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Airborne fine particulate matter induces an upregulation of endothelin receptors on rat bronchi

Rong Wang [a], Xue Xiao [a], Lei Cao [a, *], Zhen-xing Shen [b], Ying Lei [c], Yong-xiao Cao [a, **]

[a] Department of Pharmacology, School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, Xi'an, Shaanxi, China
[b] Department of Environmental Science and Engineering, Xi'an Jiaotong University, Xi'an, Shaanxi, China
[c] Department of Pharmacy, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, China

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ABSTRACT

Airborne fine particulate matter (PM2.5) is a risk factor for respiratory diseases. However, little is known about the effects of PM2.5 on bronchi. The present study investigated the effect of airborne PM2.5 on rat bronchi and the underlying mechanisms. Isolated rat bronchial segments were cultured for 24 h. Endothelin (ET) receptor-mediated contractile responses were recorded using a wire myograph. The mRNA and protein expression levels of ET receptors were studied using quantitative real-time PCR, Western blotting, and immunohistochemistry. The results demonstrated that ET_A and ET_B receptor agonists induced remarkable contractile responses on fresh and cultured bronchial segments. PM2.5 (1.0 or 3.0 μg/ml) significantly enhanced ET_A and ET_B receptor-mediated contractile responses in bronchi with a markedly increased maximal contraction compared to the DMSO or fresh groups. PM2.5 increased the mRNA and protein expression levels of ET_A and ET_B receptors. U0126 (a MEK1/2 inhibitor) and SB203580 (a p38 inhibitor) significantly suppressed PM2.5-induced increases in ET_B receptor-mediated contractile responses, mRNA and protein levels. SP600125 (a JNK inhibitor) and SB203580 significantly abrogated the PM2.5-induced enhancement of ET_A receptor-mediated contraction and receptor expression. In conclusion, PM2.5 upregulates ET receptors in bronchi. ET_B receptor upregulation is associated with MEK1/2 and p38 pathways, and the upregulation of ET_A receptor is involved in JNK and p38 pathways.

1. Introduction

Air pollution is an important public health problem worldwide because of increased industrialization, energy use, and high road traffic volumes (Kampa and Castanas, 2008). Airborne fine particulate matter (PM2.5) refers to particulate matter with an aerodynamic diameter less than 2.5 μm (Pui et al., 2014). PM2.5 contains many harmful substances that penetrate more deeply into the airway and terminal alveoli and translocate more easily into blood circulation than coarser particles (Ando et al., 1996; Kiss et al., 1998). Therefore, PM2.5 is an important air pollutant because it causes adverse health effects. Persuasive evidences demonstrate that exposure to PM2.5 is associated with bronchial asthma, acute bronchitis, and chronic obstructive pulmonary disease (Hystad et al., 2013; Kodavanti et al., 2013). PM2.5 is a risk factor for respiratory diseases (Park et al., 2011). However, little is known about the effects of PM2.5 on bronchi, and the underlying mechanisms are not clear.

Airway hyperresponsiveness is characterized by an increased sensitivity of airway smooth muscle cells to constrictor agents, which is demonstrated in most patients with symptomatic asthma bronchioles (Cockcroft and Davis, 2006). The bronchioles are the major site of airway resistance. Therefore, bronchial hyperreactivity (BHR) is the key component of airway hyperresponsiveness (Rennard, 1996), which is characterized by easily triggered bronchospasms and contraction of the bronchioles or small airways. The enhanced bronchial contractile reactivity may derive from the upregulation of specific contractile receptors and/or the downregulation of dilator receptors in bronchial smooth muscle cells.

Endothelin (ET)-1 is the most potent endogenous bronchoconstrictor (Uchida et al., 1988). Bronchial contractile responses to ET-1 are mediated through two different G-protein coupled receptors

* Corresponding author. Department of Pharmacology, School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, 76 Yanta West Road, Xi'an, Shannxi 710061, China.
** Corresponding author. Department of Pharmacology, School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, 76 Yanta West Road, Xi'an, Shannxi 710061, China.
E-mail addresses: leicao@mail.xjtu.edu.cn (L. Cao), yxy@xjtu.edu.cn (Y.-x. Cao).

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(GPCRs), the endothelin receptor type A (ET_A) and endothelin receptor type B (ET_B). Activation of the ET_A receptor induces smooth muscle cell (SMC) contraction. ET_B receptors are further divided into two subtypes, ET_{B1} and ET_{B2}. ET_{B1} receptors are located on epithelium cells and induce relaxation via nitric oxide and prostacyclin release (Naline et al., 1999). ET_{B2} receptors are located on SMCs and mediate contraction (Clozel and Gray, 1995). The ET receptors, especially ET_B receptors, demonstrate plasticity in SMC expression and contractility, and these receptors are vulnerable to environmental influences (Adner et al., 1996). ET_B receptors primarily mediate dilatation in normal physiological conditions (Haque et al., 1995). However, ET_B receptors are upregulated in pathological conditions, such as asthma (Fernandes et al., 1996) and respiratory tumors in humans (Fernandes et al., 1999) and asthma in rats (Long et al., 2009), and these receptors primarily mediate constriction. Many risk factors of respiratory diseases, such as cigarette smoke particles, increase contractility mediated by ET receptors (Granstrom et al., 2006). The present study investigated the effect of PM2.5 on the upregulation of ET receptors in airway SMCs.

