Original Paper

Pulmonary Exposure to Particulate Matter (PM_{2.5}) Affects the Sensitivity to Myocardial Ischemia/Reperfusion Injury Through Farnesoid-X-Receptor-Induced Autophagy

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Key Words

PM_{2.5} • Lung reperfusion • MI/RI • FXR • Autophagy

Abstract

Background/Aims: The purpose of this study was to investigate the effect of administering particulate matter (PM_{2.5}) to the lungs on the sensitivity to myocardial ischemia/reperfusion injury (MI/RI) and the role of farnesoid-X-receptor (FXR)-induced autophagy in this process.

Methods: Male Sprague Dawley (SD) rats were subjected to 45 min of ischemia, and the lungs were exposed to 2.0 mg of PM_{2.5} in 0.3 mL of normal saline 5 min before reperfusion. After 24 h of reperfusion, the blood and myocardium were collected, and the myocardial infarct sizes, activities of serum creatine kinase (CK) and lactate dehydrogenase (LDH), and levels of serum high sensitivity C-reactive protein (hsCRP), interleukin-4 (IL-4), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), cardiac troponin T (cTnT), P-selectin and D-dimer were measured *via* biochemical analysis. Additionally, the myeloperoxidase (MDA) content and superoxide dismutase (SOD) activity were measured in myocardial tissues *via* biochemical analysis, and the levels of reactive oxygen species (ROS), autophagosomes, microtubule-associated protein 1 light chain 3 (LC-I and II), macrophage inflammatory protein-2 (MIP-2) and farnesoid-X-receptor (FXR) were determined in myocardial tissues *via* biochemical analysis, immunohistochemical and biochemical techniques and western blot. In addition, the myocardial infarct sizes, LC-I and II expressions were determined in myocardial tissues through FXR^{-/-} and WT mice. **Results:** Levels of CK, LDH, hsCRP, IL-6, TNF-α, cTnT, P-selectin and D-dimer, MDA and infarct sizes were higher in I/R group than that in Sham group, and Levels of IL-4 and SOD were lower in I/R group than that in Sham group; ROS, autophagosomes, LC-3I, LC-3II, MIP-2 and FXR expressions were higher in I/R group than that in Sham group. Levels of CK, LDH, hsCRP, IL-6, TNF-α, cTnT, P-selectin and D-dimer, MDA and infarct sizes were higher in PM_{2.5} group than that in I/R group, and Levels of IL-4 and SOD were lower in PM_{2.5} group than that in I/R group; ROS, autophagosomes, LC-3I, LC-3II, MIP-2 and FXR expressions were

higher in PM_{2.5} group than that in I/R group. **Conclusions:** PM_{2.5} post-treatment exacerbated myocardial injury partly through upregulation of FXR to induce autophagy compared to MI/RI.

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Published by S. Karger AG, Basel

Introduction

The relationship between air particulate matter (PM_{2.5}) and angiocardiopathy is a key focus of environmental epidemiology research [1]. Numerous studies have confirmed that PM_{2.5} causes a large adverse effects on subjects in the range of 18-82 years of age who have cardiopulmonary disease [2-7]. Although the very small particles of PM_{2.5} that deposit on the alveolar epithelium of the lung tissues are partially removed through the lymphatic system, they can still affect the capillaries directly and thereby impact the inflammatory cytokines and blood vessels of the endothelium, which can result in damage to the extrapulmonary organs (particularly the cardiovascular system) [8-10]. In addition, many studies have demonstrated that PM_{2.5} can induce and promote oxidative stress-mediated damage to human lung A549 epithelial cells [11, 12]. Furthermore, Pope et al. and Schwartz have discovered that for each 10 µg/m³ increase in the fine particulate matter in the atmosphere, the incidence of acute ischemic coronary syndrome increases by 4.5% [13, 14]. A large number of studies have suggested that the cardiovascular system is an important target organ of PM_{2.5} exposure.

Autophagy is an important pathological and physiological phenomena in which external stimuli or starvation causes components or structures of eukaryotic cells to be enveloped in double membrane vesicles (autophagic vesicles) that subsequently fuse with the lysosomes and are eventually hydrolyzed by the lysosomes [15]. Autophagy, as one of the lysosomal degradation pathways, plays an important role in the half-life of protein degradation and cell renewal [16]. To a certain extent, autophagy can maintain the body's energy metabolism and reduce the external stimulation-induced damage to protect the body, and under normal circumstances, autophagy is present at low levels in the cells [17]. However, excessive levels of autophagy can cause cell death [17]. Studies have also shown that the levels of reactive oxygen species in marine mussels can be increased by herbicides and polycyclic aromatic hydrocarbons in the environment, which can lead to autophagy. These species can play an important role in the process of autophagy [18]. Some publications have shown that PM_{2.5} can induce oxidative stress and autophagy in lung epithelial MLE-12 cells [19]. Interestingly, many studies have confirmed that PM_{2.5} exposure induces autophagy in mouse aortic endothelial cells, which leads to endothelial apoptosis and dysfunction in the heart [19-21].

