

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



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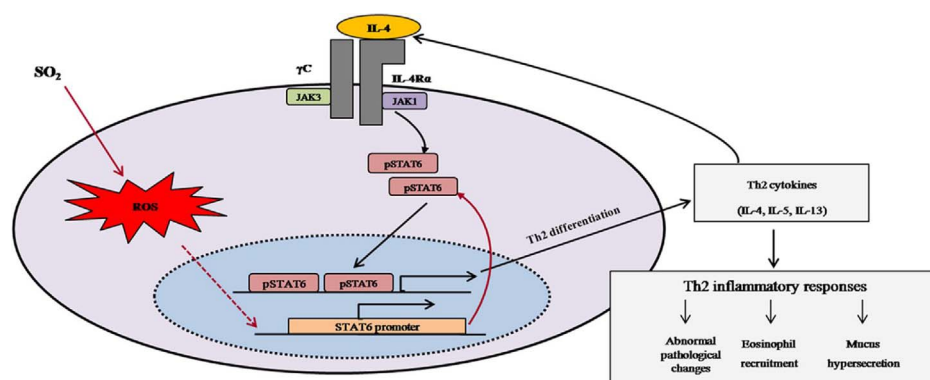
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GRAPHICAL ABSTRACT



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ABSTRACT

Sulfur dioxide (SO₂) 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 SO₂ in asthma using a classical asthmatic model with allergic airway inflammation by treating C57BL/6 mice with ovalbumin (OVA) and/or 10 mg/m³ SO₂. Our results showed that SO₂ 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 SO₂ 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 SO₂ treated mice compared with OVA alone treated mice. In addition, exposure to SO₂ alone markedly elevated STAT6 mRNA levels and hydrogen peroxide (H₂O₂) content in the lung. These findings suggest that SO₂ 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; Szeffler 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 (SO₂) is a ubiquitous gaseous pollutant that is emitted mainly from the burning of sulfur-containing fossil fuels. SO₂ 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 SO₂ exposure was significant and cannot be overlooked (Deng et al., 2015). The biological link between SO₂ exposure and asthma has been studied from 1960s (Nakazawa, 1969). Epidemiology data showed that aggravated symptoms were found in asthmatics exposed to SO₂ (Sheppard et al., 1981; Hunt and Holman, 1987; Walters et al., 1994). Recently, more and more studies revealed that exposure to SO₂ 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 SO₂ 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 SO₂ 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 SO₂ 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 SO₂-induced Th2 inflammatory responses in asthmatic mice mediated by activating STAT6 pathway.

In the present study, we investigated the role of SO₂ 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 SO₂ 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, SO₂, OVA, and OVA + SO₂. We assumed that SO₂ 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. $N \geq 6$ 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.

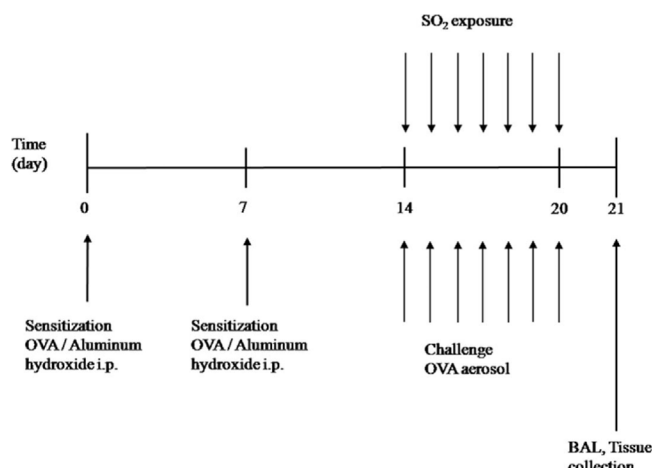


Fig. 1. Experimental protocol for OVA sensitization and challenge, and SO₂ treatment. Mice in OVA group and OVA + SO₂ group were sensitized with intraperitoneal (i.p.) injections of 100 μ g OVA, mixed with 2 mg of aluminum hydroxide in 200 μ L saline on days 0 and 7. From days 14–20, the mice were challenged by an aerosol of 1% OVA for a period of 30 min per day. Mice in SO₂ group and OVA + SO₂ group were exposed to SO₂ (10 mg/m³) for 1 h/day before OVA challenge. On day 21, bronchoalveolar lavage (BAL) and tissue collection were performed.

