile in broncho-alveolar lavage fluid (BALF). PM2.5 activated the metabolism of phospholipids and sphingolipids. Next, benzo[a]pyrene (BaP), a compound belonging to polycyclic aromatic hydrocarbon (PAH), was found to be enriched in PM2.5. A human alveolar type II cell line, A549, was employed and treated with BaP. BaP induced inflammation in A549 cells. The metabolomics results revealed that BaP promoted the metabolism of phospholipids, sphingolipids and free fatty acids (FFAs), indicating the increase in phospholipase A2 (PLA2) activity. Mechanically, BaP increased the activity of PLA2 in a Ca²⁺-dependent manner, mediating inflammation.
2. Materials and methods

2.1. Reagents

The anti-NOS2 antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The anti-β-actin antibody was purchased from Cell Signaling Technology (Boston, MA, USA). BaP was purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA). The phospholipase A2 assay kit was purchased from Thermo Fisher Scientific (Waltham, MA, USA).

2.2. PM$_{2.5}$ sampling and preparation

PM$_{2.5}$ samples were collected in March 2015 in Beijing, China for use in the present study. The PM$_{2.5}$ samples were collected onto fiber filters using a particulate sampler. The filters were weighed and cut into squares of 1–2 cm$^2$. The particulate matter in the samples was removed by agitation in ultrapure water with an ultrasonic shaker. The solution was frozen and dried using a vacuum freeze dryer. The dried PM$_{2.5}$ was weighed and kept at −20 °C before being diluted for the experiments.

2.3. Scanning electron microscopy

Size distribution of PM was measured by a field emission environmental scanning electron microscopy. Particle size was determined by Image-J software. Scanning electron microscopy was digitized and particle area measurements were made. The equivalent spherical diameter (ESD), a commonly used parameter for particle sizing, was calculated using the formula: ESD = 2 × (area / π$^{1/2}$) [18].

2.4. Animals

Male Wistar rats (180–220 g) were housed in a temperature-controlled room (22 °C) with a 12-h light/dark cycle and free access to laboratory chow and tap water. All of the animal protocols followed the “National Institutes of Health guide for the care and use of laboratory animals” and were approved by the Animal Care and Use Committee of Peking University.

2.5. Broncho-alveolar PM$_{2.5}$ instillation and lavage

Before the experiments, PM$_{2.5}$ was diluted in sterile saline at a concentration of 15 mg/ml. The rats were anesthetized and were instilled with sterile saline or PM$_{2.5}$ at a volume of 3 ml/kg into the bronchus on the first day. The intra-tracheal instillation was repeated another two times on the 3rd and 6th days. Twenty-four hours after the last intra-tracheal instillation, the rats were anesthetized. The pulmonary functional variables were then measured by flexiVent (SCIREQ, Montreal, QC, Canada). The rats were sacrificed; immediately after death, the left lung was ligated, and the trachea to the left lung was cannulated. The right lung was douchcd with 3 ml sterile saline for
The lavage fluid was centrifuged and stored at −80 °C.

2.6. Histologic assessment

The lung were harvested and fixed in buffered formaldehyde after lavage. The fixed lung were embedded in paraffin and performed the hematoxylin and eosin (H.E.) staining for light microscopy. The infiltration of polymorphonuclear neutrophils (PMNs) were counted. The lung injury score was calculated on high-power field as described[19].

2.7. Sample preparation for metabolomics analysis

To prepare BALF, 200 μl of BALF was extracted by 4-fold cold chloroform/methanol (2/1) containing Cer (19:0) (5 nM) as the lipid metabolite internal standard. For the cells, the pellets were resuspended in 100 μl of saline and were extracted in the same way as BALF. The extractions were vortexed and centrifuged. The lower organic phase and upper hydrophilic phase were collected and evaporated, respectively. The organic residue was dissolved in 50 μl of chloroform/methanol (1/1). The samples were centrifuged at 18,000 rpm for 20 min, and 5 μl was injected into the ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) system.

