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COPD rat model is more susceptible to cold stress and $PM_{2.5}$ exposure and the underlying mechanism☆

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ABSTRACT

The purpose of this study is to verify the hypothesis that chronic obstructive pulmonary disease (COPD) model rat is more susceptible to cold stress and fine particulate matter ($PM_{2.5}$) exposure than the healthy rat, and explore the related mechanism. COPD rat model, established with cigarette smoke and lipopolysaccharide intratracheal instillation, were exposed to cold stress (0 °C) and $PM_{2.5}$ (0, 3.2, 12.8 mg/ml). After that, the levels of superoxide dismutase, inducible nitric oxide synthase (iNOS), tumor necrosis factor alpha (TNF-α), monocyte chemotactic protein 1 (MCP-1) and angiotensin II (Ang-II) in lung were measured, as well as the expression levels of lung 8-hydroxy-2-deoxyguanosine (8-OHdG), nuclear factor kappa B (NF-κB), heme-oxygenase-1 (HO-1) and nuclear factor erythroid-2-related factor 2 (Nrf2). There were significant positive relationships between $PM_{2.5}$ and lung level of iNOS, TNF-α, MCP-1 and Ang-II, lung function and pathologic damage in COPD rats. The HO-1, NF-κB and 8-OHdG were found highly expressed in COPD rat lung, particularly at the higher $PM_{2.5}$ dose of cold stress groups, while Nrf2 was found declined. Thus, COPD rats may be more susceptible to cold stress and $PM_{2.5}$ exposure. Cold stress may aggravate $PM_{2.5}$-induced toxic effects in the lung of COPD rats through increasing Ang-II/NF-κB signaling pathway and suppressing Nrf2 signaling pathway.

1. Introduction

Chronic obstructive pulmonary disease (COPD), a common disease characterized by progressive and irreversible airflow limitation, is associated with enhanced chronic inflammatory response to toxic particles or gases of airway and lung (Angelis et al., 2014; Ji et al., 2015). The latest data from WHO showed that COPD is the fourth-largest cause of death in the world and will become the third major cause of death by 2020 (Nielsen et al., 2016). Previous studies reported that both particulate matter and cold weather were the critical risk factors in inducing higher incidence of COPD, and COPD subjects are also more prone to be affected by them (Balish et al., 2017; Tseng et al., 2013). However, the underlying mechanism remains unclear.

Fine particulate matter ($PM_{2.5}$), is considered as a significant respiratory hazard (Ni et al., 2015). It could cause respiratory inflammation and lead to respiratory diseases (Crobeddu et al., 2017; He et al., 2017). Both epidemiological and animal studies have found that $PM_{2.5}$ would aggravate the progression of COPD, causing decreased lung function, epigenetic changes, immune dysfunction and cell damage (Audi et al., 2017; Chu et al., 2016; Cortez-Lugo et al., 2015; Leclercq et al., 2017; Torres-Ramos et al., 2011). The incidence of COPD exacerbation in the cold season is higher than warmer season (Donaldson et al., 2012; Jenkins et al., 2012), indicating that cold stress may be one of the risk factors of COPD. Some researches reported that cold stress initiated a series of worsening procedures in patients with COPD (Li et al., 2011). Inhalation of cold air may suppress the lung immune system, increase susceptibility to respiratory viruses and induce further disease deterioration (Boonarkart et al., 2017; Hu et al., 2016). A recent study has suggested that cold stress could induce airway inflammatory response and promote more mucus production (Juan et al., 2016).

Our previous studies have also observed that there may be interactive effects on rat lung between low temperature and $PM_{2.5}$, which might be caused through the suppression of alveolar macrophage function (Luo et al., 2017; Luo et al., 2014). However,

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no study has focused on how cold stress intensified the effect of $PM_{2.5}$ on the respiratory system of COPD. We therefore conducted the current study to investigate the effects of cold stress and $PM_{2.5}$ on COPD rat and explore the underlying mechanism.

2. Materials and methods

2.1. $PM_{2.5}$ sample acquisition

By using particulate samplers (Airmetrics, Springfield, USA), $PM_{2.5}$ was continuously collected on glass fiber filters (Watman, UK) at the top of a four-story building in Lanzhou from 2015 to 2016. The filters were dried in a glass dehydrator for 24 h before sampling, then cut into 1 cm × 1 cm, placed in deionized water and sonicated 3 × 15 min with a sonicator. After filtered through twelve layers of gauze, the $PM_{2.5}$ samples were frozen dehydrated by a vacuum freeze drier and stored at −20 °C.

