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Sulfur dioxide exposure enhances Th2 inflammatory responses *via* activating STAT6 pathway in asthmatic mice

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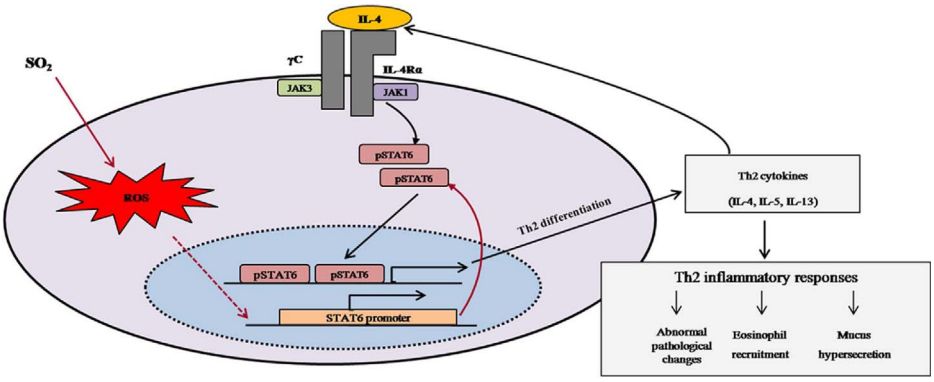
GRAPHICAL ABSTRACT



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ABSTRACT

Sulfur dioxide (SO_2) is one of potential risk factors for induction and/or exacerbation of asthma, but the underlying mechanisms are not well understood. In this study, we investigate the role of SO_2 in asthma using a classical asthmatic model with allergic airway inflammation by treating C57BL/6 mice with ovalbumin (OVA) and/or 10 mg/m^3 SO_2. Our results showed that SO_2 exposure alone induced slight pathological changes but did not significantly increase inflammatory cell counts, pro-inflammatory cytokine expression, and mucus production in the airway of mice, whereas SO_2 exposure in OVA-induced asthmatic mice caused marked pulmonary pathological changes and significantly increased the counts of eosinophil-rich leukocytes compared with OVA alone asthmatic mice. The expression of MUC5AC, TNF-α, Th2 cytokines (IL-4, IL-5, and IL-13) and STAT6 was further up-regulated in OVA plus SO_2 treated mice compared with OVA alone treated mice. In addition, exposure to SO_2 alone markedly elevated STAT6 mRNA levels and hydrogen peroxide (H_2O_2) content in the lung. These findings suggest that SO_2 amplifies Th2 inflammatory responses in OVA-induced asthmatic mice by activating STAT6, which can further induce Th2 cytokine expression. Induction of STAT6 expression might be an important mechanism underlying the increased risk for asthma after environmental exposure.

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1. Introduction

As a chronic inflammatory respiratory disease featured with mucus hyper-secretion and airway hyper-responsiveness, asthma is increasingly prevalent and has become a global health issue (Wegmann et al., 2005; Li et al., 2007; Hwang et al., 2017). It was estimated that asthma affected about 300 million people worldwide, and caused a great economic and social burden (Barnett and Nurmagambetov, 2011; Szefler et al., 2011; Pruitt and Lawson, 2011). Environmental factors, such as air pollution, may contribute to the increased occurrence of asthma (Tarlo et al., 2001; Lin et al., 2004; O'Connor et al., 2008; Smargiassi et al., 2014; Schultz et al., 2017).

Sulfur dioxide (SO_2) is a ubiquitous gaseous pollutant that is emitted mainly from the burning of sulfur-containing fossil fuels. SO_2 level in China was shown to be the highest in the world because of a coal-dominated energy structure (Watts, 2006), and the risk of high industrial SO_2 exposure was significant and cannot be overlooked (Deng et al., 2015). The biological link between SO_2 exposure and asthma has been studied from 1960s (Nakazawa, 1969). Epidemiology data showed that aggravated symptoms were found in asthmatics exposed to SO_2 (Sheppard et al., 1981; Hunt and Holman, 1987; Walters et al., 1994). Recently, more and more studies revealed that exposure to SO_2 increased the incidence of asthma among workers or children (Andersson et al., 2006; Smargiassi et al., 2009; Clark et al., 2010; Deger et al., 2012). On the other hand, several experimental studies have shown SO_2 exposure enhanced the development of allergic airway inflammation and airway hyper-responsiveness in animal asthmatic models (Riedel et al., 1988; Park et al., 2001; Song et al., 2012; Li et al., 2014). All of these studies indicate that SO_2 contributes to the induction and/or exacerbation of asthma, but the underlying mechanisms are not well understood.

Th2-mediated inflammatory responses play critical roles in the pathogenesis of asthma. It is reported that activation of STAT6 is vital for Th2 cells differentiation (Zhu et al., 2001; Walford and Doherty, 2013), and most IL-4-induced Th2 responses are actually mediated by JAK/STAT6 pathway (Kiu and Nicholson, 2012; Ashino et al., 2014). Although SO_2 is not an antigenic substance, it may increase susceptibility to allergens, induce inflammatory responses, and cause immune imbalance once exposed to animals (Cai et al., 2008; Li et al., 2014). However, to date few data are available on SO_2-induced Th2 inflammatory responses in asthmatic mice mediated by activating STAT6 pathway.

