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PM2.5 promotes abdominal aortic aneurysm formation in angiotensin II-infused apoe-/- mice

Xie Jun[a,1], Geng Jin[b,1], Chen Fu[a], Zhao Jinxuan[a], Li Xueling[a], Hu Jiaxin[a], Qiao Shuaihua[a], Shan Anqi[a], Chen Jianzhou[a], Zhou Lian[c], Xu Biao[a,*], Zhang Xiwen[b,*], Zhu Baoli[c,*]

[a] Department of Cardiology, Drum Tower Hospital, Nanjing University Medical School, Nanjing 210008, China
[b] Department of Cardiology, The Affiliated Huai'an No.1 People's Hospital of Nanjing Medical University, Huai'an 22330, China
[c] Jiangsu Provincial Center for Disease Control and Prevention, Nanjing 210008, China

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ABSTRACT

Background: Particulate matter 2.5 (PM2.5) has proven to be associated with morbidity and mortality from cardiovascular diseases. However, whether PM2.5 could promote the formation of abdominal aortic aneurysm (AAA) is unclear. Present study aimed to explore the relationship between PM2.5 exposure and AAA development.

Methods: Ang II-infused apoe$^{-/-}$ mice were treated with PM2.5 or saline by intranasal instillation. Four weeks later, histological and immunohistological analyses were used to evaluate the effect of PM2.5 on AAA formation. Human aortic smooth muscle cells (HASMCs) were also employed to further analyze the adverse effect of PM2.5 in vitro.

Results: We found that PM2.5 could significantly increase the AAA incidence, the maximal abdominal aortic diameter and could promote the degradation of elastin. Additionally, the expression of senescence markers, P21 and P16 were also enhanced after PM2.5 exposure. We also found that PM2.5 significantly increased the AAA related pathological changes, MMP2 and MCP-1 expression in HASMCs. Meanwhile, PM2.5 could increase the expression of senescence markers P21, P16 and SA-β-gal activity, also the reactive oxygen species levels in vitro.

Conclusions: PM2.5 promoted the formation of AAA in an Ang II-induced AAA model. The underlying mechanism might be cellular senescence after PM2.5 exposure.

1. Introduction

Abdominal aortic aneurysm (AAA), the most common form of aortic aneurysm, is a permanent, localized dilatation of the abdominal aorta that exceeds the normal diameter by 50% [1]. AAAs are pathologically characterized by vascular inflammation, oxidant stress, degradation of the extracellular matrix and loss of medial vascular smooth muscle cells (SMCs) [2,3]. Most AAAs are asymptomatic until rupture. AAA rupture accounts for approximately 2% of all deaths and is the tenth cause of mortality [4]. Except surgical repair, few medical approaches have been shown to prevent AAA development and rupture, partially due to the limited understanding of the pathogenic mechanisms of AAAs [5,6].

Fine particulate matter (PM) air pollution, particularly PM2.5 (aerodynamic diameter less than 2.5 μm), is considered as an important stimulus for the development of cardiovascular diseases (CVDs), leading to an increased CVD morbidity and mortality [7–11]. The underlying mechanism may be the atherosclerosis development after PM2.5 exposure [12,13]. AAA and atherosclerosis share similar risk factors [14], and vascular inflammation plays an important role both in plaque progression and AAA growth [14–16]. Smoking, an accepted risk factor of AAAs and atherosclerosis, can affect the mechanical and structural properties of the aorta [17]. Besides, cigarette exposure is reported to impair air quality and increase PM2.5 levels in homes [18]. These results indicate that PM2.5 may increase the risk of developing AAAs. However, there is no directly evidence to demonstrate the reasonable relationship between PM2.5 and AAAs. In the present study, we firstly observed that PM2.5 could predispose aortas to AAAs in apoe-/- mice and the PM2.5-induced senescence of SMCs was involved in the pathological process of AAA.

2. Materials and methods

2.1. Preparation of PM2.5

As previously described [19], PM2.5 sample was collected on glass

* Corresponding authors.
E-mail addresses: Xu Biao (xubiao@medmail.com.cn), Zhang Xiwen (zhangxiwen303@163.com), Zhu Baoli (zhubl@jscdc.cn).
[1] Xie Jun and Geng Jin contribute equally to the article.

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fiber filters, which was sonicated in double distilled water. After filtration, the extracted solution was freeze-dried in vacuum and then diluted with saline. The final concentration of PM2.5 was 1 mg/ml. The PM2.5 sample was provided by Jiangsu Provincial Center for Disease Prevention and Control, Nanjing, China.

2.2. Animal model

All animal protocols were approved by the Ethics Review Board of Nanjing Drum Tower Hospital and performed in accordance with NIH guidelines. Apoe$^{-/-}$ mice on the C57BL/6 background were obtained from Model Animal Research Center of Nanjing University (Nanjing, China).

Filled with angiotensin II (Ang II, Sigma, USA), Alzet model 2004 mini-osmotic pumps (DURECT, USA) were implanted into the subcutaneous space of eight-week old apoe$^{-/-}$ mice (n = 38) for the AAA formation model. Ang II was infused at a rate of 1000 ng/kg/min for 4 weeks. After implantation, all mice were fed a high fat diet and were randomly assigned to two groups: PM2.5 group, mice were treated with 10 μl PM2.5 twice a week by intranasal instillation as previously described [19]; control group, mice were treated with 10 μl saline as controls. A total of 20 apoe$^{-/-}$ mice were implanted with Alzet model 2004 mini-osmotic pumps filled with saline and also were randomly divided into PM2.5 and control group. To further explore the earlier effect of PM2.5 on AAA formation, Ang II-infused mice were exposed to PM2.5 or saline for two and three weeks (n = 6 per group). At last, mice were sacrificed and abdominal aortas were carefully dissected, photographs taken, and then were subjected to histological analyses. The photos were used to measure the maximal outer diameter using Image Pro Plus software. AAA formation was defined when the abdominal aorta that exceeds the normal diameter by 50%.

