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The probable roles of valsartan in alleviating chronic obstructive pulmonary disease following co-exposure to cold stress and fine particulate matter

Jiangtao Liu, Lei Guo, Kai Zhang, Quanquan Song, Qiaozhen Wei, Qin Bian, Tingting Liang, Jingping Niu, Bin Luo*

Institute of Occupational Health and Environmental Health, School of Public Health, Lanzhou University, Lanzhou, Gansu 730000, People's Republic of China

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ABSTRACT

Angiotensin II (ANG II) might play an important role in the co-effects of cold stress and fine particulate matter ($PM_{2.5}$) on chronic obstructive pulmonary disease (COPD). The purpose of this study is to evaluate the roles of valsartan in alleviating COPD following co-exposure to cold stress and $PM_{2.5}$. Both the two intervention factors are carried out upon COPD rats with the intervention of valsartan. Blockade of angiotensin receptor by valsartan decreases the levels of malondialdehyde in the normal temperature and tumor necrosis factor-α under cold stress significantly. When treated with valsartan and $PM_{2.5}$ simultaneously, the expression of 8-hydroxy-2-deoxyguanosine, nuclear factor kappa B and heme oxygenase-1 decrease significantly in the group of cold stress. In conclusion, these results indicate that valsartan might relieve the co-effects of cold stress and $PM_{2.5}$ on COPD rat lung to some degree.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death around the world and places tremendous burdens and stresses on patients and their families (Mathers and Loncar, 2006). It is a preventable and treatable disease which is characterized by the partially reversible airflow limitation (Berg and Wright, 2016). Obviously, COPD has been a severe problem of public health that we must face and deal with. Atmospheric particulates and ambient cold stress are considered critical threats to human health, particularly associated with COPD (McMichael et al., 2008; Schikowski et al., 2010). Studies showed that atmospheric particles, especially fine particulate matter ($PM_{2.5}$) could increase pneumonia risk, aggravate chronic lung diseases (Zanobetti and Schwartz, 2009) and induce the increase of asthma and allergic respiratory disease mortality (Delamater et al., 2012). $PM_{2.5}$ contributes to the systemic inflammatory process which is considered as a dangerous factor for COPD (Gutierrez-Castillo et al., 2006; Wu et al., 2016). Besides, cold stress has also been identified as a severe threat to large populations in respiratory system (Green et al., 2010). Mousa Khadadah et al. has reported that acute cold exposure could cause the contraction of tracheal sooth muscles and the decrease of pulmonary circulation (Khadadah et al., 2011). Sabnis et al. found significant inflammatory response in lung epithelial cells after a cold-stress of 18 °C for 0–4 h (Sabnis et al., 2008). In fact, our previous studies have paid a great attention on the effects of these two factors on respiratory system. We demonstrated that cold stress intensified the toxic effect of $PM_{2.5}$ on rat lung (Luo et al., 2014), which could be explained by their interactive effect over phagocytosis function suppression of alveolar macrophages (Luo et al., 2017).

Angiotensin II (ANG II) is an effector peptide of the renin-angiotensin system (RAS) which could stimulate the angiotensin receptor 1 (AT1R) and perform its main biological effects (Touyz, 2003; Schiffrin and Touyz, 2004). The activation of AT1R could lead to hypertension, vasoconstriction, fibro proliferation, oxidative stress and inflammation (Wong et al., 2012). As one of angiotensin receptor blockers (ARBs), valsartan is gradually used to treat COPD concurrently with chronic pulmonary heart disease (Churg et al., 2008; Fukuda et al., 2010). Since both $PM_{2.5}$ and cold stress have been studied to increase the secretion of ANG II in blood (Ghelfi et al., 2010; Luo et al., 2012a,b), we wonder whether the blockade of AT1R could alleviate the COPD. So far, few studies have studied the effects of cold stress and $PM_{2.5}$ in COPD rats, and no experimental studies have focused on the effects of valsartan on COPD following co-exposure to these two factors. Therefore, we hypothesized that valsartan would alleviate the progression of COPD following co-exposure to cold stress and $PM_{2.5}$.

* Corresponding author.
E-mail address: luob@lzu.edu.cn (B. Luo).

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2. Materials and methods

2.1. $PM_{2.5}$ sampling and processing

$PM_{2.5}$ was obtained from the top of QinBo building (a four-storey building) in the campus of Lanzhou University in 2016 by using particulate samplers (Airmetrics, Springfield, OR, USA). Glass fiber filters with $PM_{2.5}$ were submerged into deionized water, sonicated in an ultrasonic washer for 30 min for three times and then filtered by twelve layers of gauze. The $PM_{2.5}$ samples were achieved by frozen dehydrated by a vacuum freeze drier (Christ/ALPHA2-4 LD, Germany). Dried $PM_{2.5}$ particles were kept at 4 °C before diluted into corresponding concentrations for experimental use. Besides, organic carbon (OC)/elemental carbon (EC) composition of $PM_{2.5}$ was determined with a carbon analyzer (DRI-2001A, America).