2.2. COPD modeling

Eighty-four male Wistar rats (160–200 g, 7-week-old) were purchased from Veterinary Institute, Chinese Academy of Agricultural Sciences, China (batch number: SCXK (Gan) 2015–001). All of them were kept in an animal room maintained at 20–22 °C with a 12-hr light/dark cycle (9:00–21:00 light, 21:00–9:00 dark) and given free access to standard animal food and water. Forty-two rats were randomly picked out for COPD modeling with intratracheal instillation of lipopolysaccharides (LPS) and exposed to cigarette smoke (Gan et al., 2016) (Fig. 1) for 9 weeks. LPS (1 mg/ml, 0.2 ml) intervention was implemented every 6 days, and cigarette smoke exposure was given in a passive smoking chamber (50 cm × 40 cm × 50 cm) with 1 cigarette (tar 8 mg, nicotine of flue gas 0.9 mg, carbon monoxide of flue gas 12 mg, Gansu Tobacco Industry Co., Ltd., China) a day for 6 days a week. At the same time, we recorded the body weight of the rats every week and observed their behavioral changes. Healthy rats were placed in a room with normal temperature, similar to smoked rats and with tracheal instillation of sterile saline.

2.3. $PM_{2.5}$ and cold stress exposure

At the end of COPD modeling, all animals were grouped randomly according to Table 1. $PM_{2.5}$ particles were suspended with sterile saline into different concentrations according to study design before $PM_{2.5}$ exposure. The healthy and COPD rats were exposed to $PM_{2.5}$ (Concentration: 0, 3.2, 12.8 mg/ml; Final dose: 0, 2.4, 9.6 mg/rat) by intratracheal instillation (0.25 ml) under cold stress (0 °C) (CS) and normal temperature (20 °C) (NT) for 8 h in an environmental climate simulation chamber for three times with an interval of 1 day, respectively. Twenty-four hours after the last exposure, all rats were sacrificed under anesthesia. The experimental procedures were approved by the Institutional Animal Care and Use Committee at Lanzhou University following the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Table 1
Animal grouping method (n=7). NH/NC: normal temperature with healthy/COPD rat; CH, CC: cold stress with healthy/COPD rat.

Rat group		Healthy		COPD	
		20 °C	0 °C	20 °C	0 °C
$PM_{2.5}$ Concentration (mg/ml)	0	NH-0	CH-0	NC-0	CC-0
	3.2	NH-3.2	CH-3.2	NC-3.2	CC-3.2
	12.8	NH-12.8	CH-12.8	NC-12.8	CC-12.8

2.4. Pulmonary function test

Pulmonary function tests were performed immediately after the last exposure and were done in blind condition by technicians. Pulmonary function was measured with a noninvasive rat pulmonary function measurement system (GYD-003, Noninvasive Rat Lung Function telemetry device, Emka, France) after $PM_{2.5}$ and cold stress exposure. Peak expiratory flow (PEF) and peak inspiratory flow (PIF) were monitored to indicate the change of pulmonary function.

2.5. Lung histological and immunohistochemistry analysis

The upper lobe of the right lung was fixed in 4% paraformaldehyde, and then embedded in paraffin and cut into 4-μm paraffin sections, which were then stained with hematoxylin-eosin (HE). To investigate the effects of cold stress and fine particulate matter on respiratory system in COPD rats and to further explore the mechanism of strong susceptibility in COPD rats, we selected COPD rats for immunohistochemistry. The expression of nuclear factor kappa B (NF-κB), heme-oxygenase-1 (HO-1) and 8-hydroxy-2-deoxyguanosine (8-OHdG) in lung tissue were detected with the SP (streptavidin-perosidase) method according to the kit's instructions. The anti-NF-κB p65 antibody (1:100, Abcam, Cambridge), anti-HO-1 antibody (1:100, Abcam, Cambridge) and anti-8-OHdG antibody (1:200, Abcam, Cambridge) were applied to tissues. Then they were incubated with the corresponding secondary antibody (1:800, Zhongshan Golden Bridge Biology Company,

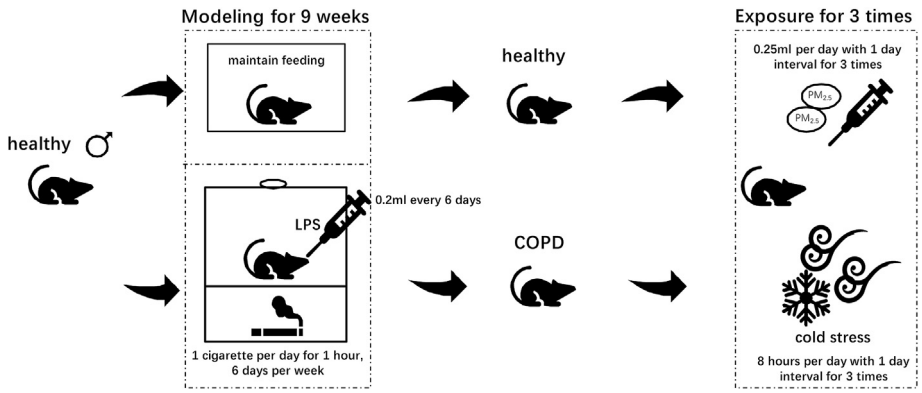

Fig. 1. Modeling and exposure details. LPS: lipopolysaccharides; COPD: chronic obstructive pulmonary disease.