In the present study, we investigated the role of SO_2 in asthma using a mouse model induced by ovalbumin (OVA). We examined the pulmonary histopathological changes, performed bronchoalveolar lavage and counted the inflammatory cells, and measured the mRNA levels of MUC5AC, TNF-α, IL-4, IL-5 and IL-13. Then, we studied the components of JAK/STAT6 pathway (JAK1, JAK3, STAT6) and oxidative damage indexes. Our findings suggest a potential mechanism for how SO_2 induces and/or aggravates asthma.

2. Materials and methods

2.1. Animal treatment

Six-week-old male C57BL/6 mice were purchased from Academy of Military Medical Sciences and housed under specific pathogen-free conditions. The mice were randomly allocated into four groups: control, SO_2, OVA, and OVA + SO_2. We assumed that SO_2 exposure could induce asthma and/or aggravate OVA-induced asthma by increasing inflammatory cell infiltration, stimulating mucus production, and causing pulmonary histopathological changes. Based on our pilot experiments and power analysis, 6 mice for each group would be sufficient to detect significant differences among them. N ≥ 6 for each group were actually used in the study in order to avoid the case that one or two mice would not respond if an intraperitoneal (i.p.) injection was not done correctly.

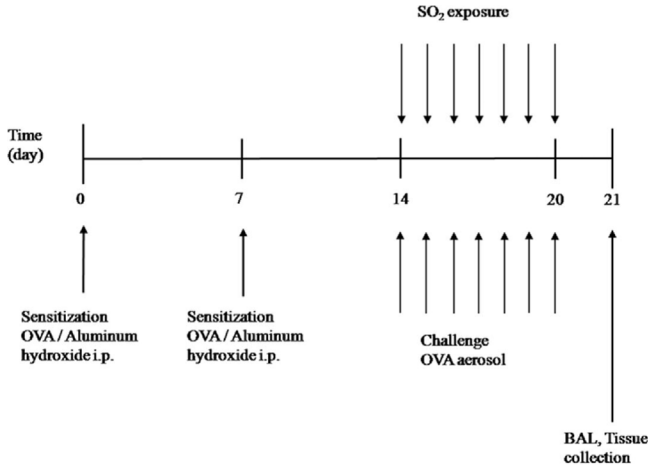

Fig. 1. Experimental protocol for OVA sensitization and challenge, and SO_2 treatment. Mice in OVA group and OVA + SO_2 group were sensitized with intraperitoneal (i.p.) injections of 100 μg OVA, mixed with 2 mg of aluminum hydroxide in 200 μL saline on days 0 and 7. From days 14–20, the mice were challenged by an aerosol of 1% OVA for a period of 30 min per day. Mice in SO_2 group and OVA + SO_2 group were exposed to SO_2 (10 mg/m^3) for 1 h/day before OVA challenge. On day 21, bronchoalveolar lavage (BAL) and tissue collection were performed.

In the OVA group and OVA + SO_2 group, the mice were sensitized by i.p. injection of 100 μg OVA (grade V; Sigma-Aldrich) and 2 mg aluminum hydroxide in 200 μL saline on days 0 and 7. In the control group and SO_2 group, mice received 200 μL of sterile saline. From days 14–20, the sensitized mice were challenged with 1% OVA aerosol for 30 min per day. On these days, mice in the SO_2 group and OVA + SO_2 group were also exposed to SO_2 for 1 h/day (10 mg/m^3 SO_2 in fumigation chambers, about 3.5 ppm) before OVA challenge. SO_2 gas was released into the chambers from a cylinder through a tube and its concentration was detected by the method of our previous study (Li et al., 2016). Control mice were exposed to fresh air during the experiment. All of the mice were provided *ad libitum* with food and water when not being treated. Combined OVA and SO_2 exposure protocol is shown in Fig. 1. Mice were anesthetized (sodium pentobarbital, 60 mg/kg, i.p.) and sacrificed 24 h after the last challenge (thus on day 21). All animal experiments were strictly performed following the protocol approved by Shanxi University to protect the welfare of animals.

2.2. Bronchoalveolar lavage and inflammatory cell counting

Under anesthesia, the right lung was ligated and the trachea was cannulated with a tracheal catheter. The left lung lobes were lavaged three times with sterile isotonic saline for a total volume of 1 mL for each mouse. Total numbers of leukocytes were counted using a hemacytometer, and differential counts were done on Wright-Gimsa stained smears. The right lung middle lobe was fixed in 10% neutral formalin for observing histopathological changes, and the rest of the right lung tissues, as well as the trachea, were stored at −80 °C for future study.

2.3. Histopathological examination

The fixed lung tissues were embedded in paraffin, cut into 4–5 μm-thick sections, and stained with hematoxylin-eosin (HE). Sections were examined using light microscopy (Olympus, Japan).

2.4. Real time quantitative PCR (qPCR)

Total RNA was isolated from lungs and tracheas using the Transzol reagent (Transgen, China) based on the manufacturer's protocol, and quantified with a spectrophotometer (Eppendorf, Germany) by measuring optical density at 260 nm. The quality was further confirmed by