2.8. Metabolomics detection by UPLC-MS/MS

Metabolites were identified and quantified by a UPLC (Waters, Milford, MA, USA) interfaced to a 5500 QTRAP hybrid triplequadrupole linear ion-trap MS (AB Sciex, Foster City, CA, USA) outfitted with a turbo ion-spray ionization source.

Hydrophilic analytes were separated using a Waters Amide XBridge HPLC column (3.5 µm; 4.6 mm inner diameter * 100 mm length) (Waters, Milford, MA, USA) maintained at 30 °C. The mobile phase was water containing 5 mM ammonium acetate (A) and methanol (B). The gradient was as follows: 0–0.5 min 20% B, 0.5–1.5 min 20–40% B, 3 min 60% B, 13 min 98% B, and 19–23 min 90% B. The flow rate was 0.5 ml/min.

Lipid analytes were separated using a Waters ACQUITY UPLC CSH C18 Column (130 Å, 1.7 µm, 2.1 mm × 100 mm, 1/μgCSH) (Waters, Milford, MA, USA) maintained at 55 °C. Mobile phase was methanol/water/acetonitrile of 1/1/1 containing 5 mM ammonium acetate (A) and isopropanol (B). The gradient was as follows: 0–0.5 min 20% B, 0.5–1.5 min 20–40% B, 3 min 60% B, 13 min 98% B, and 13.2–17 min 20% B. The flow rate was 0.3 ml/min.

The response curves were calculated using MultiQuant software. Principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA), variable importance for the projection (VIP) score,
unsupervised hierarchical clustering analysis, scatter plots and heatmap generation were carried out using MetaboAnalyst 3.0 (http://www.metaboanalyst.ca/).

2.9. Cytometric bead array

The supernatant inflammatory cytokine levels were investigated using a cytometric bead array inflammation kit (BD Biosciences, San Jose, CA, USA) and were analyzed by BD FACScalibur (BD Biosciences, San Jose, CA, USA) with Cell QuestPro software.

2.10. Measurement of the free intracellular Ca2+ concentration

The A549 cells were cultured in 96-well plates and were washed once with phosphate-buffered saline. Next, Hank’s balanced salt solution (HBSS) with Fluor3-AM (Bio-Rad Laboratories, Hercules, CA, USA) was added to the cells and was incubated for 30 min at 37 °C in a 5% CO2 atmosphere. The cells were washed once with HBSS, which was replaced with HBSS containing DMSO or BaP. The fluorescence of Ca2+/Fluo3 was monitored over time. Fmax was measured with Fluor3-AM-preloaded cells treated with 0.1% Triton X-100, and Fmin was measured with Fluor3-AM-preloaded cell-free wells. [Ca2+] was calculated using the formula: [Ca2+] = Kd × (F-Fmin) / (Fmax-F). The dissociation constant (Kd) of Fluor3 was assumed to be 400 nM.

2.11. Western blot analysis

Proteins were subjected to SDS-PAGE with a 12% running gel and were then transferred to a polyvinylidene fluoride membrane. The membrane was incubated successively with 0.1% bovine serum albumin in Tris/Tween-20-buffered saline at room temperature for 1 h, with different antibodies at 4 °C for 12 h and then with second antibody for 1 h. The immunofluorescence bands were detected by the Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE, USA).

2.12. qPCR analysis

Total RNA was isolated using Trizol reagent (Promega, Madison, WI, USA). Total RNA (2 μg) was reverse transcribed using a reverse transcription system (Promega, Madison, WI, USA). Next, 4 μl of the reaction mixture was included in the PCR samples with the Mx3000 Multiplex Quantitative PCR System (Agilent, La Jolla, CA, USA). The amount of the PCR products formed in each cycle was evaluated using SYBR Green I fluorescence. The results were analyzed using the Stratagene Mx3000 software, and the target mRNA levels were normalized to the levels of β-actin.