The farnesoid-X-receptor (FXR) is a member of the metabolic nuclear receptor superfamily [22]. It was discovered that FXR is expressed in the liver and gastrointestinal tract to regulate the homeostasis of cholesterol and bile acid [22]. More importantly, recent discoveries have suggested that FXR is present in the vasculature, where it has significant effects on the vascular physiology/pathology [23, 24]. Recent evidence has shown that expression of FXR in the myocardial tissue mediates apoptosis and that it contributes to myocardial ischemia reperfusion injury [25]. However, the potential role of FXR in the induction of autophagy and the aggravated sensitivity to myocardial ischemia/reperfusion injury (MI/IR) remains largely unknown.

In this study, we investigated FXR expression in cardiac tissues and report our findings that post ischemia exposure to particulate matter (PM_{2.5}) induces autophagy through FXR that aggravates the sensitivity to MI/IR.

Materials and Methods

Animals and reagents

Male Sprague-Dawley (SD) rats (180-200 g) were purchased from Jiaxing University Medical College, Jiaxing, China. FXR^{-/-} and wild type (WT) male mice were obtained from Laboratory of Jiaxing University Medical College, Jiaxing, China. All animal procedures were approved by the Jiaxing Association of

Accreditation of Laboratory Animal Care-certified Institutional Animal Care and Use Committee. The investigation conformed to the guide for the care and use of laboratory animals published by the US National Institutes of Health (NIH Publication updated in 2011). All reagents were purchased from Sigma.

PM_{2.5} and PM exposure through lung instillation

Air-pollution particles smaller than 2.5 µm were collected on teflon-impregnated glass fibers from an industrial area in Lanzhou, China. The handling of the air-pollution particles was described in previous documents [26]. The animals were anesthetized with 1% pentobarbital sodium (50 mg/kg), intubated with a tracheal tube, and instilled with 2.0 mg PM_{2.5} suspended in 0.3 mL normal saline.

Animal experimental design

A total of 30 SD rats were randomly assigned to one of three groups, each with 10 rats): (1) Sham; (2) I/R group: 45 min of ischemia followed by 24 h reperfusion; (3) I/R+PM_{2.5} group: 45 min of ischemia followed by the administration of 2.0 mg of PM_{2.5} in 0.3 mL normal saline 5 min before the 24 h reperfusion.

Surgical procedures of myocardial ischemia/reperfusion injury (MI/RI)

The rats were anesthetized with 1% pentobarbital sodium (50 mg/kg) by intraperitoneal injection. The rats were subjected to left thoracotomy and pericardiotomy, and the left circumflex artery was occluded for 45 min with 5-0 silk thread to induce ischemia followed by reperfusion for 24 h. Blood samples were collected and centrifuged at 3600×g for 20 min to harvest the sera. The hearts of animals were immediately excised and stored at -20 °C for the analyses described below.

Quantification of infarct size (SD rats)

The quantification of infarct size was performed using the method previously described [27, 28]. The hearts were stained by infusing 1 mL of Evan's blue via the aorta, and the unbound stain was washed out with Krebs bicarbonate buffer. The heart was then promptly removed and stored overnight at -20°C. Then, 2-mm thick sections of the hearts were cut, stained with 1% 2, 3,5-triphenyltetrazolium chloride (TTC) in phosphate buffer (pH 7.4) for 20 min at 37 °C and then fixed with 10% formalin. The viable myocardium was stained dark red, and the nonviable infarcted myocardium was pale. The slices were photographed, and the myocardial infarct sizes were measured as percentage of total area, calculated with Adobe Photoshop (CS4, Adobe Systems Inc.).

Assessment of serum lactate dehydrogenase (LDH) and creatine kinase (CK) activities

As described previously [15, 29, 30], the serum activities of LDH and CK were measured using commercial assay kits according to manufacturer's instructions. The activity of LDH was expressed as U of LDH/L and the activity of CK was expressed as U of CK/L.

Assessment of serum interleukin-4 (IL-4), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), cardiac troponin T (cTnT), high sensitivity C-reactive protein (hsCRP), P-selectin and D-dimer

Serum IL-4, IL-6, TNF-α, cTnT, hsCRP, P-selectin and D-dimer levels were performed as previously described [31-33]. The blood samples for the assessments of IL-4, IL-6, TNF-α, cTnT, hsCRP, P-selectin and D-dimer were obtained postoperatively after 24 h of reperfusion. The serum levels of IL-4, IL-6, TNF-α, cTnT, hsCRP, P-selectin and D-dimer were measured using commercial assay kits according to manufacturers' instructions. The level of IL-4 was expressed as pg of IL-4/L, the level of IL-6 was expressed as ng of IL-6/mL, the level of TNF-α was expressed as ng of TNF-α/mL, the level of cTnT was expressed as µg of TNF-α/L, the level of hsCRP was expressed as mg of hsCRP/L, the level of P-selection was expressed as ng of P-selectin/L, and the level of D-dimer was expressed as mg of D-dimer/L.

Assessment of superoxide dismutase (SOD) activity and myeloperoxidase (MDA) levels

As previously described in the literature [34-38], the levels of SOD and MDA in the tissues were measured to assess lipid peroxidation. The heart was collected after reperfusion for 24 h, and the tissue was washed to remove the blood, homogenized (100 mg) in ice-cold normal saline to produce a 10% homogenate, then centrifuged at 3600 ×g for 20 min to harvest the supernatant. The SOD activity and MDA contents were measured using the xanthine oxidase and thiobarbituric acid methods, respectively. The absorbances were

measured at 550 and 532 nm, respectively. The levels of the lipid peroxides were expressed as U of SOD/g protein and mmol of MDA/g protein.