In the OVA group and OVA + SO₂ group, the mice were sensitized by i.p. injection of 100 μ g OVA (grade V; Sigma-Aldrich) and 2 mg aluminum hydroxide in 200 μ L saline on days 0 and 7. In the control group and SO₂ group, mice received 200 μ L 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 SO₂ group and OVA + SO₂ group were also exposed to SO₂ for 1 h/day (10 mg/m³ SO₂ in fumigation chambers, about 3.5 ppm) before OVA challenge. SO₂ 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 SO₂ 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 μ m-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

Table 1
Primer sequences used for real-time PCR.

Gene	Primer sequence (5'–3')
MUC5AC	AGGGCTCTGTGACAACCTACC TGGGGTGTGGGTAGAAGAAC
TNF- α	CCTCAGACTCAGATCATCTTCTCA TGGTTGTCTTTGAGATCCATGC
IL-4	CCCCAGCTAGTTGTCATCCTCG CAAGTGATTTTGTGCGATCCG
IL-5	GCAATGAGACGATGAGGCTTC GCCCTGAAAGATTCTCCAATG
IL-13	CAGCTCCCGATACCAAAAT GCGAAACAGTTGCTTTGTGTAG
IL-4R α	AACCTGTACCCATCGAACAAC GCCGTATAGTAGACCCCTGAC
JAK1	ACGCTCCGAACCGAATCATC GTGCCAGTTGGTAAAGTAGAACC
JAK3	ACACCTCTGATCCCTCAGC GCGAATGATAAACAGGCAGGATG
STAT6	CCTGGTGGTTTCAGATGCTTT GTGCGGCAAGATGCTGTTTC
β -Actin	GATTACTGCTCTGGCTCCTA ATCGTACTCTGCTTGTCTGA

determining the A260 nm/A280 nm ratios and by 1% agarose gel electrophoresis. A PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa Bio, Japan) was used for the cDNA synthesis. Samples were analyzed using SYBR® Premix Ex Taq™ II PCR Kit (TaKaRa Bio, Japan) on the 7500 system (Applied Biosystems, USA), following the manufacturer's instructions. The final qPCR reaction volume was 20 μ L, and each reaction was performed in duplicate. Sequences of primers used are shown in Table 1. The mRNA levels of target genes were normalized to internal control β -Actin using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.5. Determination of oxidative damage indexes in lung tissues

About 100 mg of lung tissues was homogenized at 10% (w/v) in homogenization buffer (0.9% sodium chloride) on ice by a glass homogenizer. The homogenate was centrifuged at 3500 rpm for 15 min at 4 °C and the supernatant was used for analysis. Hydrogen peroxide (H_2O_2) content, malondialdehyde (MDA) content, total superoxide dismutase (T-SOD) activity, and total antioxidant capacity (T-AOC) were measured using assay kits according to the manufacturers' instructions (Nanjing, China), and protein level was measured by Coomassie brilliant blue method (Bradford, 1976).

2.6. Statistical analysis

Statistical analysis was done using SPSS 21.0 statistic software. Experimental data were subjected to one-way ANOVA and significance testing was performed by Fisher LSD and Dunnett's T3 tests. The results were expressed as mean $\pm$ SE. *P*-values less than 0.05 were accepted as statistically significant.

3. Results

3.1. Histopathological changes

The histopathological changes of lung tissues were examined by HE staining after SO_2 , OVA, and OVA + SO_2 exposure. In the control group, no histopathological abnormality was observed in the lung (Fig. 2A). After 10 mg/m³ SO_2 exposure, only minor pathological changes occurred, such as bronchial epithelial thickening, slight epithelial damage, and diminished alveolar spaces (Fig. 2B). OVA-sensitized animals showed epithelial disruption, thickened subepithelial cell layers, diminished alveolar spaces, inflammatory cell infiltration, and

slight mucus secretion (Fig. 2C). Severe pathological changes were observed in mice exposed to OVA + SO_2 , such as marked mucus secretion, strikingly increased inflammatory cells, considerably thickened airway wall, and a pronounced airway obstruction (Fig. 2D).

