Use of a Mechanism-based Toxicological Approach to Analyze the Pulmonary Pro-Fibrogenic Effects of Air Pollution PM2.5 Particulates

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Abstract

Background Airborne fine particulate matter (PM_{2.5}) has been known capable of causing acute and chronic respiratory inflammation such as chronic obstructive pulmonary disease (COPD) and lung fibrosis. With incremental experience in the predictive toxicological approach on the potential adverse effects of nanomaterials, it is logical to explore the feasibility of using such a mechanism-based approach on PM_{2.5}-induced lung toxicity.

Results In this study, we collected a panel of eight PM_{2.5} samples for a comparative analysis of their pro-fibrogenic effects in the lung. These samples were collected on filters in November 2017 in Zhengzhou, Henan, China. In vitro screening shows although the PM_{2.5} particles did not induce significant cytotoxicity, they cause potent TNF- α and interleukin-1 β (IL-1 β) production in THP-1 cells as well as TGF- β 1 in BEAS-2B lung epithelial cells. PM_{2.5} induced IL-1 β production was shown to involve NLRP3 inflammasome activation, which was evidenced by the inhibited IL-1 β production in NLRP3- and ASC-deficient cells and use of an inhibitor for cathepsin B, a known activation of the inflammasome through lysosomal damage pathway. Administration of PM_{2.5} to the lung in mice via oropharyngeal aspiration confirmed that the particles could induce TGF- β 1 production in the bronchoalveolar lavage (BAL) fluid and collagen deposition in the lung at 21 days post-exposure, suggesting PM_{2.5} has the potential to induce pulmonary fibrosis. The ranking of the pro-fibrogenic effects by the PM_{2.5} samples in vivo was consistent with that of the IL-1 β production in vitro.

Conclusions In summary, we demonstrate that the PM_{2.5} is capable of inducing NLRP3 inflammasome activation and a series of cellular responses originating from the epithelial–mesenchymal trophic cell unit in the lung to induce fibrosis in vivo.

Background

Atmospheric aerosol particles or more commonly known as particulate matter (PM) are various microscopic suspensions of solid particles or liquid droplets found within the Earth's atmosphere. It is the main source of haze and is a component of human induced air pollution.[1, 2] While sources of particulates vary from natural origins like volcanoes to grassland fires, human activities such as burning fossil fuels in vehicles and power plants can also generate copious amounts of potentially harmful particulates, especially in urban areas.[3] Depending on the size of these particulates, they can gain access to different segment of the respiratory tracts and induce detrimental effects, which can lead to both pulmonary and cardiovascular diseases. Airborne fine particulate matter (PM_{2.5}, aerodynamic diameter < 2.5 μ m), as one of the main components of air pollution, has attracted extensive attention in the aspect of toxicological properties due to the fact that they have large surface areas and they can carry high amount of organic chemicals and heavy metals and travel deep inside the lung to cause adverse effects. Once inside the lung, although some can be cleared by the mucociliary escalator, most will be phagocytosed by lung macrophages to generate pro-inflammatory cytokines and induce lung and systemic inflammation. PM_{2.5} can cause minor health complications such as irritation or coughing, or

more severe health problems such as heart disease, chronic bronchitis, chronic obstructive pulmonary disease (COPD), lung fibrosis and worsening of respiratory problems like asthma.

