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Vitamin E antagonizes ozone-induced asthma exacerbation in Balb/c mice through the Nrf2 pathway

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ABSTRACT

Millions of people are regularly exposed to ozone, a gas known to contribute significantly to worsening the symptoms of patients with asthma. However, the mechanisms underlying these ozone exacerbation effects are not fully understood. In this study, we examined the exacerbation effect of ozone in OVA-induced asthma mice and tried to demonstrate the protective mechanism of vitamin E (VE). An asthma mouse model was established, and used to identify the exacerbating effects of ozone by assessing cytokine and serum immunoglobulin concentrations, airway leukocyte infiltration, histopathological changes in lung tissues, and airway hyper-responsiveness. We then determined the amount of reactive oxygen species (ROS) accumulated, the extent to which VE induced ROS elimination, and examined the antagonistic effects of VE on the ozone-induced exacerbating effects. This study showed that 1-ppm ozone exposure could exacerbate OVA-induced asthma in mice. More importantly we found that ozone induced oxidative stress in asthmatic airways may lead to the inhibition of Nuclear factor-erythroid 2-related factor 2 (Nrf2), and may subsequently induce even more exaggerated oxidative stress associated with asthma exacerbation. Through VE induced Nrf2 activation and the subsequent increase in Nrf2 target protein expression, this study suggests a novel mechanism for alleviating ozone exacerbated asthma symptoms.

1. Introduction

Asthma is a widespread global health issue characterized by airway inflammation mediated through hyperresponsiveness (AHR), remodeling, and infiltration of eosinophils, neutrophils and mast cells into the airway wall. (Halwani et al., 2010; Kim et al., 2010). The morbidity of asthma has progressively increased over the past several decades (Barnes, 2010). Both epidemiological evidence and experimental evidence have demonstrated an association between exacerbation of asthma and levels of atmospheric pollutants (Alexis and Carlsten, 2014; Kelly and Fussell, 2011; Nastos et al., 2010). Ozone(O_3) is a robust oxidizing agent routinely affects millions of people worldwide, and significantly contributes to worsened symptoms in patients with asthma and chronic obstructive pulmonary disease (COPD) (Saingam et al., 2016; Tetreault et al., 2016). Studies in animal models have confirmed this finding, and demonstrated that ozone exposure can promote allergic sensitization and exacerbate allergic responses (Bao et al., 2014; Liang et al., 2013). However, the mechanisms underlying these ozone induced exacerbated effects in asthma patients are not fully understood. Elucidating the underlying mechanisms is pivotal in the prevention and treatment of ozone-induced asthma exacerbation.

Inhaled ozone interacts with airway lining fluid in the pulmonary system to produce reactive oxygen species (ROS) and other oxidation products, which may result in oxidative injury (Leroy et al., 2015). Oxidative damage is an important etiology in the pathophysiology of respiratory diseases, especially in pollution-

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induced exacerbations (Ciencewicki et al., 2008b). Nuclear factor kappa binding (NF-κB) is a key transcription factor that coordinates the expression of various inflammatory genes (Lawrence, 2009). ROS, created by a variety of pollutants, have various inhibitory or stimulatory roles in the NF-κB signaling system (Morgan and Liu, 2011). Various studies have reported the importance of the NF-κB pathway in ozone-induced lung inflammation and damage (Cho et al., 2007; Connor et al., 2012). Phosphorylation of NF-κB p65 (serine 536) plays an important role in activating NF-κB following ozone exposure and promoting inflammatory stimuli (Wu et al., 2010).

Nuclear factor-erythroid 2-related factor 2 (Nrf2) is a redox-sensitive transcription factor that plays an important role in the promoter region of genes, encoding antioxidant and/or detoxifying enzymes and other related stress-responsive proteins (Jang et al., 2016; Kim and Keum, 2016). Evidence suggests that there is functional cross talk between NF-κB and Nrf2 pathways. The absence of Nrf2 is associated with increased oxidative stress, exacerbated NF-κB activity and increased cytokine production, whereas NF-κB can modulate Nrf2 transcription and activity (Ganesh Yerra et al., 2013). Interventions focusing on restoring the balance between Nrf2 and NF-κB pathways in persons at risk for pollutant-induced diseases, represent a novel approach to preventing asthma exacerbation.

Given the vital role of oxidative stress in the pathophysiology of asthma exacerbation, it seems likely that antioxidants could be used as agents for the treatment of asthma caused by environmental factors such as ozone. Deficiencies in antioxidants like vitamin E have been linked to asthma exacerbation and severity (Romieu et al., 2002). Peh et al. (2015) have shown that Vitamin E acts not only as a direct free-radical scavenger, but also as an anti-inflammatory and antioxidative agent, by inhibiting NF-kB nuclear translocation, and promoting nuclear Nrf2 levels. In a previous study, we also showed that VE enhanced the expression of Nrf2, up-regulated the antioxidant genes heme oxygenase-1(HO-1) and quinone oxidoreductase 1(NQO1), and could very possibly decrease the levels of oxidative stress and alleviate ozone-induced lung injury (Zhu et al., 2016).