3.2. Inflammatory cell counts

Compared with the control, a marked influx of inflammatory cells into BALF was observed after OVA or OVA + SO_2 treatment (Fig. 3). Furthermore, OVA + SO_2 exposure significantly increased the counts for total cells, eosinophils, neutrophils, and lymphocytes, versus the OVA group. No statistical difference of inflammatory cell counts was observed in the SO_2 group.

3.3. MUC5AC mRNA expression in tracheas of mice

As shown in Fig. 4, significant increases in MUC5AC mRNA expression were observed in mice exposed to OVA or OVA + SO_2 ($P < 0.01$ or $P < 0.001$). Compared with the OVA group, MUC5AC mRNA expression was markedly higher after OVA + SO_2 exposure ($P < 0.05$). SO_2 group showed no significant change.

3.4. The expression of pro-inflammatory TNF- α and Th2 cytokines in lungs of mice

As presented in Fig. 5A, both OVA and OVA + SO_2 exposure markedly elevated TNF- α expression in lungs of exposed mice compared with its levels in the lung of control mice ($P < 0.01$ or $P < 0.001$). Furthermore, TNF- α expression was higher in mice treated by OVA + SO_2 than those in mice treated by OVA alone ($P < 0.01$). Compared with the control, TNF- α mRNA levels were not significant changed in the SO_2 group.

In Fig. 5B, both OVA and OVA + SO_2 exposure significantly increased IL-4 mRNA expression in mouse lungs ($P < 0.05$ or $P < 0.001$). Moreover, OVA + SO_2 exposure further induced IL-4 mRNA expression compared with OVA alone exposure ($P < 0.05$). IL-4R α expression was also induced by OVA + SO_2 exposure ($P < 0.05$). No statistical difference of IL-4 mRNA level was observed in mice treated by SO_2 alone. Fig. 5D and E present the mRNA levels of IL-5 and IL-13 in mice after SO_2 , OVA, and OVA + SO_2 exposure. The expression of IL-5 and IL-13 was significantly increased in mice exposed to OVA or OVA + SO_2 ($P < 0.001$), but no statistical difference was observed in the SO_2 group. Compared with the OVA group, IL-5 and IL-13 mRNA levels were significantly increased in mice after OVA + SO_2 exposure ($P < 0.05$ or $P < 0.01$).

3.5. The expression of JAK/STAT6 pathway components in lungs of mice

As presented in Fig. 6A and C, OVA exposure significantly increased the mRNA levels of JAK1 and STAT6 ($P < 0.05$ or $P < 0.01$), and OVA + SO_2 exposure also caused such effect ($P < 0.01$ or $P < 0.001$). The STAT6 mRNA expression in mice treated by OVA + SO_2 was markedly higher than that in mice treated by OVA alone ($P < 0.05$). The STAT6 mRNA level was also significantly changed in the lung of mice treated by SO_2 alone ($P < 0.01$). However, no significant difference of JAK1 expression was observed in mice after OVA + SO_2 exposure, compared with OVA group (Fig. 6A). The JAK3 mRNA expression was not significantly changed in any treatment groups (Fig. 6B).

3.6. Oxidative damage indexes in lung tissues

As shown in Fig. 7, SO_2 exposure alone significantly increased H_2O_2 content ($P < 0.01$) but did not change MDA content and SOD and T-AOC activities. SOD activity and H_2O_2 content were significantly decreased in lungs of mice exposed to OVA alone ($P < 0.05$). In OVA plus