Particulate matter especially PM_{2.5} has been shown to be able to induce lung inflammation and oxidative stress is generally considered the common mechanism, which can induce a tiered oxidative stress responses in cells and tissues. The hierarchical response pathways involve Tier 1 antioxidant responses including the expression of phase II enzymes at low levels of oxidative stress; Tier 2 pro-inflammatory effects including cytokine and chemokine productions when the oxidative stress level increases; and Tier 3 mitochondria mediated cell death when oxidative stress reaches highest levels.[4–6] Among the pro-inflammatory effects, IL-1 β , TNF- α , IL-6, IL-8 etc. are often secreted to induce lung inflammation.[7, 8] We are especially interested in IL-1 β , which is produced through the activation of the nucleotide-binding domain and leucine-rich repeat protein 3 (NLRP3) inflammasome, and this inflammasome has been shown to be involved in many chronic diseases including asbestosis (induced by asbestos fibers), silicosis (induced by quartz), gout (induced by long aspect ratio MSU crystals), Alzheimer's disease (induced by fibrillar amyloid- β), and type II diabetes (induced by oligomers of islet amyloid polypeptide).[9–11] Recent reports showed that NLRP3 inflammasome activation can be induced by PM_{2.5}. [1, 7, 12–15] It is worth noting that although there are substantial variations in terms of PM_{2.5} compositions, it has been shown that silica and black carbons are likely contained in PM_{2.5}. Recent data showed nanomaterials such as fumed silica nanoparticles and carbon nanomaterials have been shown to induce NLRP3 inflammasome activation as well as lung fibrosis.[16–25] It is found that NLRP3 inflammasome has also been shown to play a key role in lung fibrosis due to the subsequent triggering of TGF- β 1 and other pro-fibrogenic cytokine (e.g., PDGF-AA) generation and epithelial mesenchymal transition that results in myofibroblast activation, proliferation and collagen deposition. Against this background, the purpose of this paper is to study, analyze, and explore the potential chronic effects of PM 2.5 in terms of its ability to induce NLRP3 inflammasome activation and correlate that with their potential to induce lung fibrosis.

To achieve this goal, in this study, we collected a panel of PM_{2.5} particulate samples from the air in November 2017 in Zhengzhou, China and compared their potential to induce NLRP3 inflammasome activation in vitro as well as their profibrogenic effects in the mouse lung in vivo (Fig. 1). We demonstrate that the PM_{2.5} could induce NLRP3 inflammasome activation and trigger a series of pro-inflammatory responses including profibrogenic TGF- β production in the lung, leading to lung fibrosis in vivo. In addition, the in vitro ranking of the IL-1 β production correlate well with the lung fibrogenic potential in vivo, suggesting a predictive toxicology approach for assessment of the chronic lung toxicity induced by ambient PM_{2.5}.

Results

Characterization of Major Components of PM_{2.5} Particulates

Eight PM2.5 samples were collected on filters (labeled as S1 to S8, PM2.5) on different days in November 2017 in Zhengzhou, Henan, China. The control (Ctrl) is the blank filters without PM2.5 samples, (Table 1). The collected filters were cut in small pieces and gently rinsed in DI H2O for three times to wash off the PM2.5 particles on the filters. The metal and metalloid components of each sample were determined by inductively coupled plasma mass spectrometer (ICP-MS). Total 14 metal and metalloid elements (Table 2) were analyzed, where aluminum (Al), barium (Ba), zinc (Zn), iron (Fe) and sulfur (S) were the most abundant elements (Table 2). We carried out physicochemical characterization of the particle suspensions. We assessed the primary size, hydrodynamic size and zeta potential of these PM2.5 particles in DI water as well as in tissue culture medium (RPMI 1640) prior to biological experimentation (Table 3). The results showed heterogeneous sizes of these PM2.5 samples in DI water and RPMI 1640 ranging from 20.8 nm to 66.5 μ m (Table 3). All the particles showed negative zeta-potentials in DI water and the media tested ranging from -5.8 mV to -45.7 mV (Table 3). The endotoxin content of all the particles was lower than 1.2 EU/mL, as determined by the Limulus Amebocyte Lysate (LAL) assay (Figure S1).

The Ability of PM2.5 Particulates to Induce NLRP3 Inflammasome Activation and IL-1 β Production in THP-1 cells

Because our ultimate goal is to assess the profibrogenic effects of PM2.5 particles in the lung, we used a well-established in vitro platform to gain an understanding of the cooperation between macrophages and cellular elements of the epithelial–mesenchymal trophic (EMT) as the basis for pulmonary fibrosis.[26-28] The synergy between macrophage released IL-1 β and TGF- β 1 production by the EMT unit plays a major role in collagen production and deposition in the lung.[29, 30] In order to model the events leading to IL-1 β production, we used a human monomyelocytic leukemia cell line, THP-1, which differentiates into a myeloid phenotype in response to a phorbol 12-myristate 13-acetate (PMA) treatment.[31] THP-1 cells have been used successfully to look at the effect of nanomaterials, such as CNTs, graphene, GO, rare earth oxides and nanowires, on the induction of IL-1 β release by phagocytic cells.[16, 17, 19-23, 26-28, 31-38] This pro-inflammatory response is dependent on lysosomal damage and the assembly of the NLRP3 inflammasome, which is responsible for pro-IL-1 β cleavage and IL-1 β release.[12, 17, 23] While exposure of THP-1 cells to PM2.5 particles did not induce significant loss of cell viability in cells (Figure 2A and B) as determined by conducting MTS (metabolic activity) and ATP (cell energy) assay; but did trigger robust IL-1 β release into the THP-1 culture medium (Figure 2C). The overall abundance of IL-1 β production was S6 > S7 > S5 > S8 > S3 > S2 > S1 > S4 (Figure 2C). In addition, the exposure to PM2.5 particles also showed the significant increase of TNF- α level in THP-1 cells at similar trend (Figure 3). This confirmed the ability of these PM2.5 particles to induce pro-inflammatory effects in vitro.