2.13. Statistical analysis

The data were analyzed using GraphPad Prism software and were expressed as the means ± SEM. Unpaired Student’s t-test and one-way ANOVA with Tukey’s correction were used as appropriate. P < 0.05 was considered significant.

3. Results

3.1. PM2.5 physical characteristics

The morphology of PM, isolated from the fiber filters, was firstly analyzed by scanning electron microscopy. The PM displayed various size and shape (Fig. 1A). The mean ESD of the PM was 1.56 ± 0.04 μm and the ESD distribution of the PM was also shown (Fig. 1B). Over 80% of the PMs had an ESD less than 2.5 μM and the PMs that had an ESD over 2.5 μM might be due to the aggregation of small PMs. Therefore,
the PMs, used in the experiments, are PM$_{2.5}$.

3.2. Intra-tracheal instillation of PM$_{2.5}$ induces acute pulmonary injury

PM$_{2.5}$, collected from the fiber filters, was instilled to the broncho-alveolar system of Wistar rats. The instillation was performed on the 1st, 3rd, and 6th days 3 times in total. The body weight of the rats increased during the period of instillations and was not affected by PM$_{2.5}$ (45 mg/kg) (Supplementary Fig. 1A), revealing that the instillation did not cause systemic harm to the rats.

Twenty-four hours after the last instillation, the pulmonary function was examined. The inspiratory capacity (IC) and resistance of the respiratory system (Rrs) were not changed (Supplementary Fig. 1B, C). However, the elasticity of the respiratory system (Ers) was increased (Fig. 2A), and the compliance of the respiratory system (Crs) was decreased compared with those in the saline group (Fig. 2B). In addition, the area of the Pressure-Volume (P-V) loop was reduced after the instillation of PM$_{2.5}$ (Fig. 2C), suggesting the impaired pulmonary function.

Furthermore, the histological changes of the lung was examined by H.E. staining after instillation. The intra-tracheal administration of PM$_{2.5}$ thickened the alveolar wall (Fig. 2D: a, b) and induced the ebulbation and infiltration of PMNs (Fig. 2D: c, d), which was consistent with the changes in pulmonary function. The hilar lymph nodes were also enlarged after PM$_{2.5}$ instillation (Fig. 2D: e, f). Then, a combined lung injury score was generated and revealed an increased lung injury score in the PM$_{2.5}$ group (Fig. 2E). These results indicate that PM$_{2.5}$ induced acute pulmonary injury and inflammation in rats.

3.3. Intra-tracheal instillation of PM$_{2.5}$ altered lipid metabolism in the lung

To examine the PM$_{2.5}$-induced pulmonary metabolomics change, BALF from the rats receiving broncho-alveolar instillation was collected and was analyzed by UPLC-MS/MS. In general, 124 distinct lipid metabolites were identified and 33 of these metabolites were changed significantly ($P < 0.05$, fold change (FC) > 2) (Supplementary Table 1).

The BALF from rats instilled with PM$_{2.5}$ displayed a different lipid metabolic profile from that of the saline instilled rats, according to the PCA plot (Fig. 3A). The heatmap also showed a clustering of lipid metabolites between the PM$_{2.5}$ and saline groups (Fig. 3B). The PLS-DA plot and VIP score plot revealed that the phospholipid metabolism: lysophosphatidylcholine (LPC) (18:0, 18:1, 22:0, 24:0) and phosphatidylcholine (PC) (16:0, 17:0, 18:0) and the sphingolipid metabolism: sphingomyelin (SM) (17:0, 20:0, 20:4, 24:0), dihydrosphingomyelin (DHSM) (24:0), and sulfatide (12:0) had higher VIP values (Fig. 3C, D).