Reactive oxygen species (ROS) expression

As previously described [36], after 24 h of reperfusion, the rats were anesthetized with 1% pentobarbital sodium (50 mg/kg), and CM-H2DCFDA (100 µg) was injected into the myocardial circulation. At 45 min after the CM-H2DCFDA injection, the myocardium was harvested and immersed in 4% paraformaldehyde for approximately 24 h. After treatment with 20% sucrose for approximately 12 h, the myocardium was immediately frozen in liquid nitrogen. Cryostat sections were cut at -20 °C, and the sections were placed on Star-Frost adhesive slides and air-dried for approximately 3 min. The sections were washed in PBS and stained with DAPI for the qualitative evaluation of the ROS levels by fluorescence microscopy. The quantitative evaluation of the ROS in isolated mitochondria was performed using a spectrofluorometric amplex red H₂O₂/peroxidase assay kit with excitation and emission wavelengths of 544 and 590 nm, respectively.

Autophagosome estimation

Autophagosomes were measured using the previously described method [19, 39]. Briefly, under sterile conditions, the heart was removed from the rat, and the tissue was chopped and then digested with a mixture of 0.6 g/L trypsin and 2.5 g/L collagenase to produce a single cell suspension. The suspension was centrifuged for 10 min (1500 r/min), and then resuspended in 150 mL/L calf serum in DMEM. The cells were inoculated into the culture flask and cultured in a CO₂ incubator. Then, the cells were processed according to documented methods [19], and the autophagosomes were observed using laser confocal microscopy.

Immunohistochemical estimation in Sham, I/R and I/R+PM_{2.5} group

Macrophage inflammatory protein-2 (MIP-2) expression was measured using the previously described method [40]. Sections with a thickness of 5 µm were obtained from the myocardial sections and mounted on poly-l-lysine-coated slides. The sections were immersed in 0.3% H₂O₂ for 20 min and washed with PBS. The sections were then incubated in rabbit anti-MIP-2 polyclonal antiserum for 1 h (1:100 dilution). After the primary incubation and three rinses in PBS, the sections were incubated in biotinylated goat anti-rabbit IgG for 10 min (1:2000 dilution). Following an incubation in the substrate chromagen solution for 15 min, all sections were washed with PBS and distilled water, mounted in glycerol and subsequently examined microscopically. The myocardial sections that stained positively for MIP-2 were assessed and compared among the groups.

Estimation of left ventricular function

A catheter was inserted into the left ventricle, and the left ventricular ejection fraction (LVEF), left ventricular end-diastolic pressure (LVEDP) and left ventricular pressure maximal rate of rise (+dp/dt_{max}) were measured through echocardiography. The detailed method was essentially as described in the previous literature [41, 42].

Apoptosis index

The detailed method was essentially as described in the previous literature [27]. Briefly, the myocardial (5 µm) sections were stained using terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) according to the manufacturer's instruction and measured using light microscopy. The apoptosis index was counted as the percentage of stained cells, specifically, the number of apoptotic cells×100/total number of nucleated cells.

Western blot analysis

The detailed method was essentially as described in the previous literature [43, 44]. Briefly, the myocardial tissues were homogenized in protein lysis buffer. The homogenates were resolved on polyacrylamide SDS gels and electrophoretically transferred to polyvinylidene difluoride membranes. The membranes were blocked with 3% BSA, then incubated with the primary Abs against active protein 1 light chain 3I (LC3I), protein 1 light chain 3II (LC3II), or farnesoid-X-receptor (FXR) and subsequently with alkaline phosphatase-conjugated secondary Abs. They were developed using 5-bromo-4-chloro-3-

indolyl phosphate/nitroblue tetrazolium. The blots were also stained with anti- β -actin Ab, and the levels of proteins were normalized with respect to the β -actin band densities.

Immunohistochemical analysis in Sham, I/R, I/R+PM_{2.5}, I/R+Z-Gugg and I/R+PM_{2.5}+Z-Gugg group (SD rats)

50 SD rats were randomly assigned to one of five groups, each with 10 rats): (1) Sham; (2) I/R group; (3) I/R+PM_{2.5} group; (4) I/R+Z-guggulsterone (FXR antagonist, Z-Gugg) group: Z-Gugg (100 mg/kg/d) was administrated through intragastric injection for 7 days before I/R; (5) I/R+PM_{2.5}+Z-Gugg group: Z-Gugg (100 mg/kg/d) was administrated through intragastric injection for 7 days before I/R+PM_{2.5}. According to the previous literature, FXR, LC3I and LC3II expressions were measured by immunohistochemical method [45].

Quantification of infarct size and immunohistochemical analysis (FXR^{-/-} and WT mice)

Animal experimental design: a total of 30 FXR^{-/-} mice and 30 WT mice were assigned to one of six groups, each with 10 rats): (1) WT+sham group; (2) FXR^{-/-}+sham group; (3) WT+I/R group: 45 min of ischemia followed by 24 h reperfusion; (4) WT+I/R+PM_{2.5} group: 45 min of ischemia followed by the administration of 2.0 mg of PM_{2.5} in 0.3 mL normal saline 5 min before the 24 h reperfusion; (5) FXR^{-/-}+I/R group: the same as the (3); (6) FXR^{-/-}+I/R+PM_{2.5} group: the same as the (4). The surgical procedures of MI/RI was the same as the above (SD rats). The quantification measurement of infarct size was the same as the quantification of infarct size (SD rats). The measurement of LC3I and LC3II expressions were the same as the above (SD rats).