2.2. Animals

Fifty-six male Wistar rats free from any infection/pathogen and weighing 200–240 g were purchased from Veterinary Institute, Chinese Academy of Agricultural Sciences, China [Batch number: SCXK (Gan) 2015-001]. Animal room was kept at a stable environmental condition with controlled temperature (20 ± 2 °C), relative humidity (40%–60%) and an artificial dark-light cycle (light from 9:00 a.m. to 9:00 p.m.). All rats were placed in a clean air exposure chamber, free access to laboratory chow and tap water throughout the experiment. Then, 49 rats were used to develop COPD models and the other 7 were set up as healthy control. The research was conducted in accordance with the Declaration of Helsinki and with the Guide for Care and Use of Laboratory Animals as adopted and promulgated by the United National Institutes of Health. All experimental protocols were approved by the Animal Care and Use Committee of Lanzhou University.

2.3. COPD modeling

The rat COPD model was achieved by lipopolysaccharide (LPS) and smoke exposure (Antunes and Rocco, 2011; Kozma Rde et al., 2014). Each cigarette (Lanzhou Cigarette Factory, Gansu Province, China) used in this study contained 11.0 mg of total particulate matter, 9.4 mg of tar and 0.76 mg of nicotine. Each rat received 1 h of cigarette smoke exposure in a smoking exposure chamber for 6 days/week and lasted for 9 weeks. LPS (Sigma, America) was given to the rat once a week for 9 weeks at the dose of 0.2 mg/rat by intratracheal instillation under anesthesia. Rats in healthy control group were placed under room atmosphere without smoke. All rats were weighed every week to evaluate the growth and basic development conditions.

2.4. Treatment protocols

Rats were divided into seven groups (7 animals per group) after the COPD models were established. The specific group protocols were shown in Table 1. With sterile saline, $PM_{2.5}$ particle was suspended and diluted to 3.2 mg/ml, and valsartan was diluted to 6 mg/ml. The COPD rats were exposed to $PM_{2.5}$ (2.4 mg/rat) by intratracheal instillation (0.75 ml) and valsartan (15 mg/kg) by intraperitoneal injection under cold stress (0 °C) and normal temperature (20 °C) 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.

Table 1
Animal grouping protocol (n = 7).

Group	1	2	3	4	5	6	7
Cold stress	−	−	−	+	+	+	+
$PM_{2.5}$	−	+	+	−	+	+	−
valsartan	−	−	+	−	−	+	+

2.5. Blood plasma and lung tissue homogenate preparation

After the exposure, rats were sacrificed with the help of anesthesia by intraperitoneal administration of chloral hydrate (3 ml/kg, 10%). The blood was collected in the anticoagulative tube and plasma was collected by centrifuging at 3000 rpm for 10 min at 4 °C. The left lung tissue, homogenate and plasm were stored at −80 °C for the later measurements (Wei et al., 2015).

2.6. Lung histological

The middle lobe of right lung was removed, fixed with formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin for histological study. Morphological alterations in the lungs were observed under an optical microscope (DP72, Olympus, Japan).

2.7. Biomarker estimations

Enzyme linked immunosorbent assay (ELISA) kits (Elabscience, China) were applied for the detecting of tumor necrosis factor-α (TNF-α) in the lung tissue homogenate, and ANG II both in the plasma and lung tissue homogenate. Malondialdehyde (MDA) and nitric oxide synthase (iNOS) of rat lung were detected by using corresponding detecting kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China). All the detection procedures mentioned above were operated according to standard directions of kits strictly.

2.8. Immunohistochemistry analysis

Immunohistochemistry staining was used to determine the expression of 8-hydroxy-2-deoxyguanosine (8-OHdG), nuclear factor kappa B (NF-κB) and heme-oxygenase-1 (HO-1) in lungs. The lungs of rats were removed, fixed with formalin, embedded in paraffin, sectioned into 5 μm slices. The slices of lung were then placed into an incubator chamber at 65 °C for 4 h, treated with dimethylbenzene dewaxing units which was prepared to stain. The process followed the strict directions in the kits (Abcam, America). After dyeing with diaminobenzidine (DAB), washing, counterstaining, dehydration and covering slip with mounting, images of the bronchiolar epithelium were obtained under an optical microscope (DP72, Olympus, Japan). Image-Pro Plus 6.0 professional image analysis software (Media Cybernetics, America) was used for image analysis. The results were presented as the ratio of integrating optical density (IOD) between each treatment groups and the group of saline-treated under the normal temperature (Valenca and Porto, 2008).

2.9. Statistical analysis

Data were expressed as means ± S.D. The differences among groups were used One-Way ANOVA and Student T-test. Data were analyzed by using IBM SPSS Statistics for Windows, Version20.0 (Armonk, NY: IBM Corp.). All reported p values were made based on two-sided tests with a significance level of 0.05.

3. Results

3.1. The composition of $PM_{2.5}$ sample

The carbon composition of $PM_{2.5}$ sample was shown in Table 2. Organic carbon (OC) contained in the $PM_{2.5}$ sample was 2.35 mg/g and the elemental carbon (EC) was 0.95 mg/g. The total carbon (TC) was 3.30 mg/g and the ratio of OC to EC was 2.46, which was basically corresponded with the reality in the air.