3.4. Intra-tracheal instillation of PM$_{2.5}$ activates phospholipid and sphingolipid metabolism in the lung

Next, metabolite changes in phospholipids and sphingolipids were examined. The levels of phospholipid metabolites, including LPC (11:0, 14:0, 15:0, 18:0, 18:1, 22:0, 24:0) (Fig. 4A) and PC (32:0, 34:0, 36:0)
were increased in the BALF after the instillation of PM2.5. Additionally, the levels of sphingolipid metabolites, including Cer (18:0, 20:0, 22:0, 24:0, 24:1, 25:0) (Fig. 4C), glucosylceramide (GlcCer) (24:1), ceramide-1-phosphate (C1P) (Fig. 4D), SM (14:0, 17:0, 20:0, 20:4, 22:0, 24:0, 24:1) (Fig. 4E), DHSM (24:0), dihydroceramide (DHCer) (24:0), dihydroglucosylceramide (DHGlcCer) (16:0, 18:0, 24:0), dihydroceramide-1-phosphate (DHC1P) (16:0) (Fig. 4F), sulfatide (12:0, 16:0) (Fig. 4G) and sphinganine (Sa) (17:0) (Fig. 4H), were increased in the BALF of PM2.5 treated rats. However, the levels of Cer (16:0) (Fig. 4C), lactosylceramide (LacCer) (16:0) (Fig. 4D) and SM (18:0, 18:1) (Fig. 4E) were decreased in the PM2.5 group. These results suggest an increased metabolism of phospholipids and sphingolipids in response to PM2.5 instillation in the rat lung.

A series of results showed that both phospholipids and sphingolipids played an important role in pulmonary inflammation. Bioactive LPC levels were increased at the sites of inflammation, promoting chemotaxis, adherence, differentiation, activation and cytokine secretion of immune cells in the lung [11]. Cer was generated through the
upregulation of sphingomyelinase and promoted apoptosis, cytokine secretion, and decreased endothelial barrier integrity in the lung [16]. As a result, the activation of phospholipid and sphingolipid metabolism may participate in PM2.5-induced pulmonary inflammation and injury.

3.5. BaP promotes the inflammation in alveolar type II cells

To determine the exact component that is responsible for inflammation and pulmonary injury, PM2.5 was extracted from fiber filters, and the organic components were examined. PAHs were the most toxic components and were relatively enriched in PM2.5 (Fig. 5A: a). Further analysis revealed that 4-ring and 5-ring PAHs occupied the most abundant components in 4-ring PAHs (Fig. 4A: c) and was the most toxic compound among the 4-ring and 5-ring PAHs [20]. Therefore, BaP was employed to treat a human alveolar type II cell line, A549, in vitro.

The pro-inflammatory effect of BaP was examined first in A549 cells. BaP (4 μM) treatment for 24 h increased the supernatant level of IL8, a pro-inflammatory chemokine (Fig. 5B), while the levels of IL12, TNFα, IL10 and IL1β remained unchanged (Fig. 5B). The expression of NO synthase 2 (NOS2) was increased after pro-inflammatory stimulus treatment, generating NO to mediate inflammation [21]. BaP (2–8 μM) upregulated the expression of NOS2 in a time- and dose-dependent manner (Fig. 5C, D). These results indicate that BaP induced inflammation in alveolar type II cells.

3.6. BaP alters sphingolipid and FFA metabolism in alveolar type II cells

To investigate whether BaP altered lipid metabolism, A549 cells were treated with BaP (4 μM) for 6 h and underwent UPLC-MS/MS. In general, 79 distinct lipid metabolites were identified, and 25 of these metabolites were changed significantly (P < 0.05, FC > 1.5) (Supplementary Table 2).

The PCA plot displayed a disparate lipid metabolic profile in A549 cells after BaP treatment (Fig. 6A). Heatmap and cluster analysis witnessed good differentiation in metabolomics, clustering in A549 cells with or without BaP treatment (Fig. 6B). The PLS-DA and VIP score plots were further applied to choose the important variables. The score showed the change in the metabolic profile converged largely on sphingolipid metabolism: Sa (18:0), Cer (16:0, 18:0, 22:0, 24:0), sulfatide (So) (17:1), LacCer (24:0), sulfatide (12:0) and FFA (16:0, 18:0, 20:0, 20:4, 20:5, 22:6) (Fig. 6C, D).

