Biochemical Evidence of PM_{2.5} Critical Components in the Induction of Myocardial Fibrosis in Mice

Qisong Xing

Shanxi University

Meiqiong Wu

Shanxi Medical University

Zhen Xue

Shanxi University

Nan Nan

Shanxi University

Zhipeng Yan

Shanxi University

Shuyue Li

Shanxi University

Guohua Qin (✉ qinguojua@sxu.edu.cn)

Shanxi University

Nan Sang

Shanxi University

Research Article

Keywords: PM_{2.5}, critical components, CVD, myocardial fibrosis, synergy cytotoxicity.

Posted Date: February 24th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1376144/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background:

PM_{2.5} components are tightly linked to the initiation of a multitude of cardiovascular diseases (CVD). However, it is unclear that whether critical components of PM_{2.5} can induce myocardial fibrosis.

Methods:

We exposed female C57BL/6 mice to PM_{2.5} (3 mg/kg b.w.) from four different cities (Taiyuan, Beijing, Hangzhou, and Guangzhou) by oropharyngeal aspiration every other day. The varying degrees of myocardial fibrosis markers (Col1a1, Col3a1) and the possible relevant indicators, including (E/E_c), NADPH oxidase 4 (NOX4), and transforming growth factor β 1 (TGF β 1) were detected by echocardiography and / or qPCR and / or Western blot *in vivo*. Pb, Cr, Mn, Zn, and most of the polycyclic aromatic hydrocarbons (PAHs) were positive associated with diastolic cardiac function and myocardial fibrosis by using Pearson correlation. BEAS-2b cells were treated with one or more of five candidate components with or without Guangzhou PM_{2.5}, and the conditioned medium was used to culture AC16 cells. MTT assays were performed to evaluate the cytotoxicity of the treatments. ROS generation was detected by photocatalysis using 2',7'-dichlorodihydrofluorescein diacetate (DCFHDA). The expression of Col1a1, TGF β 1 were detected by qPCR *in vitro*.

Results:

The effects of PM_{2.5} from Taiyuan are the most serious, while Guangzhou group caused minor effects. Zn + Pb + Mn + BaP with PM_{2.5} from Guangzhou exposure significantly increased ROS production of BEAS-2b cells, and induced a dramatic increase of myocardial fiber-related gene expression (Col1a1 and TGF- β) in AC16 cells.

Conclusion

It indicated that the different mass concentrations of Zn, Pb, Mn, and Σ PAHs in PM_{2.5} might be the critical factor that modulated myocardial fibrosis induction by targeted. Our study provided a novel avenue for further elucidation of molecular mechanisms of PM_{2.5} components-induced myocardial fibrosis.

Background

Ambient air pollution is known to threaten people with health effects, especially fine particulate matter (PM_{2.5}, aerodynamic diameter $\leq 2.5 \mu\text{m}$). A classical cohort study of epidemiology about the *Harvard Six Cities* has shown the association between higher concentrations of PM_{2.5} exposure and adjusted mortality-rate ratios.[1] The PM_{2.5}-induced toxic effect is made up of varying compositions of toxic chemical cocktails; additional, epidemiological studies have shown that the critical components of PM_{2.5} are highly correlated with various cardiovascular diseases (CVD).[2] Long-term exposure to PM_{2.5} which

comes from coal combustion and diesel traffic-related emissions is associated with an increase in ischemic heart disease (IHD) mortality among the older adults in a cohort study of the United States with follow-up from 1982 through 2004.[3] Some typical components of PM_{2.5} have been proved to contribute CVD. Pb exposure (mean, 0.2 $\mu\text{g}/\text{m}^3$) is significantly associated with the IHD.[3, 4] Polycyclic aromatic hydrocarbons (PAHs) from combustion in PM_{2.5} exposure have shown a strong association with cardiorespiratory emergency department visit outcomes.[5] Al, Fe, Ti, Ni, and nitrate in PM_{2.5} exposure may be related to cardiovascular hospital admissions in Pakistan.[6] A cohort study in Europ has shown no statistically significant association between eight elements (Cu, Fe, K, Ni, S, Si, V, and Zn) in PM_{2.5} and total cardiovascular mortality.[7] It has been reported that the joint action of the component-adjusted model and the contributions of the components in mass PM_{2.5} can predict acute myocardial infarction (AMI) incidence better.[8] There is growing epidemiological evidence for a causal relationship between particulate components and CVD-related molecular events.

Myocardial fibrosis is considered a significant event during the beginning and development of CVD.[9, 10] Cardiac remodeling is a cellular response to a variety of stimulation of intracellular environment of kinases and signaling factors, abnormal structural remodeling of cardiomyocytes can lead to myocardial fibrosis, this scenario is accompanied by accumulation of interstitial fibrous collagen in myocardial coronary arteries and the thickening of blood vessel walls.[9–13] The cardiac tissue can be disturbed either directly or indirectly through the circulation of the blood.[14] Cytotoxicity and inflammation in cardiac tissue are critical events in CVD initiation and progression.[14–17] Furthermore, studies have indicated that water-soluble components in PM_{2.5} could activate reactive oxygen species (ROS) and release inflammatory cytokines on airway epithelial cells.[18] While the literature on different PM_{2.5} induces myocardial fibrosis is growing, it is unknown whether the PM_{2.5} components at critical concentrations can target to influence the occurrence of myocardial fibrosis.