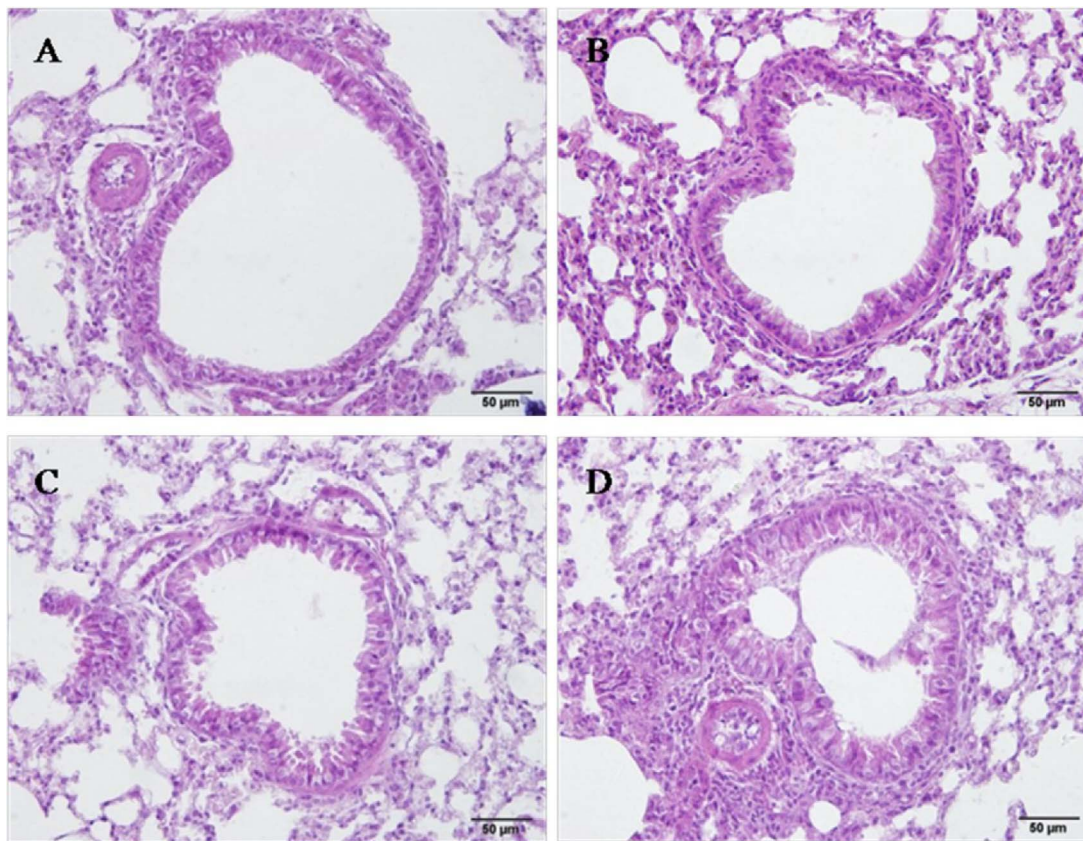


Fig. 2. Representative histopathological images of HE staining in lungs of mice from control (A), SO₂ (B), OVA (C), and OVA + SO₂ (D) group, 400 × magnification.

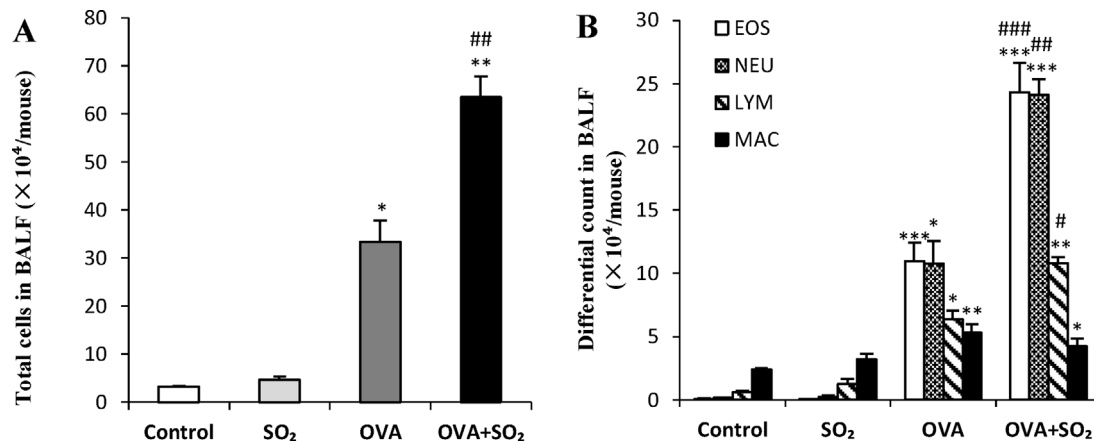


Fig. 3. The total cells (A) and differential cells (B) in BALF. EOS, eosinophils; NEU, neutrophils; LYM, lymphocytes; MAC, macrophages. Data were expressed as mean ± SE (n = 6); **P* < 0.05, ***P* < 0.01, ****P* < 0.001 versus control; #*P* < 0.05, ##*P* < 0.01, ###*P* < 0.001 versus the OVA group.