Statistical analysis

All data were expressed as the mean values $\pm$ standard deviation (SD). Analysis of variance (ANOVA) test and Student's t test were used to evaluate the statistical significance. A value of less than 0.01 ($P < 0.01$) was used for statistical significance.

Results

Infarct size (SD rats)

The TTC staining of the hearts is shown in Fig. 1. The injury to the hearts induced by I/R was significantly greater than that in the Sham rats, whereas the injury to the hearts subjected to I/R+PM_{2.5} was significantly higher than that of the I/R rats. The corresponding quantitative analysis is shown in Fig. 1.

Serum LDH, CK, hsCRP, IL-4, IL-6, TNF- α , P-selectin, D-dimer, cTnT levels and tissue SOD activity, MDA content

The results of CK and LDH presented in Fig. 2A show, the serum activity of LDH in the I/R rats was significantly higher than that of the Sham rats. The activity of LDH was 611 $\pm$ 38 U/L in the Sham rats whereas the activity of LDH reached 1398 $\pm$ 51 U/L in the I/R rats. The values following administration of I/R+PM_{2.5} were significantly higher than those for the I/R rats. The LDH activity in the I/R+PM_{2.5} rats was 1588 $\pm$ 49 U/L. The serum CK activity in the I/R rats was significantly greater than that of the Sham rats. The CK activity was 204 $\pm$ 16 U/L in the sham rats whereas the CK

Fig. 1. Infarct size estimation. Note: The sizes of the infarcts in the rats of the Sham, I/R, and I/R+PM_{2.5} groups were determined after 24 h, and the results are expressed as the means $\pm$ SD. Significant differences between the Sham and I/R groups and between the I/R and I/R+PM_{2.5} groups are indicated as * $P < 0.01$ and ** $P < 0.01$, respectively. Abbreviations: I/R: ischemia/reperfusion; PM_{2.5}: particulate matter; SD: standard deviation.

Fig. 2. Activities of CK and LDH (A); hsCRP level (B); IL-4 level (C); IL-6 level (D); TNF- α level (E); P-selectin level (F); D-dimer level (G); cTnT level (H); SOD activity and MDA levels (I). Note: The activities of CK and LDH, hsCRP level, IL-4 level, IL-6 level, TNF- α level, P-selectin level,

el, D-dimer level, cTnT level, SOD activity and MDA level of rats of the Sham, I/R, and I/R+PM_{2.5} groups were determined after 24 h, and the results are expressed as the means $\pm$ SD. (Fig. 2A) A significant increase from the sham, I/R, and I/R+PM_{2.5} group was denoted by * P <0.01 and ** P <0.01, respectively; (Fig. 2B) A significant increase from the Sham, I/R, and I/R+PM_{2.5} group was denoted by * P <0.01 and ** P <0.01, respectively; (Fig. 2C) A significant decrease from the Sham, I/R, and I/R+PM_{2.5} group was denoted by * P <0.01 and ** P <0.01, respectively; (Fig. 2D) A significant increase from the Sham, I/R, and I/R+PM_{2.5} group was denoted by * P <0.01 and ** P <0.01, respectively; (Fig. 2E) A significant increase from the Sham, I/R, and I/R+PM_{2.5} group was denoted by * P <0.01 and ** P <0.01, respectively; (Fig. 2F) A significant increase from the Sham, I/R, and I/R+PM_{2.5} group was denoted by * P <0.01 and ** P <0.01, respectively; (Fig. 2G) A significant increase from the Sham, I/R, and I/R+PM_{2.5} group was denoted by * P <0.01 and ** P <0.01, respectively; (Fig. 2H) A significant increase from the Sham, I/R, and I/R+PM_{2.5} group was denoted by * P <0.01 and ** P <0.01, respectively. (Fig. 2I) A significant decrease from the Sham, I/R, and I/R+PM_{2.5} group was denoted by * P <0.01 and ** P <0.01, respectively (SOD); A significant increase from the Sham, I/R, and I/R+PM_{2.5} group was denoted by * P <0.01 and ** P <0.01, respectively (MDA). Abbreviations: I/R: ischemia/reperfusion; PM_{2.5}: particulate matter; SD: standard deviation; CK: creatine kinase; LDH: lactate dehydrogenase; hsCRP: high sensitivity C-reactive protein; IL-4: interleukin-4; IL-6: interleukin-6; TNF- α : tumor necrosis factor- α ; cTnT: cardiac troponin T; SOD: superoxide dismutase; MDA: myeloperoxidase.

activity reached 612 ± 24 U/L in the I/R rats. The values in the rats subjected to I/R+PM_{2.5} were significantly greater than those of the I/R rats. The activity of CK was 799 ± 19 U/L in the I/R+PM_{2.5} rats.

The result of hsCRP presented in Fig. 2B show, the serum level of hsCRP in the I/R rats was significantly greater than that of the Sham rats. The level of hsCRP was 0.16 ± 0.08 mg/L in the Sham rats whereas the level of hsCRP reached 1.02 ± 0.11 mg/L in the I/R rats. The values for the I/R+PM_{2.5} rats were significantly higher than those of the I/R rats. The level of hsCRP was 1.28 ± 0.06 mg/L in the I/R+PM_{2.5} rats.