A series of reports of simulating PM_{2.5} exposure has provided strong experimental evidence support which can translocate into pulmonary and systemic circulation by using fluorogenic dyes.[19–21] However, PM_{2.5} exposure may induce different toxic endpoints, which may be related to components interactions at different doses. As stated in the British Committee on the Medical Effects of Air Pollutants (COMEAP) report (2018) that sulfates could play a role in the capacity of PM_{2.5} to drive pulmonary inflammation via either increasing metal mobilization from PM, and hence increasing the ability to drive oxidative stress leading to inflammation. In addition to molecular epidemiology research, the assessment of toxicity of the simulated PM_{2.5} was focused on adding one or more components to the calculated black carbon model, and evaluating the toxic contribution of critical components theoretically by using the concentration addition (CA) concept.[22–24] However, few systematic studies have been conducted on the toxicity of critical components in actual air samples that induced the occurrence of myocardial fibrosis. It is proved that the carbon nucleus is essential to the toxicity in different PM_{2.5}. [25] Thus, it is necessary to consider the structural integrity of the PM_{2.5} samples. In addition, our previous study had

shown that Zn and acenaphthene (AC) in PM_{2.5} might be critical components that induce myocardial hypertrophy by direct lung inflammation and indirect cardiac inflammation.[14]

The objective of this study is to determine which critical components in PM_{2.5} can induce the occurrence of myocardial fibrosis. To illustrate the toxicity of different PM_{2.5}, we chose four cities (Taiyuan, Beijing, Hangzhou, and Guangzhou) in China, which have significant differences in socioeconomics, geographical location, and urban development strategy. In the present study, we exposed ten-month-old female C57BL/6 mice to PM_{2.5} from different cities, and initially investigated the differential manifestation in myocardial fibrosis. Second, we screened the critical components of PM_{2.5} which might induce myocardial fibrosis. Finally, myocardial fibrosis indirectly influenced by the combination of essential components were verified *in vitro*.

Results

Exposure to PM_{2.5} From Different Cities Induces Myocardial Fibrosis in Mice

After 4-week PM_{2.5} from different city exposure, we evaluated the heart function of mice. A significant increase in E/E' was observed in mice after exposure to PM_{2.5} from Taiyuan compared with control group (2.62-fold) (Fig. S1). As shown in Fig. 1A and B, PM_{2.5} from Taiyuan exposure led to the occurrence of myocardial fibrosis which were manifested as a significant increase in collagen deposition (1.82-fold compared with the control group), and the expression of collagen, type I, alpha 1 (Col1a1), type III, alpha 1 (Col3a1), and transforming growth factor β (TGF- β) were significantly increased (2.23-, 9.36- and 1.57-fold) (Fig. 1C). The protein levels of NADPH Oxidases 4 (NOX4) and Col1a1 were also significantly increased in the hearts of mice after exposure to PM_{2.5} from Taiyuan compared with control group (1.44- and 1.22-fold) (Fig. S2). PM_{2.5} from Hangzhou exposure showed the same tendency which were manifested as a significant increase in collagen deposition (1.62-fold compared with the control group) and the expression of Col1a1 and TGF- β (5.21- and 1.34-fold) (Fig. 1). No signs of collagen deposition or pathological changes were observed in the hearts of mice exposed to PM_{2.5} from Beijing or Guangzhou.

The Critical Components in PM_{2.5} Associated with Myocardial Fibrosis-Related Index

We performed a Pearson correlation analysis to examine the possible association of PM_{2.5} critical components (heavy metals and PAHs) with diastolic cardiac function and myocardial fibrosis-related indicators *in vivo*. [14, 26] As shown in Fig. 2A, the relative changes of E/E' appeared positive correlation significantly with 16 PAHs and 9 metals (including Li, Cr, Mn, Mo, Cs, Pb, Bi, Th, and U). The relative expression of Col1a1 demonstrates a positive correlation significantly with 6 PAHs (including AC, FL, Bghip, IP, DBahA, and COR) and Cr, Pb, and Bi. The three metals (including Pb, Cr, and Mn) were screened for further study *in vitro* based on two rules, a strong positive correlation (Fig. 2A) and higher

concentrations ($> 0.1 \mu\text{g}/\text{mg}$, Table 1). All three metals have an excellent linear regression with E/E'. Pb and Cr have an excellent linear regression with the relative expression of Col1a1 ($R^2 > 0.90$). (Fig. 2B-D).

Critical Components in PM_{2.5} Directly Induce Intracellular ROS production in BEAS-2b cells

To determine which critical components are correlated strongly with myocardial fibrosis, we choose five candidate critical components. In addition to the three metals mentioned above (Pb, Cr, and Mn), we added two additional critical components for validation *in vitro* (Zn and BaP). Myocardial fibrosis is a hallmark of cardiac hypertrophy. Based on our previous study, Zn was selected as a heavy metal associated with myocardial hypertrophy.[14] Furthermore, Zn concentrations were associated with cardiovascular mortality and morbidity in a meta-analysis.[30] BaP was selected as a typical representative PAHs.

To assess which combination of multiple compounds significantly induced ROS production in BEAS-2b cells, one or more water-soluble compounds of five candidate components were added with or without PM_{2.5} from Guangzhou. Firstly, we determined the effective concentration exposed to PM_{2.5} in different cities (200 $\mu\text{g}/\text{mL}$) and exposure time (24h), which were illustrated by intracellular ROS in BEAS-2b cells (Fig. S3, S4). A significant difference was presented between Taiyuan and Guangzhou groups in ROS production after treatment for 24h (Fig. S4). Next, we prepared exposed suspension by adding the different concentration of compounds between PM_{2.5} from Taiyuan and Guangzhou (including Zn, Pb, Cr, Mn, and BaP). As indicated in Fig. 3A, the ROS production of BEAS-2b cells after being treated with Taiyuan PM_{2.5}, and five candidate components with and without PM_{2.5} increase to 1.21-, 1.20- and 1.13-fold compared with that of the vehicle control group ($P < 0.001$, $P < 0.001$ and $P < 0.01$), respectively. However, adding a single candidate component with or without Guangzhou PM_{2.5} could not induce a significant increase in ROS production (Fig. 3D). Two kinds of combinations of four candidate components can induce ROS increase significantly. Treatment with Zn + Pb + Mn + BaP with or without PM_{2.5} from Guangzhou and Zn + Cr + Mn + BaP with PM_{2.5} from Guangzhou to increased 1.16-, 1.17- and 1.12-fold ROS production of the vehicle control ($P < 0.01$, $P < 0.001$ and $P < 0.05$), respectively (Fig. 3B). Furthermore, in the absence of Cr, three or two candidate components could not elevate the ROS levels of BEAS-2b cells (Fig. 3C). Overall, Zn + Pb + Mn + BaP and Zn + Cr + Mn + BaP might be the effective combinations to induce intracellular ROS in BEAS-2b cells.