SO₂ group, MDA content significantly increased (*P* < 0.05), whereas SOD and T-AOC activities significantly decreased (*P* < 0.01 or *P* < 0.05).

4. Discussion

In this study, we use OVA-induced asthma model in mice to investigate how SO₂, a major environmental risk factor, exacerbates asthma. Our results showed that SO₂ exposure alone induced STAT6 mRNA expression, increased H₂O₂ content, and caused slight airway injury. The inflammatory phenotype of asthma, such as inflammatory cell counts, pro-inflammatory cytokine expression, and mucus production, appeared to be not significantly changed in mice exposed to SO₂ alone. However, SO₂ markedly enhanced OVA-induced Th2 airway

inflammation and increased the expression of downstream components of the JAK/STAT6 pathway. Our results revealed a potential mechanism, involving the IL-4/JAK/STAT6 pathway, for SO₂ induced or aggravated asthma.

SO₂ at 10 mg/m³ (about 3.5 ppm) level was used to examine the effects of its exposure in this study. The latest 24-h average standard of SO₂ in China is 0.15 mg/m³ (about 0.0525 ppm). Although SO₂ exposure concentration in the experiments may be considered as beyond normal atmospheric value, two important points could justify the use of this exposure protocol. First, the mice in the present study were exposed to SO₂ for 1 h per day, with an 23 h interval between exposures, therefore the 24-h average exposure concentration was 0.417 mg/m³ (about 0.146 ppm), which is 2.78 times higher than the air quality standard in China. However, it must be noted that this conversion is

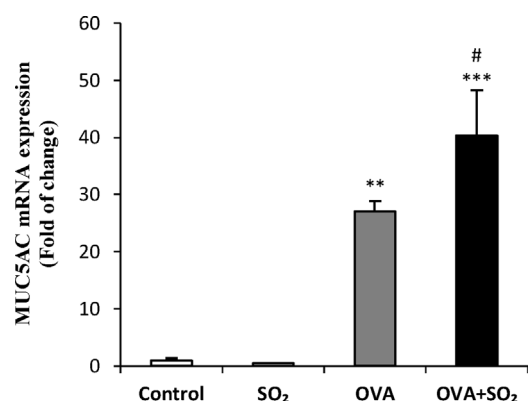


Fig. 4. qPCR analysis of mRNA levels of MUC5AC in tracheas of different groups of mice. The level of MUC5AC mRNA in each treatment group was expressed as a fold change compared to the mean value in the control group, which has been ascribed as an arbitrary value of 1. Data were expressed as mean $\pm$ SE (n = 6); ***P* < 0.01, ****P* < 0.001 versus control; #*P* < 0.05 versus the OVA group.

nonequivalent, because the two modes of exposure (*i.e.* low-level life-time exposure and higher-level of short-term exposure) may have differential biological effects. Second, the National Institute for Occupational Safety and Health (NIOSH) acceptability standards vary from 5 ppm SO₂ exposure for 15 min to 2 ppm exposure for 10 h, the short term exposure limit (STEL for 15 min) recommended by the Scientific Committee on Occupational Exposure Limits (SCOEL) for SO₂ is 1 ppm, and the threshold limit value (TLV)-STEL standards recommended by American Conference of Governmental Industrial Hygienists (ACGIH) is 0.25 ppm. The SO₂ exposure in this study is 3.5 ppm, which is close to the NIOSH standards and 3.5 times higher

than SCOEL recommended standards and 14 times higher than ACGIH recommended standards. Considering the exposure concentration and the 23 h interval between exposures, the SO₂ exposure model used in this study is suitable for mimicking short-term environmental exposure in seriously polluted areas and occupational exposures under some conditions.