3.7. BaP activates sphingolipid, FFA and phospholipid metabolism in alveolar type II cells

BaP decreased the levels of Cer (16:0, 18:0, 20:0, 22:0, 24:0, 24:1, 25:0) (Fig. 7A), SM (16:0, 18:0, 18:1, 20:0, 22:0, 24:0) (Fig. 7C), DSHM (18:0) (Fig. 7D), sulfatide (Fig. 7E), So (17:1) and Sa (17:0, 18:0) (Fig. 7F), but BaP increased the levels of GlcCer (24:1), DHSM (24:0), DHcer (24:1), DHGlcCer (16:0, 18:0) and DHClP (24:0) in A549 cells (Fig. 7F); these results partially contrast with the results from the in vivo experiments.

The levels of FFA were examined. BaP treatment decreased the levels of different species of FFA (12:0, 14:0, 16:0, 18:0, 18:1, 19:0, 20:0, 20:1, 20:4, 20:5, 22:0, and 22:6) in A549 cells (Fig. 7G), especially the proportion of polysaturated fatty acid (PUFA) (Fig. 7H). Within the different species of PUFA, the ratio of ω-3/ω-6 PUFA was also decreased (Fig. 7I), suggesting that the anti-inflammatory effect driven by...
ω-3 PUFA was decreased after BaP treatment.

Phospholipids are the major component of pulmonary surfactant secreted by alveolar type II cells and are involved in pulmonary inflammation [22]. In addition, there was a marked change in phospholipids in the BALF of rats after PM2.5 instillation in vivo (Fig. 3D). Therefore, the change in phospholipids after BaP treatment was also examined. The levels of LPC (9:0, 14:0, 15:0, 17:0, 18:0, 18:1, and 20:0) were increased after BaP treatment in A549 cells (Fig. 7J), which was in line with the results from the in vivo experiments. However, the levels of PC (32:0, 34:0, and 36:0) were decreased (Fig. 7K), suggesting the dysfunction of pulmonary surfactant generation. The hydrolysis of PC to LPC depends on phospholipase A2 (PLA2) [23]. BaP treatment markedly decreased the ratio of LPC/PC (Fig. 7L), suggesting the increased activity of PLA2 in these cells.

3.8. The activation of PLA2 mediates BaP-induced inflammation in a Ca²⁺-dependent manner

PLA2 catalyzes the hydrolysis of the sn-2 position of glycerophospholipid to liberate lysophospholipid and FFA [11]. To determine whether PLA2 was involved in BaP-induced inflammation in A549 cells, PLA2 activity was first examined. BaP increased the activity of total PLA2, as early as 4 h, in a time-dependent manner, in A549 cells (Fig. 8A). PLA2 was mainly classified into three families: cytosolic PLA2 (cPLA2), Ca²⁺-independent PLA2 (iPLA2) and secretory PLA2 (sPLA2) [24]. Different inhibitors were added to A549 cells to distinguish different subtypes of PLA2. The BaP-induced secretion of IL8 was partially reversed by p-bromophenacyl bromide (PBB), a general PLA2 inhibitor and methyl arachidonil fluorophosphonate (MAPF), a cPLA2 and iPLA2 inhibitor (Fig. 8B). Varespladib (VPLB), a sPLA2 inhibitor, had only a mild inhibitory effect on IL8 secretion and was not statistically significant (Fig. 7B), indicating that cPLA2 or iPLA2 contributed most to the inflammation induced by BaP in the A549 cells.