In this study we established an allergic asthma mouse model, and used it to identify the exacerbating effects of ozone by assessing cytokine and serum immunoglobulin concentrations, airway leukocyte infiltration, histopathological changes in lung tissue, and AHR. We then determined the amount of reactive oxygen species (ROS) accumulated, the extent to which vitamin E (VE) induced ROS elimination, and examined the antagonistic effects of VE on the ozone-induced exacerbating effects. Furthermore, by detecting the expression of Nrf2 and NF-κB in lung tissues, we tried to elucidate the potential molecular mechanisms of VE in attenuating the effect on ozone exacerbated allergic asthma.

2. Materials and methods

2.1. Animals

5-6 weeks SPF male Balb/c mice were purchased from the Hubei Province Experimental Animal Center (Wuhan, China). All mice were housed in pathogen-free cages at (24–26) °C and 12 h light-dark cycle with 55%–75% humidity. A commercial diet (Hubei Province Experimental Animal Center) and filtered water was provided *ad libitum*. All experiments were performed according to relevant guidelines and regulations. The experimental procedures were approved by the Office of Scientific Research Management of Central China Normal University (ID: CCNU-SKY-2011-008).

2.2. Ozone exposure and experimental design

O_3 was produced using a KTB portable ozone generator (Guangzhou, China) from ambient air. An ozone dosimeter inside the chamber was used to monitor the different concentrations of ozone. The experiment comprised 4 groups: (A) Control group, (B) Ovalbumin(OVA) group, (C) OVA+1.0 ppm ozone exposure group, (D) OVA + 1.0 ppm ozone and VE (α-tocopherol, 100 mg VE/kg via intraperitoneal injection) group. The mice in the control group were exposed to filtered air (FA). In this study, the concentration of ozone and quantity of vitamin E were determined according to our previous studies (Li et al., 2014a; Zhu et al., 2016).

The mice were sensitized with OVA + $Al(OH)_3$ (83.33 μg OVA and 2.92 mg $Al(OH)_3$ in 500 μL saline) or saline on days 1, 8 and 15 by intraperitoneal injection. Then the mice were exposed to an aerosol challenge of 1% OVA or saline (30 min/d) using an ultrasonic nebulizer (Yuyue 402A type I, China) on days 19 through 25. At the same time, mice were exposed to clean air or 1.0-ppm ozone for 3 h/day from days 19–25. VE was administered as an antioxidant 3 h after exposure to 1.0-ppm ozone. The detailed protocols are shown in Fig. 1.

2.3. Quantitative analyses of total serum IgE

On day 26, all mice were anaesthetized with 100 mg/(kg bw) pentobarbital. Heart blood was centrifuged at 3000 rpm for 10 min at room temperature, after which the serum was collected. The concentration of total-IgE in the serum was detected using eBioscience (San Diego, CA, USA) ELISA kits according to the manufacturer's instructions.

2.4. Examination of bronchoalveolar lavage fluid (BALF)

Mice were sacrificed and subjected to BAL after serum collection. BALF was obtained by lavaging three times with saline via a tracheal cannula while the mouse's chest was gently massaged. The volume of total lavage fluid was about 1.5 mL. The collected BALF was centrifuged at 1500 rpm at 4 °C for 10 min, and the cell sediment was then resuspended in 0.5 mL saline. Lymphocytes, eosinophils and neutrophils were counted using an automatic hematology analyzer (Matenu, MTN-21, China).

2.5. Tissue homogenate preparation and detection of cytokines

After collecting bronchoalveolar lavage fluid, the lung tissue was excised and washed in ice-cold phosphate-buffered saline (PBS). 10% lung tissue homogenate samples were prepared by homogenizing on ice using 10 mL/g of ice-cold PBS (pH 7.5). Afterwards, tissue samples were centrifuged at 12,000 rpm for 10 min at 4 °C. Finally, the supernatant was collected for evaluating the concentration of lung cytokines (IL-4, IL-5, IL-13 and IFN-γ) and TSLP using eBioscience ELISA kits. A modified BCA protein assay kit was purchased from Sangon Biotech (Shanghai, China) to determine total protein.

2.6. Analysis the level of glutathione (GSH) and malonaldehyde (MDA)

In accordance with the manufacturer's instructions, GSH content was measured by assay kit (Nanjing Jiancheng Bioengineering Institute, China). Malondialdehyde (MDA) concentration was determined using the thiobarbituric acid (TBA) method (Li et al., 2014a). The protein concentration was determined using the modified BCA protein assay kit (Sangon Biotech, China).