The result of IL-4 presented in Fig. 2C show, the serum level of IL-4 in the I/R rats was significantly lower than that of the Sham rats. The level of IL-4 was 2.15 ± 0.19 pg/L in the Sham rats but reached 1.82 ± 0.14 pg/L in the I/R rats. The values for the I/R+PM_{2.5} rats were significantly lower than those of the I/R rats. The level of IL-4 was 1.59 ± 0.16 pg/L in the I/R+PM_{2.5} rats.

The result of IL-6 presented in Fig. 2D show, the serum level of IL-6 in the I/R rats was significantly greater than that of the Sham rats. The level of IL-6 was 127.5 ± 13.2 ng/mL in the Sham rats whereas the level of IL-6 reached 298.6 ± 18.5 ng/mL in the I/R rats. The values for the I/R+PM_{2.5} rats were significantly greater than those of the I/R rats. The level of IL-6 was 403.8 ± 20.4 ng/mL in the I/R+PM_{2.5} rats.

The result of TNF- α presented in Fig. 2E show, the serum level of TNF- α on I/R rats was significantly greater than that of the Sham rats. The level of TNF- α was 1.18 ± 0.16 ng/mL in the Sham rats whereas the level of TNF- α reached 3.24 ± 0.35 ng/mL in the I/R rat. The values for the I/R+PM_{2.5} rats were significantly greater than those of the I/R rats. The level of TNF- α was 5.08 ± 0.29 ng/mL in the I/R+PM_{2.5} rats.

The result of P-selectin presented in Fig. 2F show, the serum level of P-selectin in the I/R rats was significantly greater than that of the Sham rats. The level of P-selectin was 4.08 ± 1.86

Fig. 3. LVEF (A), LVEDP (B) and +dp/dt_{max} (C). Note: The LVEF, LVEDP and +dp/dt_{max} in the rats of the Sham, I/R, and I/R+PM_{2.5} groups were determined after 24 h, and the results are expressed as the means ± SD. (Fig. 3A) A significant decrease from the Sham, I/R, and I/R+PM_{2.5} group was denoted by *P<0.01 and **P<0.01, respectively; (Fig. 3B) A significant increase from the Sham, I/R, and I/R+PM_{2.5} group was denoted by *P<0.01 and **P<0.01, respectively; (Fig. 3C) A significant decrease from the sham, I/R, and I/R+PM_{2.5} group was denoted by *P<0.01 and **P<0.01, respectively. Abbreviations: I/R: ischemia/reperfusion; PM_{2.5}: particulate matter; SD: standard deviation; LVEF: left ventricular ejection fraction; LVEDP: left ventricular end-diastolic pressure; +dp/dt_{max}: left ventricular pressure maximal rate of rise.

ng/L in the Sham rats whereas the level of P-selection reached 16.24±3.41 ng/L in the I/R rats. The values for the I/R+PM_{2.5} rats were significantly greater than those of the I/R rats. The level of P-selectin was 21.19±2.87 ng/L in the I/R+PM_{2.5} rats.

The result of D-dimer presented in Fig. 2G show, the serum level of D-dimer in the I/R rats was significantly greater than that of the Sham rats. The level of D-dimer was 0.24±0.06 mg/L in the Sham rats whereas the level of D-dimer reached 1.18±0.09 mg/L in the I/R rats. The values for the I/R+PM_{2.5} rats were significantly greater than those of the I/R rats. The level of D-dimer was 1.36±0.04 mg/L in the I/R+PM_{2.5} rats.

The result of cTnT presented in Fig. 2H show, the serum level of cTnT in the I/R rats was significantly higher than that of the Sham rats. The level of cTnT was 0.11±0.04 µg/L in the sham rats whereas the level of cTnT reached 0.33±0.09 µg/L in the I/R rats. The values for the I/R+PM_{2.5} rats were significantly greater than those of the I/R rats. The level of cTnT was 0.51±0.07 µg/L in the I/R+PM_{2.5} rats.

The results of SOD and MDA presented in Fig. 2I show, the activity of SOD in the I/R rats was significantly lower than that in the Sham rats. The activity of SOD was 13.7±3.1 U/g in the Sham rats whereas the activity of SOD reached 8.5±2.2 U/g in the I/R rats. The values for the I/R+PM_{2.5} rats were significantly lower than those of the I/R rats. The activity of SOD was 6.2±1.8 U/g in the I/R+PM_{2.5} rats. The serum level of MDA in the I/R rats was significantly higher than that of the Sham rats. The level of MDA was 1.5±0.2 mmol/g in the Sham rats whereas the level of MDA reached 4.5±1.3 mmol/g in the I/R rat. The values in the I/R+PM_{2.5} rats were significantly greater than those of the I/R rats. The level of MDA was 6.1±0.7 mmol/g in the I/R+PM_{2.5} rats.

Left ventricular function

The results presented in Fig. 3A show, the LVEF in the I/R rats was significantly lower than that of the Sham rats. The LVEF was 63.21±10.21% in the Sham rats whereas the LVEF reached 40.26±8.74% in the I/R rats. The values in the I/R+PM_{2.5} rats were significantly lower than those of the I/R rats. The LVEF was 34.27±12.33% in the I/R+PM_{2.5} rats.