Critical Components in PM_{2.5} Induce Indirectly Response of ROS in AC16 cells

To simulate the effects of PM_{2.5} on human health in a natural environment, AC16 cells were exposed to BEAS-2b cells-CM which were exposed to the mixture of Taiyuan PM_{2.5}, Zn + Pb + Mn + BaP, and Zn + Cr + Mn + BaP with or without Guangzhou PM_{2.5} for 24h. PM_{2.5} from Taiyuan increased the expression of Col1a1 and TGF- β by 1.8 and 1.21 fold in AC16 cells ($P < 0.001$ and $P < 0.01$). Compared with the vehicle group, the expression of Col1a1 and TGF- β of AC16 cells after Zn + Pb + Mn + BaP with Guangzhou PM_{2.5} treatment were elevated significantly to 1.60- and 1.21-fold ($P < 0.05$ and $P < 0.05$). It suggested that the

different concentrations in the combination of Zn, Pb, Mn, and BaP might contribute to myocardial fibrosis between PM_{2.5} from Taiyuan and Guangzhou (Fig. 4A, B).

Discussion

There are increasing concerns about the correlation between air pollution and human health indicators. [31–33] PM can enter the lungs through breathing, and cause damage to various organs through blood circulation.[2, 34, 35] PM_{2.5} can induce inflammation in 6-week-old BALB/c wild male mice by activating the TGF- β /Smad signaling pathway, leading to lung injury.[36] Meanwhile, PM_{2.5} organic components play an essential role in inflammation, oxidative stress, cell differentiation, cell cycle regulation, and signal pathway.[18] As described by Lin et al., the transition from a more-polluted to a less-polluted city has led to changes in the pro-oxidative and pro-inflammatory metabolic indicators of cardiovascular disease in human blood, which are related to exposure to PAH metabolites.[37] Our previous researchers found that PM_{2.5} from Taiyuan induced lung and myocardial inflammation in ten-month-old female C57BL/6 mice by up-regulated the expression of IL-6 and TNF- α in the lung and heart tissues.[14, 38] Indirect myocardial inflammation-induced myocardial hypertrophy through IL-6/JAK2/STAT3/ β -MHC signaling pathway.[14] In this study, we identified the disparity response of myocardial fibrosis in mice exposed to PM_{2.5} from different cities. We found that PM_{2.5} induced the occurrence of myocardial fibrosis by accumulating collagen and up-regulating fibrosis markers in the levels of mRNA (Col1a1 and Col3a1) (Fig. 1C) and proteins (Col1a1) (Fig. S2). Compared with PM_{2.5} from Taiyuan in winter 2012, some components of PM_{2.5} in 2017 decreased by 1 to 2 orders of magnitude.[26, 38, 39] Myocardial fibrosis could be observed in Taiyuan and Hangzhou group in the present study.[14] No significant changes in myocardial fibrosis were observed in the hearts of mice exposed to PM_{2.5} from Beijing or Guangzhou (Fig. 1B). As typical markers of myocardial fibrosis, the mRNA levels of Col1a1 and Col3a1 were all upregulated in the hearts of mice after exposure to PM_{2.5} from Taiyuan. PM_{2.5} from Hangzhou induced myocardial fibrosis by up-regulating the expression of Col1a1 protein levels and Col3a1 mRNA levels; however, the mRNA levels of Col1a1 were not significantly increased (Fig. 1 and Fig. S2).[14] It might be due to a post-transcriptional regulation such as microRNAs, which miR-29 might be involved regulating of Col1a1.[40] According to the above mentioned, our present study demonstrated that PM_{2.5} from different cities induced different degrees of effects in myocardial fibrosis.

The observed different effects of myocardial fibrosis might be due to the various components of PM_{2.5} from different cities. To get information about the relevant components which play roles in fibrosis, we use indicator-components dependency to filter components. We found a highly positive correlation between the relative expression of diastolic cardiac function (E/E') and myocardial fibrosis-related indicators (Col1a1) and heavy metals (Pb, Cr, and Mn) and PAHs in PM_{2.5} (Fig. 2). Based on the positive correlation between the indexes and the concentration of components in the present and previous studies and the mass of components, it is suspected that Zn, Pb, Cr, Mn, and PAHs might play essential roles in PM_{2.5}-induced cardiac dysfunction and myocardial fibrosis. The average concentrations of Zn, Pb, Cr, and

Mn in PM_{2.5} between Taiyuan and Guangzhou were ranged from 0.368 to 0.856 $\mu\text{g}/\text{mg}$. A previous study pointed out that the concentrations of heavy metals in the lung (Pb and Cd), and heart (Zn, Mn and Cd) tissues of 10-month-old mice were increased significantly after exposure to PM_{2.5} from Taiyuan in the 2014 winter.[27] Others noted that the PM_{2.5}-bound metal contents (Pb and Cu) were increased in the placenta after being exposed to PM_{2.5} from Taiyuan in 2014 winter.[28] We next examined the four water-soluble compounds containing heavy metals (ZnSO₄, Pb(CH₃COO)₂, K₂Cr₂O₇, MnCl₂) and BaP (dissolved in DMSO), a typical PAHs, for further validation. Considering that the introduced heavy metal may cause varying degrees of toxic effects by changing the pH of the culture medium,[41] however, we did not observe a significant change in the color of the culture medium after being exposed to different component compounds.