Many studies demonstrated that increased receptor expression was connected with the mitogen-activated protein kinase (MAPK) pathway. The MAPK pathway plays an important role in the intracellular signaling in response to extracellular stimuli, and it regulates various cellular activities (Pearson et al., 2001). The MAPK pathway includes the extracellular signal-regulated protein kinase 1 and 2 (MEK1/2), c-Jun N-terminal kinase (JNK) and p38 pathways. Different MAPKs are involved in different important cellular functions, such as proliferation, differentiation and survival (Lei et al., 2011). Previous studies suggested that MAPK pathway activation contributed to BHR (Duan and Wong, 2006). MAPK also tightly regulate GPCRs in airway smooth muscles (Duan et al., 2005). Our previous studies demonstrated that MAPK activation participated in contractile receptor upregulation of trachea and bronchi in different models (Lei et al., 2008; Cao et al., 2012a).

PM2.5 exposure is strongly associated with BHR. However, the underlying mechanisms of PM2.5 that induce bronchial alterations are not well established. The present study established an *in vitro* bronchus culture model and investigated the hypothesis that PM2.5 induces an upregulation of ET receptors on rat bronchi through the MAPK pathway to subsequently result in BHR.

2. Materials and methods

2.1. Animals and reagents

Male Sprague–Dawley rats weighting 200–250 g were obtained from the Experimental Animal Center of Xi'an Jiaotong University College of Medicine, China. The Ethics Committee of Xi'an Jiaotong University approved the experimental protocol. The Institutional Animal Care and Use Committee of Xi'an Jiaotong University approved all experiments. ET-1 and sarafotoxin 6c (S6c) were purchased from NeoMPS (NeoMPS SA. Strasbourg, France) and dissolved in 0.1% bovine serum albumin. ET_B receptor antagonist (BQ-788) was purchased from Medchemexpress (Shanghai, China). Other reagents were purchased from Sigma (St. Louis, MO, USA). MAPK inhibitors (SP600125, SB203580 and U0126) were dissolved in dimethylsulfoxide (DMSO).

2.2. PM2.5 sources and characterization

PM2.5 samples were collected from October to December 2012 on the roof surface of a 15 m-high building using quartz microfiber filters (Whatman Ltd., Maidstone, UK). The sampling site is located in the southeastern part of downtown Xi'an. Inorganic ions concentrations were analyzed using an ion chromatograph. Carbonaceous aerosols were analyzed using thermal and optical carbon analyzers. Metal elements were determined using inductively coupled plasma-atomic emission spectrometry. Polycyclic aromatic hydrocarbons (PAHs) was analyzed using gas chromatography-mass spectrometry. Detailed methods for sample collection and analyses were previously described (Shen et al., 2010; Xiao et al., 2015). Detached particles were dried, weighed, and resuspended in 1 ml DMSO at a series of concentrations (0.3, 1.0, and 3.0 mg/ml), and 1 μl samples were added to 1 ml culture medium for final concentrations of 0.3, 1.0, and 3.0 μg/ml, respectively.

2.3. Preparation and culture of bronchial segments

Rats were anesthetized with CO_2 and sacrificed. The lungs were removed gently and immersed in an ice-cold MOPS solution (mM): NaCl 140, MOPS 2.0, $Na_2HPO_4 \cdot 12H_2O$ 1.2, EDTA 0.02, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl_2$ 1.6, and glucose 5.6 (Ping et al., 2014). The bronchi were dissected free of adhering tissue under a dissection microscope and cut into 1–2 mm-long ring segments. Individual bronchi segments were placed into wells of a 24-well plate with 1 ml of serum-free Dulbecco's modified Eagle's medium (DMEM) supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml). The bronchus segments were divided into different groups according to the following experiments: fresh group (without culture), control group (DMSO), PM2.5 group, PM2.5 + inhibitor groups, and DMSO + inhibitor groups. The concentration of inhibitors was 10^{-5} M in the wells. The plates were incubated at 37°C in humidified 5% CO_2 and 95% air for 24 h. Some of the bronchial segments were snap-frozen at −80°C or fixed in paraformaldehyde for mechanism studies.

2.4. Contractile function studies

A wire myograph (Danish Myo Technology A/S, Aarhus, Denmark) was used to record bronchial contraction. Each bronchial segment was threaded through two thin wires (40 μm in diameter) and mounted in myograph baths. One wire was connected to a force displacement transducer attached to an analog-to-digital converter unit (AD Instruments, Hastings, UK). The other wire was connected to a movable displacement device that allowed adjustment of the distance between the two parallel wires. Data were recorded using the software program Chart™ (AD Instruments, Hastings, UK) (Cao et al., 2012b; Sun et al., 2013). The segments were immersed in temperature-controlled (37°C) baths containing 5 ml MOPS solution (pH 7.4). The MOPS solution was continuously gassed with O_2. The segments were given an initial tension of 1 mN (Lei et al., 2010) and allowed to stabilize for 1.5–2 h.The contractile capacity of each bronchial segment was determined by exposure to a K^+-rich MOPS solution, which had the same composition as MOPS solution, except NaCl was replaced by an equimolar concentration of KCl. The K^+-induced contraction was used as a reference, and the segments were used only if K^+ elicited reproducible responses >2 mN with a variation <10%.

ET_A receptor-mediated contraction experiments began with the desensitization of the ET_B receptors by inducing a concentration response curve for the selective ET_B receptor agonist S6c (Cao et al., 2012b). The maximal contraction (E_{max}) induced by S6c was reached, and the segments were maintained with the highest concentration of S6c for an additional 1 h until the contractile curves fell to a baseline level, which was considered as a total desensitization. Therefore, ET-1-induced concentration-contraction curves were mediated ET_A receptors. The selective ET_B receptor antagonist BQ-788 (10^{-6} M) was added to confirm the