As a respiratory inflammatory disease, asthma is characterized by recruiting various inflammatory cells into the airway (Busse and Lemanske Jr, 2001). We demonstrated the effects of SO₂ on airway inflammation in OVA-induced asthmatic mice. Consistent with previous investigations (Li et al., 2014; Cai et al., 2008), SO₂ exposure markedly enhanced OVA-induced inflammatory cell infiltration, which was demonstrated by HE staining and inflammatory cell counts in BALF. The pro-inflammatory cytokine TNF- α plays a critical role in amplifying asthmatic inflammation (Barnes, 2008). It not only can directly trigger inflammation in tissues, but also has the capacity to induce the expression of many other cytokines, chemokines, adhesion molecules, and mucins (Matera et al., 2010). Thus, we investigated the mRNA levels of TNF- α in the lung and found that SO₂ significantly up-regulated TNF- α expression in OVA-induced mice. These findings indicated that SO₂ enhanced OVA-induced airway inflammation by inducing inflammatory cell infiltration and pro-inflammatory cytokine expression. Although no obvious inflammatory reactions were observed, slight airway epithelial injury was detected in the lung of mice exposed to SO₂ alone. This airway epithelial injury could probably promote the access of the inhaled OVA, which may be an important reason why OVA + SO₂ exposure can incite a stronger inflammatory response than OVA exposure alone.

Airway mucins play critical roles in host defense response, but mucus overproduction represents a major cause of the airway obstruction (Wang et al., 2017; Knowles and Boucher, 2002). Mucus

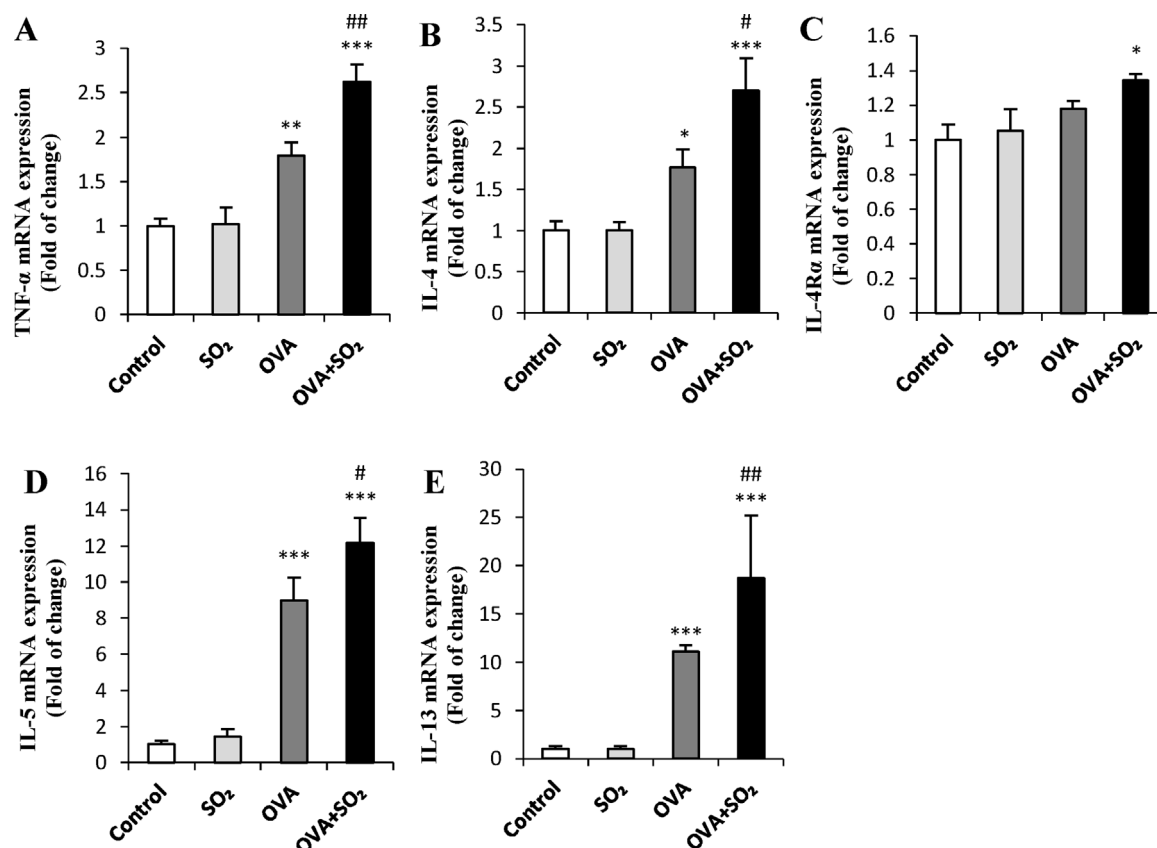


Fig. 5. qPCR analysis of mRNA levels of TNF- α (A), IL-4 (B), IL-4R α (C), IL-5 (D), and IL-13 (E) in lungs of mice. mRNA levels in each treatment group were expressed as fold changes compared to the mean values in the control group, which have been ascribed as an arbitrary value of 1. Data were expressed as mean $\pm$ SE (n = 6); **P* < 0.05, ***P* < 0.01, ****P* < 0.001 versus control; #*P* < 0.05, ##*P* < 0.01 versus the OVA group.