Over the past few decades, the ability to generate ROS demonstrated to be a good predictor of adverse health effects, and oxidative stress-mediated cytotoxicity has dominated our understanding of the inflammatory and health effects mediated by PM_{2.5}. [42–44] Meanwhile, ROS are essential regulators in PM_{2.5} induced myocardial fibrosis which is probably regulated via the ROS/TGF- β 1/Smad3 signaling pathway.[45] We found that PM_{2.5} from Taiyuan and Guangzhou induced a disparity significant in ROS production of BEAS-2b cells at 200 $\mu\text{g}/\text{mL}$ which dose is the same as used in other studies (Fig. S3, S4). [46, 47] A large body of literature showed that PM_{2.5} as an environmental mixture, the extraction of organic and inorganic components in PM_{2.5} could not well predict its biotoxicity.[46, 48–51] It suggests that PM_{2.5} biotoxicity should consider the fit between the carbon nuclear with its various loaded synergy components. It is necessary to add critical components to PM_{2.5} from Guangzhou in this way of exposure. PM_{2.5}-induced cytotoxicity is closely related to heavy metals and organic pollutants, and it is found little correlation with water-soluble ions.[16] Similarly, our results suggested that the combined effects of five components (Zn + Pb + Mn + Cr + BaP) with or without Guangzhou PM_{2.5} can induce ROS production in BEAS-2b cells (Fig. 3A). It has been reported that both PM_{2.5-0.3} inorganic components and PM_{2.5-0.3} organic components can activate toxic effects, but their combination is lower than the entire PM_{2.5-0.3}. [51] Our results showed that Zn + Pb + Mn + BaP and Zn + Cr + Mn + BaP might be the reason for a disparity significant between BEAS-2b cells exposed to PM_{2.5} from Taiyuan and Guangzhou in ROS production (Fig. 3B). Pb has a strong negative correlation with cell vitality and intracellular ROS content, and Cu, Cd, and Mn are negatively correlated with cell vitality.[16] The Mn ion catalyzes by the aerosol surface interface can explain 92.5% sulfate formation.[52] Silicon dioxide nanoparticles (SiNPs), carbon black nanoparticles (CBNPs), and heavy metal (Pb(AC)₂) induce cytotoxicity, oxidative stress, oxidative damage of AC16 cells, and PM_{2.5} from Beijing can directly trigger more severe oxidative stress of AC16 cells.[53] The inflammatory cytokines, and ROS exposed by PM_{2.5} are first produced through the bronchi or alveoli by the nasal cavity, then enter the bloodstream and arrive in the heart leading to cardiotoxicity in natural biological systems. We speculate that the toxicity changes in PM_{2.5} after adding critical components and the cytotoxicity indirectly produced by BEAS-2b cells may be the source of toxicity of AC16 cells.[54] PM_{2.5} from Beijing in winter 2013 induces the expression and secretion of IL-6, TNF- α , and

monocyte chemoattractant protein-1(MCP-1) in macrophages, and hepatocellular insulin-stimulated glucose consumption is weakened by macrophage-CM[55]. We used an indirect exposure method to evaluate the toxicity of PM_{2.5} and its candidate components on AC16, and our results implicated that the expression of myocardial fibrosis marker Col1a1 and critical regulator of fibrosis TGF-β were dramatically increased in PM_{2.5} from Guangzhou + Zn + Pb + Mn + BaP group (Fig. 4).

Conclusion

In summary, the present study proved that city-specific PM_{2.5} could induce varying degrees of myocardial fibrosis. The different mass concentrations of Zn, Pb, Mn, and PAHs in PM_{2.5} might be the essential information to the targeted induction of myocardial fibrosis. More systematic and comprehensive toxicology research is needed to characterize the effects of critical components on the occurrence of CVD. The timing and concentration of toxicology exposure need to be reconsidered and explore the relationship between toxicology and exposure of real people. Our present study has some limitations, secondary derivatives of PAHs such as oxygenated PAHs, Nitro-PAHs in PM_{2.5} need to be considered. This study provides essential proof of risk factors for toxicity hazard of regional PM_{2.5}, while the detection of unknown components in PM_{2.5} and assessment of related toxicity needs to be improved in our future work. In view of the significant differences in particulate composition between cities and regions, this result of the study may extend our knowledge of which critical components in PM_{2.5} induce myocardial fibrosis, as well as provide a relevant scientific basis for the treatment of PM_{2.5} pollution in different cities.

Methods

PM_{2.5} Samples Collection and Metal Compositions Measurement.

PM_{2.5} samples were collected from 4 urban cities in China (Taiyuan (112°34.882'E, 37°47.713'N), Beijing (116°0'E, 39°99'N), Hangzhou (112°09'E, 37°25'N), and Guangzhou (113°17'E, 23°05'N).) during November 2017 to January 2018. Detailed descriptions of samples procedure and samples characterization were given in our previous paper.[14, 26] After sampling, PM_{2.5} was extracted from the quartz membrane, freeze-dried in vacuum, and stored at - 80°C in the dark.

Alkali-melting elements compositions concentrations in PM_{2.5} samples (Mg, Al, Ca, and Fe) were characterized by inductively coupled plasma optical emission spectrometry (ICP-OES, Varian MPX, USA). A total of 30 acid-soluble elements (Li, Be, Na, P, K, Sc, V, Cr, Mn, Co, Ni, Cu, Zn, As, Rb, Y, Mo, Cd, Sn, Sb, Cs, La, Ce, Sm, W, Tl, Pb, Bi, Th, and U) were characterized by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7500a, USA). Details are shown in Table 1 SI (Supporting Information). PAHs in PM_{2.5} from different cities analyses were performed in our previous paper.[26] The results for each composition are expressed as μg/mg which the different concentrations of compositions were subtracted with the blank filter.

Animals Treatment And Echocardiographic Assessment

Female C57BL/6 mice of 10-week-old were supplied by Junke Biological Engineering Co., Ltd. (Nanjing, China). The mice were randomly divided into the PM group (Taiyuan, Beijing, Hangzhou, and Guangzhou group) and the vehicle control group (treated with ultrasonic oscillation of the blank membrane). Detailed descriptions of PM_{2.5} exposure (3 mg/kg b.w.) procedure by oropharyngeal aspiration every other day for four weeks are given in our previous paper.[14, 27, 28] PM_{2.5} exposure concentrations were used to about threefold greater with a PM_{2.5} (75 µg/m³) of China's National Ambient Air Secondary Quality Standard (GB 3095 – 2012). All animals were treated humanely throughout the experimental period according to the instructions of the Committee of Scientific Research Shanxi University.

After four weeks of exposure, the Vevo770™ echocardiography system was used to detect mice heart function (Visual-Sonics, Toronto, Ontario, Canada). Detailed information on cardiac function could be seen in the previous paper.[14] Left ventricle filling parameters were assessed from the ends of mitral leaflets in the apical quadrilocular image, and peak early (E) and peak early motion (E') were measured. The ratio of the blood flow velocity of the mitral valve to the velocity of the mitral valve annulus in the early diastole (E/E') was obtained using defined calculations.

