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Research Paper

Effects of particulate matter from straw burning on lung fibrosis in mice

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ABSTRACT

Objective: To investigate the impacts of particulate matter 2.5 (PM2.5) from straw burning on the acute exacerbation of lung fibrosis in mice and the preventive effects of N-acetylcysteine (NAC).
Methods: The composition, particle size, and 30-min concentration change in an exposure system of the PM2.5 from straw-burning were determined. Forty C57BL male mice were equally randomized to two groups: bleomycin (BLM)-induced lung fibrosis with an exposure to air (BLM + air) and BLM + PM2.5 groups. On day 7 after receiving intratracheal injection of BLM, mice were exposed to air or PM2.5 in an exposure system for 30 min twice daily and then sacrificed after one-week or four-week exposure (10 mice/group). Mouse survival, lung histopathology, macrophage accumulation in the lung, and pro-inflammatory cytokine levels in alveolar lavage fluid (ALF) were determined.
Results: PM2.5 from straw burning were mainly composed of organic matter (74.1%); 10.92% of the inorganic matter of the PM2.5 were chloride ion; 4.64% were potassium ion; other components were sulfate, nitrate, and nitrite. Particle size was 10nm–2 μm. Histopathology revealed a greater extent of inflammatory cell infiltration in the lung, widened alveolar septum, and lung fibrosis in the BLM + PM2.5 group than in the BLM + air group and a greater extent of those adverse effects after four-week than after one-week exposure to PM2.5. The BLM + PM2.5 group also showed macrophages containing particular matter and increased pulmonary collagen deposition as the exposure to PM2.5 increased. Interleukin (IL)-6 and TNF-α levels in ALF were significantly higher in the BLM + PM2.5 group than in the BLM + air group ($P < 0.05$) and significantly higher after four-week exposure than after one-week exposure to PM2.5 ($P < 0.05$). TGF-β levels in ALF after four-week exposure were significantly higher in the BLM + PM2.5 group than in the BLM + air group ($P < 0.05$). The levels of IL-6, TNF-α, and TGF-β in peripheral serum were not significantly different in the BLM + PM2.5 and BLM + air groups. Lung hydroxyproline contents increased as the exposure to PM2.5 increased and were significantly higher after four-week than after one-week exposure ($P = 0.019$). Exposure to PM2.5 did not affect the survival of normal mice (100%) but reduced the survival of mice with BLM-induced IPF (30%), whereas NAC extended the survival (70%, vs. BLM + PM2.5, $P = 0.032$).
Conclusion: Exposure of mice with BLM-induced IPF to PM2.5 from straw burning exacerbated lung inflammation and fibrosis and increased mortality; NAC increased the mouse survival, indicating protective effects.

1. Background

Particulate matter 2.5 (PM2.5), which are fine particles with an aerodynamic diameter smaller than 2.5 μm, are the main component of atmospheric pollutants (smog). Because of their small diameter, large specific surface area, complex chemical composition, and their capability to easily enrich toxic and harmful substances, PM2.5 can reach the respiratory fine bronchi and alveolar cavity after entering the respiratory track, harming human healthy at substantially greater extent than particulate matter with a diameter bigger than 2.5 μm.

Abbreviations: PM2.5, Particulate matter less than 2.5 micrometers in diameter; BLM, Bleomycin; NAC, Acetylcysteine; AM, Alveolar macrophages; ALF, Alveolar lavage fluid; IL-6, Interleukin-6; TNF-α, Tumor necrosis factor-α; TGF-β, Transforming growth factor-β; IPF, Interstitial pulmonary fibrosis; COPD, Chronic obstructive pulmonary disease

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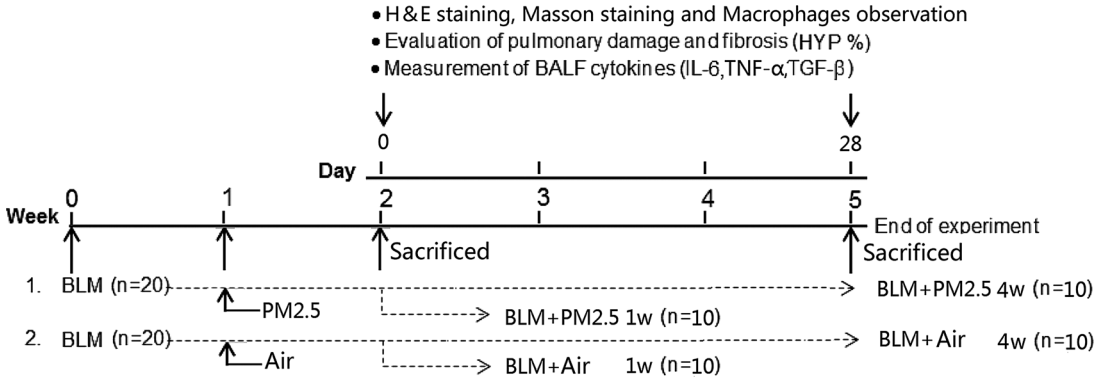

Fig. 1. Animal experiment protocol.
Mice were randomized into bleomycin-induced IPF + exposure to air (BLM + air) group and bleomycin-induced IPF + exposure to PM2.5 (BLM + PM2.5) group (20 mice per group). Mice in both groups received intratracheal injection of bleomycin (4 mg/mL, dissolved in 50 μL saline) at experiment day 0 to establish lung fibrosis. On day 7 after the bleomycin injection, the mice were exposed to air or PM2.5. Each group was further divided into one-week and four-week groups, which were sacrificed after one-week and four-week exposure, respectively, and mouse specimens were collected.