The results presented in Fig. 3B show, the LVEDP on I/R rats was significantly greater than that of the Sham rats. The LVEDP was 3.21±1.86 mmHg in the Sham rats whereas the LVEDP reached 10.87±2.32 mmHg in the I/R rats. The values in the I/R+PM_{2.5} rats were

Fig. 4. ROS and autophagosome and MIP-2 expression. Note: The levels of ROS and the autophagosome and MIP-2 expression in the rats of the Sham, I/R, and I/R+PM_{2.5} groups were measured after 24 h. The levels of ROS, autophagosomes and MIP-2 in the I/R rats were significantly higher than those on Sham rats; the levels of ROS, autophagosomes and MIP-2 of the I/R+PM_{2.5} rats were significantly higher than those of the I/R rats. Abbreviations: I/R: ischemia/reperfusion; PM_{2.5}: particulate matter; ROS: reactive oxygen species; MIP-2: macrophage inflammatory protein-2.

significantly greater than those of the I/R rats. The LVEDP was 14.51 ± 1.78 mmHg in the I/R+PM_{2.5} rats.

The results presented in Fig. 3C show, the $+dp/dt_{\max}$ on I/R rats was significantly lower than that of the Sham rats. The $+dp/dt_{\max}$ was 7025.16 ± 134.56 mmHg/s in the Sham rats whereas the $+dp/dt_{\max}$ reached 4819.65 ± 145.67 mmHg/s in the I/R rat. The values in the I/R+PM_{2.5} rats were significantly lower than those of the I/R rats. The $+dp/dt_{\max}$ was 4211.23 ± 165.38 mmHg/s in the I/R+PM_{2.5} rats.

Fig. 5. Apoptosis index. Note: The apoptosis index in the rats of the Sham, I/R, and I/R+PM_{2.5} groups were determined after 24 h, and the results are expressed as the means $\pm$ SD. A significant increase from the Sham, I/R, and I/R+PM_{2.5} group was denoted by * $P < 0.01$ and ** $P < 0.01$, respectively. Abbreviations: I/R: ischemia/reperfusion; PM_{2.5}: particulate matter; SD: standard deviation.

ROS, autophagosome and MIP-2 estimations

The results presented in Fig. 4 show, the levels of ROS, autophagosomes and MIP-2 in the I/R rats were significantly greater than those of the Sham rats. The levels of ROS, autophagosomes and MIP-2 in the I/R+PM_{2.5} rats were significantly greater than those of the I/R rats.

Apoptosis index

The results presented in Fig. 5 show, the apoptosis index in the I/R rats was significantly greater than that of the Sham rats. The value of the apoptosis index in the I/R+PM_{2.5} rats was significantly greater than that of the I/R rats. The corresponding quantitative analysis is shown in Fig. 5.

Fig. 6. FXR, LC3I and LC3II expressions Sham, I/R, and I/R+PM_{2.5} groups. Note: The FXR, LC3I and LC3II expressions of the rats of the Sham, I/R, and I/R+PM_{2.5} groups were determined after 24 h, and the results are expressed as the means $\pm$ SD. A significant increase from the Sham, I/R, and I/R+PM_{2.5} group was denoted by * P <0.01 and ** P <0.01, respectively. Abbreviations: I/R: ischemia/reperfusion; PM_{2.5}: particulate matter; SD: standard deviation; FXR: farnesoid-X-receptor; LC3I: protein 1 light chain 3I; LC3II: protein 1 light chain 3II.

Fig. 7. FXR, LC3I and LC3II expressions in Sham, I/R, I/R+PM_{2.5}, I/R+Z-Gugg, I/R+PM_{2.5}+Z-Gugg groups. Note: (A-C) The FXR, LC3I and LC3II expressions of the rats of the Sham (a), I/R (b), I/R+PM_{2.5} (c), I/R+Z-Gugg (d) and I/R+PM_{2.5}+Z-Gugg (e) groups were determined after 24 h. The quantitative analysis was showed in “A-f, B-f, and C-f”. The FXR, LC3I and LC3II expressions in the I/R rats were significantly higher than those on Sham rats (P <0.01); the FXR, LC3I and LC3II expressions in the I/R+PM_{2.5} rats were significantly higher than those of the I/R rats (P <0.01); the FXR, LC3I and LC3II expressions in the I/R+Z-Gugg rats were lower than those of the I/R rats (P <0.01); the FXR, LC3I and LC3II expressions in the I/R+PM_{2.5}+Z-Gugg rats were lower than those of the I/R+PM_{2.5} rats (P <0.01). Abbreviations: I/R: ischemia/reperfusion; PM_{2.5}: particulate matter; Z-Gugg: Z-guggulsterone; FXR: farnesoid-X-receptor; LC3I: protein 1 light chain 3I; LC3II: protein 1 light chain 3II.

FXR, LC3I and LC3II expression in Sham, I/R and I/R+PM_{2.5} groups

The results presented in Fig. 6 show, the expression of FXR, LC3I and LC3II on I/R rats was significantly greater than the values for the Sham rats. The values for the expression of FXR, LC3I and LC3II in the I/R+PM_{2.5} rats were significantly greater than those in the I/R rats. The corresponding quantitative analysis is shown in Fig. 6.