Histopathological Analysis Of Masson Staining And Western Blot

The apical tissue of the left ventricle was fixed in 10% formaldehyde for paraffin embedding and frozen sectioning. The tissue sections were stained with Masson's trichrome staining. The histopathology of the tissue sections was observed and photographed at 400×. The collagen fibers staining analysis was performed by the image processing software IPWIN32. Detailed operating procedures for Western Blot are shown in Text1 SI.

Cell Treatment

BEAS-2b cells. BEAS-2b cells were cultured in a Dulbecco's modified Eagle's medium (DMEM, 10% heat-inactivated fetal bovine serum and 1% penicillin – streptomycin antibiotics) at 37°C in a humidified atmosphere with 5% CO₂. BEAS-2b cells were cultured in 96-well plates, and were exposed to 100 µL of DMEM with PM_{2.5} samples (200 µg/mL) or pure compounds with critical components (ZnSO₄, Pb(CH₃COO)₂, K₂Cr₂O₇, MnCl₂, and benzo(a)pyrene (BaP)) at an effective dose (200 µg/mL) by changing the medium for 24h. All heavy metal were dissolved in ultrapure water, BaP was dissolved in 1% DMSO, and then diluted in DMEM. The target concentrations of critical components in PM_{2.5} were calculated by using Eq. 1

$$TC_i = ED \times CC \times R_i$$

where TC_i is the target concentration of pure compound (mg/mL), ED is the effective doses *in vitro* ($\mu\text{g/mL}$), CC is the difference concentration between the component in $\text{PM}_{2.5}$ from Taiyuan and Guangzhou (mg/mL), and R_i is the ratio of the relative molecular mass of the compound to element.

AC16 cells. AC16 cells were plated under the same conditions. AC16 cells were indirectly treated with BEAS-2b cells-conditioned medium (CM) for 24h.

Cells Viability And Ros Detection

Cell viability was assessed by MTT assays. After 24h treatment, the MTT solution was added to the wells ($10\ \mu\text{L/well}$, 0.5% MTT). The cells continued to be incubated for four hours. The culture medium was removed and added dimethyl sulfoxide (DMSO, $150\ \mu\text{L/well}$) in 96-well plates. The absorbance was determined by Varioskan LUX (Thermo Fisher Scientific) at 490 nm.

After 24h treatment, intracellular ROS production was determined using a 2',7'-dichlorofluorescein diacetate (DCFH-DA, 10mM, Beyotim, China) assay following the manufacturer's instructions. Fluorescence was measured at 530 nm emission wavelength and 488 nm excitation wavelength by Varioskan LUX (Thermo Fisher Scientific).

Qrt-pcr

Total RNA was extracted from mice heart tissue and cells using TRIzol Reagent (TaKaRa, Dalian, China), and cDNA was synthesized. The target genes expression were performed by qPCR and determined by dividing the quantities of the target sequence by the quantity obtained for GAPDH.[14, 29] qPCR program: 10min at 95°C; 45 cycles (for 20s at 95°C; 20s at 55°C; 20s at 72°C); melting curve 10min at 75°C. The primer sequences are summarized in Table 2 SI.

Statistical Analysis

All data were evaluated the homogeneity of variance (HV) between different groups and processed by using one-way repeated analysis of variance (ANOVA) followed by Tukey's post hoc test and LSD test for significance. For non-normally distributed data, non-parametric Kruskal–Wallis one-way ANOVA test was used. The results were considered significant when $P < 0.05$.

Abbreviations

CVD:cardiovascular diseases; Col1a1: Collagen I; Col3a1: Collagen III;TGF β 1: Transforming growth factor β 1;NOX4: NADPH oxidase subunit 4;DCFHDA: 2',7'-dichlorodihydro fluorescein diacetate; PAHs: thepolycyclic aromatic hydrocarbons;DMEM: Dulbecco's modified eagle's medium; E/E ϕ : Ratio of peak

early diastolic flow velocities and peak early motion wave values; GAPDH: Glyceraldehyde 3-phosphatede hydrogenase; mRNA: Messenger ribonucleic acid; PM_{2.5}: Particulate matter with a diameter of 2.5 μm or less; qRT-PCR: Quantitative real time polymerase chain reaction; ROS: Reactive oxygen species

Declarations

Acknowledgements

Not applicable.

Funding

This study was supported by National Science Foundation of China (No. 22076109, 21777091, 22036005), the Natural Science Foundation of Shanxi Province (201901D211331), and Open Foundation of Beijing Key Laboratory of Occupational Safety and Health (2021).

Availability of data and materials

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contributions

Qisong Xing: Writing - Original Draft, Data Curation, Visualization, Investigation; Meiqiong Wu: Writing - Original Draft, Methodology, Investigation; Zhen Xue: Investigation; Nan Nan: Investigation; Zhipeng Yan: Investigation; Shuyue Li: Investigation; Guohua Qin: Conceptualization, Writing - Original Draft, Supervision, Project administration, Resources, Funding acquisition; Nan Sang: Resources, Funding acquisition