Next, the expression of PLA2 in A549 cells was further examined. BaP upregulated the mRNA level of Pla2g2a, a sPLA2 gene. However, the expression of Pla2g4a and Pla2g10 remained unchanged (Fig. 8C). In addition, the upregulation of Pla2g2a occurred posterior to increased PLA2 activity (Supplementary Fig. 2A-C), suggesting that the increased PLA2 activity was due to the activation, not the expression, of PLA2. The activation of cPLA2 depends on Ca²⁺. BaP was found to increase intracellular Ca²⁺ in A549 cells (Fig. 8D). To determine whether the BaP-induced activation of PLA2 depended on Ca²⁺, different Ca²⁺ chelators were added to A549 cells. The BaP-induced IL8 secretion was partially inhibited by BAPTA-AM, an intracellular Ca²⁺ chelator, and EGTA, an extracellular Ca²⁺ chelator (Fig. 8E), suggesting that BaP induces inflammation by increasing extracellular Ca²⁺ influx in A549 cells.

4. Discussion

In this study, metabolomics was employed to examine the lipid profile of PM2.5-induced pulmonary injury. PM2.5 was given to rats through intra-tracheal instillation in vivo. PM2.5 impaired pulmonary function and induced pulmonary inflammation. Metabolomics analysis revealed increased metabolism of phospholipids and sphingolipids in the BALF of PM2.5-treated rats. BaP was one of the most abundant pollutants in PM2.5 particles. To explore the mechanisms, A549 cells, a human alveolar type II cell line, were employed and treated with BaP. BaP promoted inflammation in A549 cells and activated the metabolism of phospholipids, sphingolipids and FFAs. BaP increased the activity of PLA2. BaP-induced inflammation was reversed by general PLA2 and cPLA2 inhibitors. BaP mainly activated cPLA2 and promoted LPC generation by elevating intracellular Ca²⁺, promoting inflammation.

Previous studies have reported that acute exposure to low dose PM2.5 induces inflammation, oxidative stress and impairment of pulmonary function [25,26]. In consistent with previous studies, intra-tracheal instillation of PM2.5 induced acute pulmonary injury, indicated by higher lung injury score. The increased infiltration of neutrophils into the interstitium was observed after PM2.5 instillation, which is considered to play a vital role in the acute pulmonary injury [27]. PM2.5 has been reported to induce the production of reactive oxygen species in neutrophils of asthmatic patients, which is involved in neutrophil activation and pulmonary injury [28]. The expression of adhesion molecules in endothelium is also up regulated after treatment of PM2.5 [29,30], facilitating the migration of neutrophils into the lung.

PM2.5 promotes the expression and secretion of IL8 [31], which is a powerful chemokines to neutrophils and has been shown to play an important role in the onset of airway inflammation [32]. BaP increases the secretion of pro-inflammatory cytokines, including IL8, inducing pulmonary inflammation [33,36]. In accordance with previous studies, the secretion of IL8 was increased in A549 cells by BaP. As BaP is one of the components of PM2.5, BaP-induced IL8 secretion may be involved in the infiltration of neutrophils in the lung after PM2.5 instillation.

The detrimental effect of PM2.5 on the body is closely associated with lipid metabolism. Long-term PM2.5 exposure has been shown to accelerate the pathogenesis of lipid-associated metabolic disease, including atherosclerosis and type II diabetes [37,38], by inducing dyslipidemia [39,40], vascular inflammation [37], adipose dysfunction [41,42] and insulin resistance [38]. The lipid metabolic profile of plasma has been delineated after the exposure of PM2.5 [43,44]. However, as the first barrier following exposure to a high level of PM2.5, pulmonary global lipid metabolomics has not been carefully studied. In this study, the metabolomics of both BALF from rats instilled with PM2.5 in vivo and A549 cells treated with BaP were examined.

The metabolomics profile revealed that PM2.5 instillation mainly increased LPC and PC levels in BALF. However, another study found that PM2.5 decreased the levels of these metabolites in pulmonary tissue [17]. In that study, PM2.5 was given to rats through ambient air for 8 months and histopathology revealed emphysema [17]; however, in our study, PM2.5 was given to rats through acute instillation, and histopathology only exhibited edema and infiltration of PMNs in the alveolar septum, which is a different stage of PM2.5-induced pulmonary injury. In the early stage of pulmonary injury, the elevation of LPC and PC levels was a response to PM2.5 stimulation. In the late stage, however, the LPC and PC levels might be decreased due to compensation. This contradiction displayed a dynamic change in the pulmonary lipid profile in different stages of PM2.5-induced pulmonary injury.