FXR, LC3I and LC3II expressions in Sham, I/R, I/R+PM_{2.5}, I/R+Z-Gugg, I/R+PM_{2.5}+Z-Gugg groups (SD rats)

The FXR, LC3I and LC3II expressions in the I/R rats were significantly higher than those on sham rats; the FXR, LC3I and LC3II expressions in the I/R+PM_{2.5} rats were significantly higher than those of the I/R rats; the FXR, LC3I and LC3II expressions in the I/R+Z-Gugg rats were lower than those of the I/R rats; the FXR, LC3I and LC3II expressions in the I/R+PM_{2.5}+Z-Gugg rats were lower than those of the I/R+PM_{2.5} rats. The results presented in Fig. 7 show.

Infarct size (FXR^{-/-} and WT mice)

The TTC staining of the hearts is shown in Fig. 8A. The injury to the hearts induced by WT+I/R was significantly greater than that in the WT+sham and FXR^{-/-}+sham mice, whereas the injury to the hearts subjected to WT+I/R+PM_{2.5} was significantly higher than that of the WT+I/R mice. The injury to the hearts subjected to FXR^{-/-}+I/R was lower than that of the WT+I/R mice, the injury to the hearts subjected to FXR^{-/-}+I/R+PM_{2.5} was lower than that of the WT+I/R+PM_{2.5} mice. The corresponding quantitative analysis is shown in Fig. 8A.

The LC-3I and LC-3II expressions (FXR^{-/-} and WT mice)

The LC3I and LC3II expressions in the WT+I/R rats were significantly higher than those on WT+sham and FXR^{-/-}+sham mice; the LC3I and LC3II expressions in the WT+I/R+PM_{2.5} mice were significantly higher than those of the WT+I/R mice; the LC3I and LC3II expressions in the FXR^{-/-}+I/R mice were lower than those of the WT+I/R mice; the LC3I and LC3II expressions in the FXR^{-/-}+I/R+PM_{2.5} mice were lower than those of the WT+I/R+PM_{2.5} rats. The results presented in Fig. 8B-C show.

Fig. 8. Infarct size estimation, LC3I and LC3II expressions in WT+sham, FXR^{-/-}+sham, WT+I/R, WT+I/R+PM_{2.5}, FXR^{-/-}+I/R, FXR^{-/-}+I/R+PM_{2.5} groups. Note: (A) The sizes of the infarcts in the rats of the WT+sham, FXR^{-/-}+sham, WT+I/R, WT+I/R+PM_{2.5}, FXR^{-/-}+I/R, FXR^{-/-}+I/R+PM_{2.5} groups were determined after 24 h, and the results are expressed as the means ± SD. Significant differences between the WT+sham, FXR^{-/-}+sham and WT+I/R groups, between the WT+I/R and WT+I/R+PM_{2.5} groups and between the WT+I/R, WT+I/R+PM_{2.5} and FXR^{-/-}+I/R, FXR^{-/-}+I/R+PM_{2.5} groups are indicated as *P<0.01, **P<0.01, #P<0.01 and ##P<0.01, respectively; (B-C) The LC3I and LC3II expressions in the rats of the WT+sham, FXR^{-/-}+sham, WT+I/R, WT+I/R+PM_{2.5}, FXR^{-/-}+I/R, FXR^{-/-}+I/R+PM_{2.5} groups were determined after 24 h, and the results are expressed as the means ± SD. Significant differences between the WT+sham, FXR^{-/-}+sham and WT+I/R groups, between the WT+I/R and WT+I/R+PM_{2.5} groups and between the WT+I/R, WT+I/R+PM_{2.5} and FXR^{-/-}+I/R, FXR^{-/-}+I/R+PM_{2.5} groups are indicated as *P<0.01, **P<0.01, #P<0.01 and ##P<0.01, respectively. Abbreviations: I/R: ischemia/reperfusion; PM_{2.5}: particulate matter; LC3I: protein 1 light chain 3I; LC3II: protein 1 light chain 3II.

Discussion

Currently, individuals are involuntarily exposed to many sources of air pollution. Most diseases, including angiocardopathy, are associated with acute or chronic exposure to air pollution [46-49]. The effect of atmospheric particulate matter (PM_{2.5}) on extrapulmonary organs has received attention in recent years, and considerable numbers of epidemiological studies have confirmed that PM_{2.5} can increase the morbidity and mortality from cardiovascular disease and lung disease in aging populations [1]. Inhaled PM_{2.5} can be deposited in the alveoli, and particles with sizes <0.1 μm can enter the blood circulation [1]. The human health hazards of atmospheric PM_{2.5} are closely associated with the particulate characteristics and pollution levels. Epidemiological findings have shown that people with heart or lung diseases or myocardial ischemia/reperfusion injury (MI/RI) and immunocompromised patients are more sensitive to PM_{2.5} [50]. WHO recently determined that over 800,000 deaths worldwide every year are associated with PM_{2.5} [51]. Pope et al. have demonstrated that for every 10 μg/m³ increase in the atmospheric PM_{2.5} levels, cardiopulmonary mortality is increased by

6% [50]. In addition, the same levels of increases in the levels of atmospheric PM_{2.5} are associated with a 2.1% increase in the quantity of deaths associated with ischemic heart disease [52].