2.3. Histological analyses

Collected abdominal aortas were fixed with formalin, embedded with paraffin and cut into 4 μm slices for hematoxylin and eosin (H&E) and elastin van Gieson (EVG) stainings. As previously described [20,21], a standard score was used for the semi-quantification of elastin degradation: score 1, no degradation; score 2, mild degradation; score 3, severe degradation; score 4, aortic rupture. All images were assessed using Image Pro Plus software, and analyzed by two investigators who were blinded to treatments.

2.4. Immunohistochemical staining

Paraffin-embedded slides were incubated with primary antibodies for overnight at 4℃ and then were incubated with the secondary antibodies for 1 h and subsequently counterstained with hematoxylin. The primary antibodies used were MMP2 (#ab37150, Abcam), MCP-1 (#sc-52701, Santa), P16 (#sc-377412, Santa) and P21 (#ab109199, Abcam). The medial MMP2, MCP-1, P16 or P21 content was analyzed by calculating the integration optical density value of positive staining within the aortic wall. Images were evaluated using Image Pro Plus software and analyzed by two investigators who were blinded to treatments.

2.5. Cell culture

Human aortic smooth muscle cells (HASMCs) were purchased from ScienCell (#6110, ScienCell) and cultured with Smooth Muscle Cell Medium (SMCM, #1101, ScienCell). For the initial experiments, HASMCs were divided into the following groups: (1) control group (only SMCM); (2) PM2.5 group (SMCM with 100 μg/ml PM2.5 for 24 h); (3) Ang II group (SMCM with 10 μ mol/L Ang II for 24 h); (4) Ang II+PM2.5 group (SMCM with 10 μ mol/L Ang II plus 100 μg/ml PM2.5 for 24 h). Cells in Ang II+PM2.5 group were additionally treated with different PM2.5 concentrations (50, 100 and 200 μg/ml) for further analysis.

2.6. SA-β-gal activity assay

HASMCs were stained to determine SA-β-gal activity using Senescence Detection Kit (#ab65351, Abcam) as previously described [22]. Briefly, HASMCs were fixed with 4% paraformaldehyde for 15 min and then were incubated with 1 mg/ml X-gal for overnight at 37 ˚C. HASMCs were counterstained with DAPI. The cells appeared green color were considered SA-β-gal positive and the proportion of SA-β-gal positive cells were calculated using light and fluorescence microscopy.

2.7. Reactive oxygen species (ROS) levels

To assess the ROS production after treatment with PM2.5 and/or Ang II, HASMCs were incubated with 10 μ mol/l fluorescent dye dihydroethidium (#KGAF019, KeyGEN BioTECH) for 30 min at 37 ˚C. The cells then were fixed with 4% paraformaldehyde for 15 min and counterstained with DAPI. The ROS level was quantified by the mean red fluorescence intensity of each cell. All images were assessed using Image Pro Plus software by two investigators who were blinded to treatments.

2.8. Western blotting

Western blotting was performed as previously described [23]. The primary antibodies used were GAPDH (Bioworld Technology), MMP2 (#ab37150, Abcam), P16 (#sc-377412, Santa) and P21 (#ab109199, Abcam). MCP-1 (#sc-52701, Santa) and MCP-1 (#sc-32819, Santa) were respectively used to detect the expression of MCP-1 in aortas and HASMCs. Proteins were visualized using enhanced chemiluminescence kit (Millipore Corporation) and quantified by Image Pro Plus software.

2.9. Statistical analysis

Fisher's exact test and log-rank test were employed for the comparisons of AAA incidence and survival rate between groups. Other data were presented as mean ± standard deviation and were determined using ANOVA test. SPSS version 22.0 was used for the analyses and a 2-tailed p value < 0.05 was considered statistically significant.

3. Results

3.1. PM2.5 increases Ang II-induced AAA formation in mice

Saline infusion did not induce any AAA formation both in control and PM2.5 group (Fig. 1A and B). In Ang II-infused groups, the AAA incidence in PM2.5 group was 94.7% (18/19), which is significantly higher than the 57.9% (11/19) in control group (Fig. 1A and B). No mice died in the saline-infused groups. A total of 6 mice (31.6%) died from aortic rupture in Ang II-infused PM2.5 group, whereas only 5.3% (1/19) of Ang II-infused control mice died. The Kaplan-Meier curve was shown in Fig. 1C and the log rank p value was 0.037. Saline-treated mice showed normal morphology and elastin layers of suprarenal aortas based on the results of histological analysis with H&E and EVG staining (Fig. 1D and E). Infusion of Ang II resulted in AAA formation (Fig. 1D) and elastin degradation (Fig. 1E). In addition, compared with the control group, PM2.5 treatment remarkably increased the maximal abdominal aortic diameter and the elastin degradation score in Ang II-treated mice (Fig. 1D–G).

3.2. PM2.5 aggravates Ang II-induced AAA related pathological changes in vivo

Matrix metalloproteinase (MMP) and monocyte chemoattractant protein (MCP) play crucial roles in AAA initiation and development [20,21]. We therefore detected the expressions of MMP2 and MCP-1