Ethics approval and consent to participate

All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Shanxi University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Dockery DW, Pope CA, Xu X, Spengler JD, Ware JH, Fay ME, et al. An Association between Air Pollution and Mortality in Six U.S. Cities. *N Engl J Med* [Internet]. Massachusetts Medical Society; 1993;329:1753–9. Available from: <https://doi.org/10.1056/NEJM199312093292401>
2. Martinelli N, Olivieri O, Girelli D. Air particulate matter and cardiovascular disease: A narrative review. *Eur J Intern Med* [Internet]. European Federation of Internal Medicine.; 2013;24:295–302. Available from: <http://dx.doi.org/10.1016/j.ejim.2013.04.001>
3. Thurston GD, Burnett RT, Turner MC, Shi Y, Krewski D, Lall R, et al. Ischemic Heart Disease Mortality and Long-Term Exposure to Source-Related Components of U.S. Fine Particle Air Pollution. *Environ Health Perspect*. 2016;124:785–94.
4. Thurston GD, Ito K, Lall R. A source apportionment of U.S. fine particulate matter air pollution. *Atmos Environ* [Internet]. Elsevier Ltd; 2011;45:3924–36. Available from: <http://dx.doi.org/10.1016/j.atmosenv.2011.04.070>
5. Sarnat SE, Winkler A, Schauer JJ, Turner JR, Sarnat JA. Fine particulate matter components and emergency department visits for cardiovascular and respiratory diseases in the St. Louis, Missouri-Illinois, metropolitan area. *Environ Health Perspect*. 2015;123:437–44.
6. Lu Y, Lin S, Fatmi Z, Malashock D, Hussain MM, Siddique A, et al. Assessing the association between fine particulate matter (PM_{2.5}) constituents and cardiovascular diseases in a mega-city of Pakistan. *Environ Pollut* [Internet]. 2019;252:1412–22. Available from: <https://www.sciencedirect.com/science/article/pii/S0269749119300247>
7. Wang M, Beelen R, Stafoggia M, Raaschou-Nielsen O, Andersen ZJ, Hoffmann B, et al. Long-term exposure to elemental constituents of particulate matter and cardiovascular mortality in 19 European cohorts: Results from the ESCAPE and TRANSPHORM projects. *Environ Int* [Internet]. Elsevier Ltd; 2014;66:97–106. Available from: <http://dx.doi.org/10.1016/j.envint.2014.01.026>
8. Chen H, Zhang Z, Van Donkelaar A, Bai L, Martin R V., Lavigne E, et al. Understanding the Joint Impacts of Fine Particulate Matter Concentration and Composition on the Incidence and Mortality of Cardiovascular Disease: A Component-Adjusted Approach. *Environ Sci Technol*. 2020;54:4388–99.
9. Brilla CG, Janicki JS, Weber KT. Impaired diastolic function and coronary reserve in genetic hypertension: Role of interstitial fibrosis and medial thickening of intramyocardial coronary arteries. *Circ Res*. 1991;69:107–15.
10. Weber KT, Brilla CG. Pathological Hypertrophy and Cardiac Interstitium. *Circulation*. 1991;83:1849–65.
11. Bansal SS, Ismahil MA, Goel M, Zhou G, Rokosh G, Hamid T, et al. Dysfunctional and Proinflammatory Regulatory T-Lymphocytes Are Essential for Adverse Cardiac Remodeling in Ischemic Cardiomyopathy. *Circulation*. 2019;139:206–21.
12. Li L, Zhang Q, Zhang X, Zhang J, Wang X, Ren J, et al. Microtubule associated protein 4 phosphorylation leads to pathological cardiac remodeling in mice. *EBioMedicine* [Internet]. The Authors; 2018;37:221–35. Available from: <https://doi.org/10.1016/j.ebiom.2018.10.017>

13. Brilla CG, Janicki JS, Weber KT. Cardioreparative effects of lisinopril in rats with genetic hypertension and left ventricular hypertrophy. *Circulation*. 1991;83:1771–9.
14. Xing Q, Wu M, Chen R, Liang G, Duan H, Li S, et al. Comparative studies on regional variations in PM_{2.5} in the induction of myocardial hypertrophy in mice. *Sci Total Environ* [Internet]. Elsevier B.V.; 2021;775:145179. Available from: <https://doi.org/10.1016/j.scitotenv.2021.145179>
15. Lind L, Araujo JA, Barchowsky A, Belcher S, Berridge BR, Chiamvimonvat N, et al. Commentary Key Characteristics of Cardiovascular Toxicants. 2021;129.
16. Zhang HH, Li Z, Liu Y, Xinag P, Cui XY, Ye H, et al. Physical and chemical characteristics of PM_{2.5} and its toxicity to human bronchial cells BEAS-2B in the winter and summer. *J Zhejiang Univ Sci B*. 2018;19:317–26.
17. Qi Z, Zhang Y, Chen ZF, Yang C, Song Y, Liao X, et al. Chemical identity and cardiovascular toxicity of hydrophobic organic components in PM_{2.5} [Internet]. *Ecotoxicol. Environ. Saf.* Elsevier Inc.; 2020. p. 110827. Available from: <https://doi.org/10.1016/j.ecoenv.2020.110827>
18. Jia YY, Wang Q, Liu T. Toxicity research of PM_{2.5} compositions in vitro. *Int J Environ Res Public Health*. 2017;14.
19. Nemmar A, Vanbilloen H, Hoylaerts MF, Hoet PHM, Verbruggen A, Nemery B. Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. *Am J Respir Crit Care Med*. 2001;164:1665–8.
20. Burch WM. Passage of inhaled particles into the blood circulation in humans. *Circulation*. 2002;106:411–4.
21. Li D, Li Y, Li G, Zhang Y, Li J, Chen H. Fluorescent reconstitution on deposition of PM 2.5 in lung and extrapulmonary organs. *Proc Natl Acad Sci U S A*. 2019;116:2488–93.
22. Pan X, Yuan X, Li X, Gao S, Sun H, Zhou H, et al. Induction of Inflammatory Responses in Human Bronchial Epithelial Cells by Pb²⁺-Containing Model PM_{2.5} Particles via Downregulation of a Novel Long Noncoding RNA Inc-PCK1-2:1. *Environ Sci Technol*. 2019;53:4566–78.
23. Backhaus T, Faust M. Predictive environmental risk assessment of chemical mixtures: A conceptual framework. *Environ Sci Technol*. 2012;46:2564–73.
24. Jin L, Xie J, Wong CKC, Chan SKY, Abbaszade G, Schnelle-Kreis J, et al. Contributions of City-Specific Fine Particulate Matter (PM_{2.5}) to Differential in Vitro Oxidative Stress and Toxicity Implications between Beijing and Guangzhou of China. *Environ Sci Technol*. 2019;
25. Kim YH, Tong H, Daniels M, Boykin E, Krantz QT, McGee J, et al. Cardiopulmonary toxicity of peat wildfire particulate matter and the predictive utility of precision cut lung slices. *Part Fibre Toxicol*. 2014;11:1–17.
26. Nan N, Duan H, Yang X, Wang L, Liu A, Chen R, et al. Atmospheric PM_{2.5}-bound polycyclic aromatic hydrocarbons in China's four cities: Characterization, risk assessment, and epithelial-to-mesenchymal transition induced by PM_{2.5}. *Atmos Pollut Res* [Internet]. Elsevier B.V.; 2021;101122. Available from: <https://doi.org/10.1016/j.apr.2021.101122>