In the lung, the alveolar epithelium consists of type I and type II epithelial cells. Alveolar type II cells occupy about 4% of the alveolar surface but play an important role in maintaining the normal structure and function of the alveolus. Alveolar type II cells can trans-differentiate into alveolar type I cells to repair the alveolus and secrete cytokines to induce pulmonary inflammation [22,45–47]. Additionally, alveolar type II cells synthesize and secrete pulmonary surfactant (90–95% phospholipid) to reduce the surface tension of the alveolus [22]. Previous results have suggested the participation of phospholipid and sphingolipid metabolites in the inflammation of alveolar type II cells [48–51]. Therefore, the A549 alveolar type II cell line was used.

The levels of PC and sphingolipid metabolites were increased in BALF after PM2.5 instillation in vivo but were decreased in A549 cells after BaP treatment in vitro. These findings may be due to the difference in stimulation that was used and involvement of other cells in vivo. The FFA levels were decreased after BaP treatment in A549 cells, a finding that seemed inconsistent with the increased PLA2 activity. This contradiction may be due to the increased catabolism of FFA in A549 cells. BaP and LPC upregulated the expression of cyclooxygenase-2 (COX-2) [52,53], which is responsible for the metabolism of ω-3 and ω-6 PUFA. In line with this, the proportion of PUFA was decreased after BaP treatment. ω-3 PUFA has been shown to have a markedly anti-inflammatory effect on different diseases by competing with the pro-inflammatory metabolism of the ω-6 PUFA cascade [54]. However, the ratio of ω-3/ω-6 PUFA was decreased after BaP treatment in these
cells, suggesting a weakened anti-inflammatory effect. In line with this, exogenous supplementation of ω-3 PUFA inhibited the inflammation induced by both PM2.5 and BaP in vivo and vitro [55,56].

Activation of both cPLA2 and sPLA2 was found to mediate BaP-induced inflammation in A549 cells, a finding that was consistent with previous results [57]. The activation of cPLA2 was dependent on intracellular Ca²⁺ [24], and BaP increased intracellular Ca²⁺ in an aryl hydrocarbon receptor (AHR)-dependent manner [58], a finding that was also observed in this study. PLA2g2a was up regulated by BaP. PLA2g2a is often referred to as an “inflammatory sPLA2” and was markedly up regulated by pro-inflammatory stimuli and was correlated with the severity of inflammatory diseases [24]. However, the upregulation of PLA2g2a was not responsible for the increased generation of LPC because its upregulation occurred posterior to the increased PLA2 activity [59]. There is cross-talk between sPLA2 and cPLA2, and the upregulation of PLA2g2a may be due to the activation of cPLA2 and amplification of the inflammation induced by BaP. The expression of PLA2g2a has been proven to be inducible by the activation of cPLA2 in a peroxisome proliferator-activated receptor α-dependent manner [60].

This study indicated the participation of lipid metabolism in PM2.5-induced pulmonary injury. BaP was further identified as one of the components of PM2.5 and activated lipid metabolism in alveolar type II cells. BaP induced inflammation in A549 cells by activating phospholipid metabolism in a PLA2-dependent manner, suggesting that PLA2 may be a putative drug target for improving PM2.5-induced pulmonary injury.

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Author contributions

SY.Z. and D.S. designed and performed the experiments and analyzed the data. H.L. performed the HPLC-MS/MS and analyzed the data. All of the authors approved the final manuscript. The authors declare no competing financial interests. W.X. and H.W. are the guarantors of this work and, as such, had full access to all of the data in the study and hold the responsibility for the integrity of the data and the accuracy of the analysis.

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The authors have no conflicts of interest to declare.

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