In order to confirm the involvement of the NLRP3 inflammasome, we used NLRP3-deficient (NLRP3^{-/-}) as well as ASC-deficient (ASC^{-/-}) THP-1 cells to demonstrate that gene knockout leads to abrogation of the IL-1 β response to PM2.5 particles (Figure 2D and 2E). The initiation of this response by PM2.5 particles is dependent on lysosomal damage, which leads to cathepsin B release and provides a robust signal for assembly of the NLRP3 subunits in phagocytic cells. The role of cathepsin B was confirmed by using a cathepsin B inhibitor, CA-074 methyl ester, to show interference in IL-1 β production (Figure 2F).

The Ability of PM2.5 particles to Induce TGF- β 1 Production in BEAS-2B Cells

The epithelial cell is an important component of the trophic cell unit undergoing epithelial–mesenchymal transition in the lung during development of fibrosis.[29, 30] The production of TGF- β 1 by the epithelial cells contributes in synergistic fashion to the establishment of a matrix synthesis phenotype and collagen synthesis by the participating mesenchymal cellular elements and other pro-fibrogenic cytokines such as PDGF-AA.[29, 30] We chose BEAS-2B cells, a human lung epithelial cell line, to determine whether the PM2.5 particles impact the synthesis of this growth factor, as determined by conducting an ELISA of the supernatant. The results showed that similar to THP-1 cells, none of the PM2.5 samples were associated with cytotoxicity in BEAS-2B cells (Figure 4A). Moreover, all these PM2.5 samples induced TGF- β 1 production irrespective of the particle types (Figure 4B). Taken together with the IL-1 β data, these results show that PM2.5 particles could induce pro-inflammatory and profibrogenic cytokine production in vitro.

Exposure to Mouse Lung to Determine the Fibrogenic Potential of PM2.5 Particulates

We have previously demonstrated for silica and carbonaceous nanomaterials that the in vitro profibrogenic responses in THP-1 and BEAS-2B cells largely reflect their ability to generate similar biomarker responses in the bronchoalveolar lavage fluid (BALF) of mice receiving oropharyngeal aspiration.[22, 26, 32] The C57BL/6 mice were used for exposure to S1, S3, S5 and S6 PM2.5 particles, which corresponded to low, medium and high response level of pro-inflammatory and profibrogenic effects in vitro. The mice were sacrificed at 21 days after the oropharyngeal inspiration of the PM2.5 particles. Aspiration of Min-U-Sil (quartz or crystalline SiO₂) was used as positive control. Examination of the BALF fluid showed that all the particles could induce an increase in eosinophil cell counts at the order of S1 and S3 < S5 and S6, with an association with allergic response. The eosinophil cell counts for S5 and S6 were significantly higher than S1 and S3 (Figure 4A and B). Although this effect was accompanied by a nonresponsive effect in macrophage cell count (Figure 4C), neutrophil cell count (Figure 4D), LIX levels for all particles tested (Figure 4E) as well as IL-6 and TNF- α (Figure S2), there was a significant increase of TGF- β 1 for all PM2.5 particles tested (Figure 4F) with an order of S1 < S3 < S5 < S6. This particle dependent profibrogenic effect was consistent with the IL-1 β production data in vitro (Figure 2A). The trend toward more inflammation by S6 was confirmed by haematoxylin and eosin (H&E)

staining, which showed visual evidence of more inflammatory infiltrates around small- and medium-sized airways in the lung (Figure 5). Again, the effect of the S6 was most pronounced (Figure 5). SiO₂ induced significant increases in neutrophil counts, LIX and TGF- β 1 levels in the BALF along with inflammatory changes in the lung (Figure 4 and 5).