27. Ku T, Zhang Y, Ji X, Li G, Sang N. PM_{2.5}-bound metal metabolic distribution and coupled lipid abnormality at different developmental windows. *Environ Pollut* [Internet]. Elsevier Ltd; 2017;228:354–62. Available from: <http://dx.doi.org/10.1016/j.envpol.2017.05.040>
28. Yue H, Ji X, Li G, Hu M, Sang N. Maternal Exposure to PM_{2.5} Affects Fetal Lung Development at Sensitive Windows. *Environ Sci Technol*. 2020;
29. Qin G, Xia J, Zhang Y, Guo L, Chen R, Sang N. Ambient fine particulate matter exposure induces reversible cardiac dysfunction and fibrosis in juvenile and older female mice. *Part Fibre Toxicol. Particle and Fibre Toxicology*; 2018;15:1–14.
30. Yang Y, Ruan Z, Wang X, Yang Y, Mason TG, Lin H, et al. Short-term and long-term exposures to fine particulate matter constituents and health: A systematic review and meta-analysis. *Environ Pollut* [Internet]. 2019;247:874–82. Available from: <https://www.sciencedirect.com/science/article/pii/S0269749118328847>
31. Hu X, He L, Zhang J, Qiu X, Zhang Y, Mo J, et al. Inflammatory and oxidative stress responses of healthy adults to changes in personal air pollutant exposure. *Environ Pollut* [Internet]. 2020;263:114503. Available from: <https://www.sciencedirect.com/science/article/pii/S026974911936717X>
32. Sinharay R, Gong J, Barratt B, Ohman-Strickland P, Ernst S, Kelly FJ, et al. Respiratory and cardiovascular responses to walking down a traffic-polluted road compared with walking in a traffic-free area in participants aged 60 years and older with chronic lung or heart disease and age-matched healthy controls: a randomised, crossover. *Lancet* [Internet]. The Author(s). Published by Elsevier Ltd. This is an Open Access article under the CC BY 4.0 license; 2018;391:339–49. Available from: [http://dx.doi.org/10.1016/S0140-6736\(17\)32643-0](http://dx.doi.org/10.1016/S0140-6736(17)32643-0)
33. Chen H, Zhang X, Zhang T, Li X, Li J, Yue Y, et al. Ambient PM Toxicity Is Correlated with Expression Levels of Specific MicroRNAs. *Environ Sci Technol*. 2020;54:10227–36.
34. Chen R, Hu B, Liu Y, Xu J, Yang G, Xu D, et al. Beyond PM_{2.5}: The role of ultrafine particles on adverse health effects of air pollution. *Biochim Biophys Acta - Gen Subj* [Internet]. 2016;1860:2844–55. Available from: <http://www.sciencedirect.com/science/article/pii/S0304416516300745>
35. Peled R. Air pollution exposure: Who is at high risk? *Atmos Environ* [Internet]. 2011;45:1781–5. Available from: <https://www.sciencedirect.com/science/article/pii/S1352231011000033>
36. Gu LZ, Sun H, Chen JH. Histone deacetylases 3 deletion restrains PM_{2.5}-induced mice lung injury by regulating NF- κ B and TGF- β /Smad2/3 signaling pathways. *Biomed Pharmacother* [Internet]. Elsevier Masson SAS; 2017;85:756–62. Available from: <http://dx.doi.org/10.1016/j.biopha.2016.11.094>
37. Lin Y, Ramanathan G, Zhu Y, Yin F, Rea ND, Lu X, et al. Pro-Oxidative and Proinflammatory Effects after Traveling from Los Angeles to Beijing: A Biomarker-Based Natural Experiment. *Circulation*. Lippincott Williams and Wilkins; 2019;140:1995–2004.
38. Qin G, Xia J, Zhang Y, Guo L, Chen R, Sang N. Ambient fine particulate matter exposure induces reversible cardiac dysfunction and fibrosis in juvenile and older female mice. *Part Fibre Toxicol. Particle and Fibre Toxicology*; 2018;15:1–14.