One of the key sources of air pollutants in China is the smoke (containing large amounts of PM2.5 (Li et al., 2016; Shi et al., 2016; Chen et al., 2015)) from straw burning in rural area. A previous study has demonstrated that long-term inhalation of biofuel smoke contributes predominantly to the high prevalence of chronic obstructive pulmonary disease (COPD) in rural area (Guan et al., 2016). PM2.5 are also found to correlate closely with the increased incidences of common chronic airway diseases, such as COPD and bronchial asthma (Kelly and Fussell, 2011). Our previous study has shown that exposure to PM2.5 exacerbates the pulmonary injury and increases the mortality of mice with emphysema (Wang et al., 2015). However, the association between PM2.5 and the development and progression of idiopathic pulmonary fibrosis (IPF) has not been studied. Whether PM2.5 can exacerbate IPF remains unclear. In the current study, to simulate the exposure of patients with IPF to severe air pollution, we exposed mice with bleomycin (BLM)-induced IPF to PM2.5 generated from straw burning and investigated the effects of air pollution on IPF deterioration and the underlying mechanisms. Our study also explored potential preventive approaches.

2. Materials and methods

2.1. Preparation of PM2.5 pollutants and the exposure system

Smoke generated from rice straw burning was used as the source of PM2.5 pollutants. Rice straw was cut into 5 cm-length small pieces, washed, and dried at 80 °C in an oven for 4 h. The dried rice straw was aliquoted into 5 g per sealed bag. The whole body inhalation exposure system (Tianjin Hope Industry & Trade Co., Ltd, HOPE-MED8050 exposure controlling system) has a volume of 0.3 m^3 and allows to expose a maximum of 20 mice simultaneously. The exposure temperature was 25 °C; the relative humidity of exposure was 40%. After exposure, the pollutants in the exposure system were replaced with air (≥30 min). The exposure system was cleaned daily.

2.2. Monitoring PM2.5 in the exposure system

Rice straw (5 g) was burned inside the exposure system; the mini fan inside the system was switched on to allow the PM2.5 distribute evenly. The sampling port was connected to two aerosol monitors (Model: AM510, Product No.: #32 and #27). The mass concentrations of PM2.5 and PM 1.0 inside the exposure system were monitored for a 30-min period and the monitoring was repeated 4 times. The time course of the mass concentration of PM2.5 and PM 1.0 of the aerosol in the exposure system was then determined. The particle size of particulate matter was analyzed by wide-range particle (WPS) Sizer (Model: 1000XP, TSI Inc, USA). The distributions of concentration, surface area, and volume of particles with a size between 10 nm and 10 μm were also determined. Particles were collected by a particulate matter sampler, and then the size and composition of the particles were analyzed by a transmission electron microscope (TEM, Model: JEOL-2100F).

2.3. Animals

The protocol for handling mice has been approved by the Institutional Animal Care and Use Committee of Shanghai Pulmonary Hospital of Tongji University School of Medicine (Approval No. SYXK [SH] 2012-0031). A total of C57BL male 40 mice aged 8-week and weighted 16g–18 g were randomized into two groups (20 mice/group): bleomycin-induced IPF + exposure to air (BLM + air) group and bleomycin-induced IPF + exposure to PM2.5 (BLM + PM2.5) group. Mice in both groups received intratracheal injection of bleomycin (4 mg/mL, dissolved in 50 μL saline) at experiment day 0 according to our previous description (Wei et al., 2016). Mice in the control group were injected with 50 μL saline intratracheally. On day 7 after bleomycin injection, the mice were exposed to air or PM2.5. Each group was further divided into one-week and four-week groups, which were sacrificed after one-week and four-week exposure (10 mice/group), respectively, and mouse specimens were collected (Fig. 1).

To determine mouse survival, 1) mice in the control groups (Control + PM2.5, 15 mice) were exposed to PM2.5 twice daily (30 min every exposure) for 28 days; 2) mice in the BLM + PM2.5 group (20 mice) were exposed to PM2.5 after BLM-induced IPF was established, and the exposure was twice daily (30 min every exposure) for 28 days; 3) mice in the BLM + air group (12 mice) were observed for survival for 28 days; 4) mice in the *N*- Acetylcysteine (NAC) intervention group (BLM + PM2.5 + NAC, 20 mice) were exposed to PM2.5 twice daily (30 min every exposure) for 28 days after BLM-induced IPF was established, and NAC (150 mg/kg, dissolved in 2 mL saline) was injected intraperitoneally daily before exposure to PM2.5 (Demiralay et al., 2013).

2.4. Exposure to PM2.5

Mice were housed in standard cages before being exposed to PM2.5. Rice straw (5 g) was burned completely in the exposure system, and then the cages containing mice were placed inside the exposure system. The temperature and humidity of the exposure system was 25 °C and 40%, respectively. Each exposure lasted 30 min, and 1L air was supplied to the exposure system every 15 min. The exposure was twice daily and the time interval between daily exposures was less than 4 h. Mice in the BLM + air group were exposed to air in the similar manner.