Moreover, the S6 but not S1, S3 or S5 induced apparent increase in collagen deposition around airways and alveolar spaces of mice, as determined by the Masson's trichrome staining (Figure 6A). In contrast, no trichrome staining was seen in the lungs of animals receiving S1, S3 or S5. This was confirmed by the Sircol collagen assay, which showed that the exposure to S6 significantly increased the collagen production in the mouse lung (Figure 6B). The exposure to S1, S3 or S5 could also induce an increase in collagen production, but was not statistically significant if compared to the none-treated control; while SiO₂ (positive control) could induce a significant effect both in the Masson's trichrome staining and Sircol collagen assay (Figure 6A and B). The data above demonstrate that PM_{2.5} particles can cause pro-inflammatory and profibrogenic effects in vitro and in vivo. In addition, the ranking of in vitro IL-1 β productions relatively correlate with the in vivo TGF- β 1 production and collagen deposition.

Discussion

In this study, we explored the cytotoxic potential and effect of air pollution (PM_{2.5}) particulates can have on human cells, particularly looking at human lung bronchial epithelial and human macrophages, which are the first line of defense against air pollution in the lung. The overall toxicity as measured by cell viability of the PMs is minimal. Past research has shown that the surface of PM_{2.5} is rich in copper, zinc, manganese, iron, and other transition elements; while in this study we showed that aluminum (Al), barium (Ba), zinc (Zn), iron (Fe) and sulfur (S) were the most abundant elements (Table 2). The presence of iron is interesting because it is known to trigger Fenton reaction and produce reactive oxygen species. We did not determine the content of organic chemicals due to the limitations on the filter samples, which may lose many of the volatile organic chemicals after long time storage. However, the different components such as transition metals (iron) and organic chemical (polycyclic aromatic hydrocarbon, PAHs) on PM_{2.5} could increase the production of free radicals may cause oxidative stress in lung tissues.[39] Among the different cells, alveolar macrophage is a major cell type at risk. For example, Jalava et al. collected real-life air particulates from 6 cities in Europe and cultured them with mice macrophages in vitro for 24 hours [40], they found the alveolar macrophage viability significantly decreased when exposed to different dose levels of PM 0.2- PM 2.5 (300 pg/mL to 150 g/mL), and showed an increased in TNF- α expression.[40] Chronic toxicological effects of PM_{2.5} are well explored but the effect on NLRP3 inflammasome activation was only recently explored. An important note to mention is that the compositions of air pollution particles are not constant as they have huge variations among different cities, local environment, vehicle types and fleet compositions, temperature, time of sample collection, etc., which will cause differences in the toxic potential in cells in vitro, animal lung in vivo, and risk of pulmonary diseases in humans. [41] [42]

Although the PMs showed a less degree of decreased cell viability, IL-1 β secretion using ELISA assay indicated significant increase by PMs at different levels. Figure 2B and C denote significant decrease in cytokine release when the NLRP3 and ASC pathways are modified with knockout forms, potentially providing evidence towards the importance of NLRP3 inflammasome pathway in inducing toxicity in pulmonary systems. This pathway also includes the internalization of PM_{2.5} by phagocytic or endocytic processes and lysosomal damage, which releases the lysosomal enzyme, cathepsin B. Cathepsin B release is known to activate NLRP3 inflammasome.[1] We showed that the use of a cathepsin B inhibitor significantly reduced the IL-1 β production in THP-1 cells. TNF- α production follows the similar trend for different PMs. We then evaluated the sub-chronic toxicity of PM_{2.5} in animal lung in vivo.[1] In terms of cytokine and growth factor presence in lungs, only the TGF- β 1 production was statistically significant as well as the collagen deposition for sample S6. Eosinophil showed increase after PM exposures while lymphocyte and neutrophil cell counts were not significant in the total cell count suggesting the lack of lung inflammation. It worth mentioning that the ranking of in vitro IL-1 β productions relatively correlate with the in vivo TGF- β 1 production and collagen deposition, which agrees with our previous results on lung fibrosis induced by nanomaterials including carbon nanotubes, fumed silica, graphene and graphene oxides, suggesting a similar mechanism on inducing sub-chronic lung inflammation and fibrosis.[16–22, 26, 28, 32] It is also interesting that the fibrogenic potential of the PM samples do not correlate with the overall PM_{2.5} levels reported on those sample collecting day (data not shown), suggesting that overall PM_{2.5} levels are not a good indicator of the chronic lung toxicity potential of the PMs. While the compositions of the PMs play a dominant role that needs to be fully characterized and analyzed for their toxicological potential. For example, Table 3 showed that among the metal components, iron levels correlate well with the NLRP3 inflammasome activation and IL-1 β production in vitro and the profibrogenic potential in vivo. Although we do not know the valence of the Fe, Fe²⁺ is known to be able to trigger Fenton reaction to generate hydroxyl radicals and oxidative stress, which plays an important role to facilitate the activation of NLRP3 inflammasome.