39. Chen M, Li B, Sang N. Particulate matter (PM_{2.5}) exposure season-dependently induces neuronal apoptosis and synaptic injuries. 2016;54.
40. Van Rooij E, Sutherland LB, Thatcher JE, DiMaio JM, Naseem RH, Marshall WS, et al. Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. *Proc Natl Acad Sci U S A*. 2008;105:13027–32.
41. Spurgeon DJ, Loft S, Hankard PK, Toal M, McLellan D, Fishwick S, et al. Effect of pH on metal speciation and resulting metal uptake and toxicity for earthworms. *Environ Toxicol Chem*. 2006;25:788–96.
42. Hellack B, Yang A, Cassee FR, Janssen NAH, Schins RPF, Kuhlbusch TAJ. Intrinsic hydroxyl radical generation measurements directly from sampled filters as a metric for the oxidative potential of ambient particulate matter. *J Aerosol Sci* [Internet]. Elsevier; 2014;72:47–55. Available from: <http://dx.doi.org/10.1016/j.jaerosci.2014.02.003>
43. Li XY, Gilmour PS, Donaldson K, MacNee W. Pro-inflammatory effect of particulate air pollution (PM₁₀) in vivo and in vitro. *Thorax*. 1996;51.
44. Newby DE, Mannucci PM, Tell GS, Baccarelli AA, Brook RD, Donaldson K, et al. Expert position paper on air pollution and cardiovascular disease. *Eur Heart J*. 2015;36:83–93.
45. Jiang J, Li Y, Liang S, Sun B, Shi Y, Xu Q, et al. Combined exposure of fine particulate matter and high-fat diet aggravate the cardiac fibrosis in C57BL/6J mice. *J Hazard Mater* [Internet]. Elsevier; 2020;391:122203. Available from: <https://doi.org/10.1016/j.jhazmat.2020.122203>
46. Jin L, Xie J, Wong CKC, Chan SKY, Abbaszade G, Schnelle-Kreis J, et al. Contributions of City-Specific Fine Particulate Matter (PM_{2.5}) to Differential in Vitro Oxidative Stress and Toxicity Implications between Beijing and Guangzhou of China. *Environ Sci Technol*. 2019;
47. Liu Y, Zhou T, Sun L, Wang H, Zhou L. The effect of Notch signal pathway on PM_{2.5}-induced Muc5ac in Beas-2B cells. *Ecotoxicol Environ Saf* [Internet]. Elsevier Inc.; 2020;203:110956. Available from: <https://doi.org/10.1016/j.ecoenv.2020.110956>
48. Badran G, Ledoux F, Verdin A, Abbas I, Roumie M, Genevray P, et al. Toxicity of fine and quasi-ultrafine particles: Focus on the effects of organic extractable and non-extractable matter fractions. *Chemosphere*. 2020;243:1–11.
49. Lepers C, André V, Dergham M, Billet S, Verdin A, Garçon G, et al. Xenobiotic metabolism induction and bulky DNA adducts generated by particulate matter pollution in BEAS-2B cell line: geographical and seasonal influence. *J Appl Toxicol*. 2014;34:703–13.
50. Rao X, Zhong J, Brook RD, Rajagopalan S. Effect of Particulate Matter Air Pollution on Cardiovascular Oxidative Stress Pathways. *Antioxidants Redox Signal*. 2018;28:797–818.
51. Badran G, Verdin A, Grare C, Abbas I, Achour D, Ledoux F, et al. Toxicological appraisal of the chemical fractions of ambient fine (PM_{2.5-0.3}) and quasi-ultrafine (PM_{0.3}) particles in human bronchial epithelial BEAS-2B cells. *Environ Pollut*. 2020;263:1–12.
52. Wang W, Liu M, Wang T, Song Y, Zhou L, Cao J, et al. Sulfate formation is dominated by manganese-catalyzed oxidation of SO₂ on aerosol surfaces during haze events. *Nat Commun* [Internet]. Springer

US; 2021;12:1–10. Available from: <http://dx.doi.org/10.1038/s41467-021-22091-6>

53. Feng L, Yang X, Asweto CO, Wu J, Zhang Y, Hu H, et al. Low-dose combined exposure of nanoparticles and heavy metal compared with PM_{2.5} in human myocardial AC16 cells. *Environ Sci Pollut Res. Environmental Science and Pollution Research*; 2017;24:27767–77.
54. Wende AR, Young ME, Chatham J, Zhang J, Rajasekaran NS, Darley-Usmar VM. Redox biology and the interface between bioenergetics, autophagy and circadian control of metabolism. *Free Radic Biol Med* [Internet]. Elsevier; 2016;100:94–107. Available from: <http://dx.doi.org/10.1016/j.freeradbiomed.2016.05.022>
55. Li R, Qiu X, Xu F, Lin Y, Fang Y, Zhu T. Macrophage-Mediated Effects of Airborne Fine Particulate Matter (PM_{2.5}) on Hepatocyte Insulin Resistance in Vitro. *ACS Omega*. 2016;1:736–43.

Figures

Figure 1

PM_{2.5} induces myocardial fibrosis in mice. (A) Representative images (400× objective) of heart sections stained with Masson's Trichrome from the control and Taiyuan, Beijing, Hangzhou, and Guangzhou PM_{2.5} groups. Scale bar = 50 μ m. (B) The collagen deposition area was quantified. (C) The mRNA levels of Col1a1, Col3a1, and TGF- β in the heart were detected by qPCR. The data are expressed as the means $\pm$ SEM (n = 3). *, $P < 0.05$ **, $P < 0.01$ ***, $P < 0.001$ vs. control group by one-way ANOVA and Tukey's post hoc tests. When $P < 0.05$, the differences were considered significant. Groups not sharing the same letter are significantly different at $P < 0.05$.

Figure 2

Correlation between the components of PM_{2.5} from four cities and the relative index in mice. (A), Correlation between PAHs and heavy metals in PM_{2.5} and the relative heart function-related index of the ratio of the blood flow velocity of the mitral valve to the velocity of the mitral valve annulus in the early diastole (E/E') and relative mRNA expression of Col1a1, Col3a1, and TGF-β by Pearson correlation coefficient. Green represents a positive correlation between gene expression and components, and red represents a negative correlation. The relative levels of E/E' and Col1a1 were concentration-dependent manner with the concentration of Pb (B), and Cr (C), respectively. The E/E' was the concentration-dependent manner with the concentration of Mn (D). *, represents significant positive correlations, $P < 0.05$, and $0.95 < r < 0.99$; **, $P < 0.01$, and $0.99 < r < 1.00$; #, represents significant negative correlations, $P < 0.05$, and $0.95 < r < 0.99$; ##, $P < 0.01$, and $0.99 < r < 1.00$.

Figure 3

Direct effects of critical components on ROS production in BEAS-2b cells. (A) Effects of five candidate critical components with or without PM_{2.5} from Guangzhou on ROS in BEAS-2b cells (n=6). (B), Effects of four of the five candidate critical components with or without PM_{2.5} from Guangzhou on ROS in BEAS-2b cells (n=8). (C), Effects of three or two of the five candidate critical components with or without PM_{2.5} from Guangzhou on ROS in BEAS-2b cells (n=6). (D), Effects of a single candidate critical component with or without PM_{2.5} from Guangzhou on ROS in BEAS-2b cells (n=6). Statistical was analyzed using one-way ANOVA, followed by Tukey's post hoc tests. Groups not sharing the same letter are significantly different at $P < 0.05$.

Figure 4

Indirect effects of critical components on mRNA expression of myocardial fibrosis and vital regulatory factors in AC16 cells. The mRNA expression of Col1a1 (A) and TGF- β (B) in the vehicle control and Taiyuan, Guangzhou PM_{2.5} and candidate critical components with or without PM_{2.5} from Guangzhou groups (n=6). The significance of the differences between the data was analyzed using one-way ANOVA, followed by a LSD test. Groups not sharing the same letter are significantly different at $P < 0.05$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GraphicalAbstract.png](#)
- [Supplementfile.docx](#)