Inhaled PM_{2.5} can lead to inflammation in the heart that is characterized by neutrophil and macrophage activation [50]. In addition, PM_{2.5} causes increases in high sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and macrophage inflammatory protein-2 (MIP-2) and decreases in interleukin-4 (IL-4). These factors are considered to be risk factors for cardiovascular and lung disease [52, 53]. Studies have also found that PM_{2.5} induces P-selectin- and D-dimer-mediated white blood cell and platelet association with endothelial cells and the adhesion, aggregation, activation and release of inflammatory mediators that are involved in the inflammatory process [54]. Furthermore, PM_{2.5} inhalation can result in the risk of cardiovascular stress [55]. Thus, exposure to PM_{2.5} increases inflammatory parameters and oxidative stress, which are closely associated with cardiovascular disease [17, 56].

Recently, many researches have affirmed that autophagy plays an important effect on MI/RI in both animal models and cellular models through producing progressive degeneration of the heart [57]. Autophagy is a dynamic process which turns over organelle and protein by a lysosome-associated degradation process, and plays an important role on cellular homeostasis *via* modulating cell survival and cell death pathway [58]. Autophagy is closely related to MI/RI [59]. In previous studies, MI/RI model showed that F2 exerted a protective effect against I/R-induced injury, and which the mechanism included F2-mediated autophagy inhibition. In addition, autophagy was closely related to MI/RI, although the accurate functional effect of autophagy on cell survival and death pathway related to heart injury remained unclear. Many literatures showed that autophagy could augment cell survival *via* removing the cell of damaged protein to product the intracellular building block needed to keep important effects during nutrient-limiting conditions [60, 61]. Autophagy could also augment cells death by excessive self-digestion and degradation of momentous cellular constituent or it could interact with the apoptosis in the heart [62], showing a double-edged sword for the biological action of autophagy on MI/RI. Some literatures showed that autophagy could augment cell apoptosis, and autophagy inhibition could reduce apoptosis [63, 64]. Most studies have shown that PM_{2.5} exposure can induce autophagy, which leads to endothelial apoptosis and dysfunction [19-21, 65]. In the process of autophagy, the expression of protein 1 light chain 3I (LC3I) and protein 1 light chain 3II (LC3II) is increased, and these changes reflect the extent of the autophagy [66]. Many studies have confirmed that PM_{2.5} exposure leads to excessive autophagy, which causes tissue damage as indicated by measurements of the expression of LC3I and LC3II [56]. Additionally, since farnesoid-X-receptor (FXR) is activated after feeding bile acids and FXR is expressed in the heart, recent evidence suggests that that FXR promotes myocardial ischemia-reperfusion injury [25]. Previous studies have shown that FXR knockdown decreases cardiac remodeling following MI/RI [67]. FXR knockdown appears to decrease infarct size and heighten cardiac function following MI/RI. Furthermore, FXR knockdown alleviates cell apoptosis following MI/RI [67]. Interestingly, FXR modulates autophagy under physiological conditions [17]. In our study, FXR was shown to play an important role in heart-associated MI/RI by inducing autophagy and enhanced the damage induced by administration of PM_{2.5} to the lungs. On the other hand, a significant reduction in infarct size, C-3I and LC3-II expressions in FXR^{-/-} mice compared with in WT mice on MI/RI are determined, thus suggesting that the absence of FXR may be associated with the amelioration of cardiac remodeling and cardiac function following MI/RI. These findings suggest that FXR participates in MI/RI, partly by activating autophagy, and that PM_{2.5} lung administration aggravates this effect. Therefore, the existence of potential feedback modulation between FXR and autophagy following PM_{2.5} exposure requires further exploration.

In the present study, PM_{2.5} was confirmed to induce the expression and activation of LC3I and LC3II and to stimulate autophagosome formation. Furthermore, it was shown that FXR has a positive regulatory role in the PM_{2.5}-induced autophagy following MI/RI. The

results of the study indicated that PM_{2.5} exposure upregulates FXR to stimulate autophagy following MI/RI, as indicated by the biochemical features of FXR and autophagy. However, the molecular mechanism remains to be further elucidated.

Conclusion

The present results suggested a central role: pulmonary exposure to PM_{2.5} aggravates the sensitivity to MI/RI through farnesoid-X-receptor-induced autophagy.

Abbreviations

PM_{2.5} (Particulate matter); FXR (Farnesoid-X-receptor); MI/RI (Myocardial ischemia/reperfusion injury); TTC (2, 3,5-triphenyltetrazolium chloride); CK (Creatine kinase); LDH (Lactate dehydrogenase); hsCRP (High sensitivity C-reactive protein); IL-4 (Interleukin-4); IL-6 (Interleukin-6); TNF- α (Tumor necrosis factor- α); cTnT (Cardiac troponin T); MDA (Myeloperoxidase); SOD (Superoxide dismutase); ROS (Reactive oxygen species); LC3I (Protein 1 light chain 3I); LC3II (Protein 1 light chain 3II); MIP-2 (Macrophage inflammatory protein-2); LVEF (Left ventricular ejection fraction); LVEDP (Left ventricular end-diastolic pressure); +dp/dt_{max} (Left ventricular pressure maximal rate of rise).

Acknowledgements

This work was financed through the Natural Science Foundation of China (41573125) and the Science and Technology Planning Project of Guiyang, Guizhou Province.

Disclosure Statement

The authors declare no competing interests.

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