Conclusions

In summary, we showed PM_{2.5} is capable to induce lung fibrosis. The mechanism involves the NLRP3 inflammasome and IL-1 β production, which triggers a series of responses in epithelial–mesenchymal trophic cell unit in the lung. This leads to TGF- β 1 production, myofibroblast activation, and collagen deposition, which results in lung fibrosis. In addition, we found the ranking of the pro-fibrogenic effects by the PM_{2.5} samples in vivo was consistent with that of the IL-1 β production in vitro. Thus, this predictive toxicological approach may be useful to serve as a rapid screening tool to determine the fibrogenic potential of the ambient PMs.

Materials And Methods

Preparation of PM_{2.5} samples.

PM2.5 samples were collected on various days in November 2017 in Zhengzhou, Henan, China. KC-120H intelligent medium-flow TSP sampler was used to collect PM2.5 samples continuously for 24 hours with the flow rate is 30 L/min. Sampling filters are all quartz filters. Before sampling, the filters are balanced for more than 24 hours under constant temperature and humidity (temperature 20 °C, relative humidity 35%) environment, and then samples were weighed and numbered separately. After sampling, it was put into the same constant temperature and humidity environment to balance for more than 24 hours, weighed again, and recorded the weight changes. Aluminum foil is used for shading during the transportation, storage and weighing of filter membranes. Samples were stored at 4 °C.

The particles were extracted from sampling Quartz Fiber Filtration Membrane by immersing in 1 mL of distilled water followed by 30 min of sonication for three times. The extracted samples were then lyophilized and weighted for preparation of PM2.5 suspension. Each sample was labeled with the corresponding labels (S1 to S8 on circular Quartz Fiber Filtration Membrane for collecting PM2.5 particles).

Analysis for metal elements using ICP-MS.

For the total heavy metal content detection, the PM2.5 suspensions were transferred into an 8 mL mixture of hydrochloric acid, nitric acid and hydrofluoric acid (2:1:1 ratio by volume). The above samples were digested using a microwave digestion/extraction system. Microwave digestion was carried out at 135 °C for 1 h. The digested samples were diluted to 15 mL with de-ionized water and then filtered (Whatman No. 42) to remove any solid residues. The obtained samples were analyzed for metal elements quantification (V, Mn, Fe, Co, Ni, Cu, Zn, Ge, Ba, and Ce) by inductively coupled plasma-mass spectrometry (ICP-MS).

Cell Culture.

Human bronchial epithelial cells (BEAS-2B) and Human monomyelocytic leukemia (THP-1) cell lines were obtained from ATCC (Manassas, VA). BEAS-2B cells were cultured in bronchial epithelial basal medium (BEBM) (Lonza, Walkersville, MD), supplemented with growth factors from the Single Quots™ Kit (Lonza) to reconstitute bronchial epithelial growth medium (BEGM). THP-1 cells were cultured in RPMI1640 medium containing 10% fetal bovine serum, 100 units/mL penicillin and 100 mg/mL streptomycin at 37 °C in a humidified 5% CO₂ atmosphere, and passaged at 70–80% confluency every 2–4 days.

Cellular Exposure to PM2.5 suspension for Assessing Cytotoxicity and Proinflammatory Effects.

The PM2.5 stock suspensions were prepared in DI H₂O at 1 mg/mL. THP-1 cells were pretreated with 1 µg/mL phorbol 12-myristate acetate (PMA) overnight and primed with 10 ng/mL lipopolysaccharide (LPS). Aliquots of 3×10^4 primed cells were cultured in 0.1 mL medium with PM2.5 suspensions in 96-well plates (Costar, Corning, NY, USA) at 37 °C for 24 h. In order to provide less aggregated particles that can be suspended in biological culture media, all PM2.5 suspensions were freshly prepared by adding a desired amount of stock solutions to RPMI 1640 media at 12.5–100 µg/mL in the presence of 10% fetal bovine serum (FBS). After 24 h, the supernatants were collected for the measurement of IL-1β, TNF-α (BD

Biosciences, San Diego, CA) and TGF- β 1 (Promega, Madison, WI), using ELISA kits according to manufacturer's instructions. Concentrations are expressed as pg/mL. The remaining cells were treated with 120 μ L culture medium containing 16.7% of MTS (CellTiter 96 Aqueous, Promega Corp) for 0.5 h at 37 °C. The plates were centrifuged at 2000 g for 10 min to remove the cell debris and the particles. 85 μ L of the supernatant was transferred into a new 96 well plate. The absorbance was read at 490 nm on a SpectraMax M5 microplate reader (Molecular Devices Corp., Sunnyvale, CA, USA).

Assessment of NLRP3 inflammasome activation by small molecule inhibitors and gene knockdown. Differentiated THP-1 cells were seeded into 96-well plates at the density of 3×10^4 in 0.1 mL complete RPMI 1640 medium. The cells were incubated with 10 μ M CA-074 methyl ester (cathepsin B inhibitor) for 45 min. The medium was subsequently changed to fresh RPMI 1640 into which 100 μ g/mL PM2.5 suspensions were suspended. Following the addition of one of the chemical inhibitors (CA-074 methyl ester), the cells were incubated for a further 5 h and the supernatant collected to measure the IL-1 β production by ELISA. For the NLRP3 and ASC deficient assay, NLRP3 $^{-/-}$ THP-1 cell and ASC $^{-/-}$ THP-1 cell were obtained from Invivogen (San Diego, California, USA). The cells were derived by siRNA knockdown of NLRP3 and ASC genes. The siRNA were delivered by lentiviral vectors. Cells were cultured in complete RPMI 1640 supplemented with 200 μ g/mL HygroGold, and 100 μ g/mL Normocin before exposure to PM2.5 suspensions for 5 h. The supernatant was collected to measure the IL-1 β production by ELISA.

Oropharyngeal Aspiration to Assess Pulmonary Effects in Mice.

Eight-week-old male C57Bl/6 mice were purchased from Charles River Laboratories (Hollister, CA). All animals were housed under standard laboratory conditions according to UCLA guidelines for care and treatment as well as the NIH Guide for the Care and Use of Laboratory Animals in Research (DHEW78-23). The animal experiments were approved by the Chancellor's Animal Research Committee at UCLA and include standard operating procedures for animal housing (filter-topped cages; room temperature at 23 ± 2 °C; 60% relative humidity; 12 h light, 12 h dark cycle) and hygiene status (autoclaved food and acidified water). Animal exposure was carried out according to the oropharyngeal aspiration method developed at NIOSH. The animals received oropharyngeal aspiration of PM2.5 suspended in saline at 2 mg/kg. The mice were sacrificed after 21 d to assess the subchronic effects. BALF and lung tissue were collected for measurement of TGF- β 1 (Promega, Madison, WI) and TNF- α (BD Biosciences, San Diego, CA) levels and performance of hematoxylin and eosin or Masson's trichrome staining.

Statistical Analysis.

Mean and standard deviations (SD) were calculated for each parameter. Results were expressed as mean $\pm$ SD of multiple determinations. Comparisons within each group were conducted by a two-sided Student's t test. A statistically significant difference was assumed with $p < 0.05$.

Abbreviations

PM2.5
fine particulate matter
COPD
chronic obstructive pulmonary disease
TNF- α
Tumor Necrosis Factor- α
IL-1 β
Interleukin-1 β
TGF- β 1
Transforming Growth Factor- β 1
NLRP3
nucleotide-binding domain and leucine-rich repeat protein 3
PDGF-AA
Platelet-Derived Growth Factor-AA
ICP-MS
inductively coupled plasma mass spectrometer
LAL
Limulus Amebocyte Lysate
EMT
epithelial–mesenchymal trophic
H&E
haematoxylin and eosin
MSU
Monosodium Urate Crystal
PMA
phorbol 12-myristate acetate
BALF
bronchoalveolar lavage fluid

Declarations

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Availability of data and materials

The datasets analyzed during this study will be made available from the corresponding author upon a reasonable request.

Authors' contribution

XW, WC and TX designed the study; CHC, JJ, PH and PL collected and characterized the PM2.5 samples used in this study; MY and JK performed most of the cellular experiments; XW and YL performed the animal experiments. The writing of the paper was led by XW, WC and TX. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interest.

Consent for publication

"Not applicable".

Ethics approval

All procedures performed on animals were approved by UCLA guidelines as well as the NIH Guide for the Care and Use of Laboratory Animals (DHEW78-23).

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Tables

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Figures

Figure 1

A schematic describing the collection and preparation of PM2.5 samples as well as the in vitro/in vivo study design.

Figure 2

Cytotoxicity and IL-1 β production in THP-1 cells exposed to PM2.5 particles. The MTS assay was used to assess the cytotoxic effects of PM2.5 particles in A) THP-1 cells. The cells were exposed to 12.5, 25, 50, and 100 μ g/mL for each PM2.5 particles 1-1 through 8-1 for 24 h. The absorbance was read at 490 nm in a microplate reader (SpectroMax M5e, Molecular Devices, Sunnyvale, CA, USA). All the MTS values were normalized with respect to the non-treated control, which was regarded as representing 100% viability. ZnO nanoparticles were used as a positive control. * $p < 0.05$ compared to non-treated control. (C) The supernatants from Figure 1A were collected to measure the IL-1 β level by ELISA. * $p < 0.05$, comparing non-treated control; (D and E) The involvement of the NLRP3 inflammasome activation was confirmed by using NLRP3 deficient (NLRP3 $-/-$) and ASC deficient (ASC $-/-$) THP-1 cells. (F) The cathepsin B inhibitor, CA-074 methyl ester, was used to demonstrate the involvement of cathepsin B pathway in inflammasome activation. * $p < 0.05$, comparing non-treated control; # $p < 0.05$ for pairwise comparisons as shown.

Figure 3

TNF- α production in THP-1 cells exposed to PM_{2.5} particles. The supernatants from Figure 1A were collected to measure the TNF- α level by ELISA. *p < 0.05, comparing non-treated control.

Figure 4

Cytotoxicity and TGF-β1 production in BEAS-2B cells exposed to PM2.5 particles. A) The MTS assay was used to assess the cytotoxic effects of PM2.5 particles in BEAS-2B cells. The cells were exposed to 12.5, 25, 50, and 100 μg/mL for each PM2.5 particles S1 through S8 for 24 h. The absorbance was read at 490 nm in a microplate reader (SpectroMax M5e, Molecular Devices, Sunnyvale, CA, USA). All the MTS values were normalized with respect to the non-treated control, which was regarded as representing 100% viability. ZnO nanoparticles were used as a positive control. *p < 0.05 compared to non-treated control. B) The supernatant from Figure S1A was collected to measure the TGF-β1 level by ELISA. *p < 0.05, comparing non-treated control.

Figure 5

Assessment of the lung effects of the PM2.5 particles in mice. C57BL/6 mice were exposed to 2.0 mg/kg bolus doses of PM2.5 particles delivered by one-time oropharyngeal aspiration. Animals were euthanized after 21 d, and BALF was collected to determine A) Differential cell count; B) Eosinophil cell count; C) Macrophage cell count; D) Neutrophil cell count; E) LIX and F) TGF- β 1 levels. *p < 0.05 compared to control.

Figure 6

Assessment of subchronic inflammatory effects of the PM2.5 particles in the lung. The lung tissue from the same animals as in Figure 5 was stained by H&E method. Representative histological images (100 ×) of the lung were taken under 100 × magnification. Animals exposed to SiO₂ (quartz) served as a positive control.

Figure 7

Assessment of collagen deposition and total collagen content in the lung. A) Collagen deposition in the lung was assessed by staining lung slices with Masson's trichrome. Collagen is stained blue in the images viewed under 100 × magnification. B) The total collagen content of the lung tissues was determined by the Sircol collagen kit (Biocolor Ltd., Carrickfergus, U.K.). SiO₂ (quartz) at 5.0 mg/kg was treated as a positive control. *p < 0.05 compared to control.

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