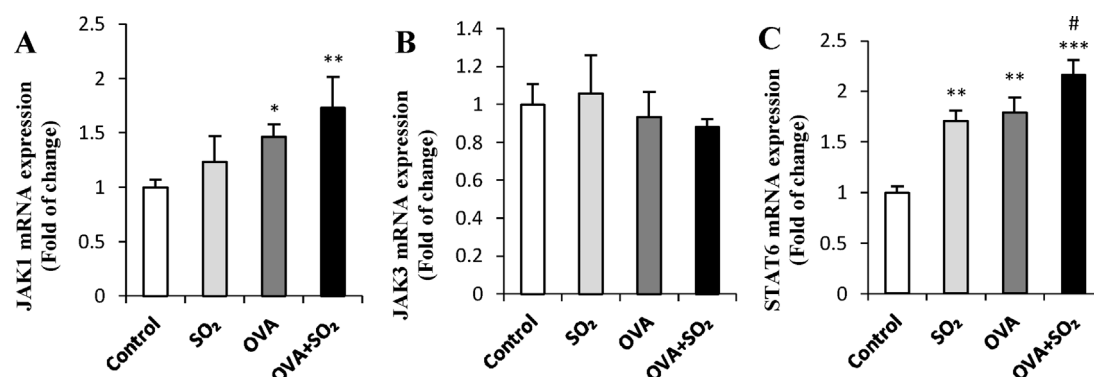


Fig. 6. qPCR analysis of mRNA levels of JAK1 (A), JAK3 (B), STAT6 (C) in lungs of mice. mRNA levels in each treatment group were expressed as fold changes compared to the mean values in the vehicle control group, which have been ascribed as an arbitrary value of 1. Data were expressed as mean $\pm$ SE (n = 6); * P < 0.05, ** P < 0.01, *** P < 0.001 versus control; # P < 0.05 versus the OVA group.

hyper-secretion is an important pathological feature in asthma (Ulrik et al., 2005; Lai and Rogers, 2010). MUC5AC is a major mucin in airway and is known to be overproduced in asthmatics (Alimam et al., 2000; Wang et al., 2017). Our present studies showed that OVA exposure markedly increased MUC5AC mRNA levels in mouse tracheas, and OVA + SO₂ exposure enhanced this effect. These results illustrated that SO₂ exposure aggravated the symptoms of asthma by inducing excess mucus production, which was demonstrated by HE staining.

Th2 lymphocytes play important roles in initiation and progression of asthma by releasing characteristic cytokines such as IL-4, IL-5, and IL-13 (Bosnjak et al., 2011), which mediate allergic inflammatory responses in airway (Guo et al., 2014; Paul and Zhu, 2010). IL-4 regulates Th2 cell differentiation and may be involved in the initial allergen sensitization (Barnes, 2008; Paul and Zhu, 2010). IL-5 contributes to

airway eosinophilia, and plays a critical role in allergic airway inflammation mediated by eosinophils (Molfino et al., 2012). IL-13 and IL-4 have both been implicated in asthmatic mucus production (Kuperman et al., 2005). Airway eosinophil recruitment and mucus hyper-secretion enhanced by SO₂ in OVA-induced mice may result from the increased levels of Th2 cytokines. Indeed, our findings showed that SO₂ significantly increased the transcriptional levels of IL-4, IL-5 and IL-13 in OVA-induced asthmatic mice. These findings implied that SO₂ could amplify Th2 inflammatory responses by inducing Th2 cytokine expression in asthmatic mice, which might be an important mechanism for how SO₂ increases the risk of asthma.

STAT6 has been shown to regulate many pathological characteristics of asthma, including Th2 cell differentiation, airway eosinophilia, and mucus secretion, in animal models (Walford and Doherty, 2013;

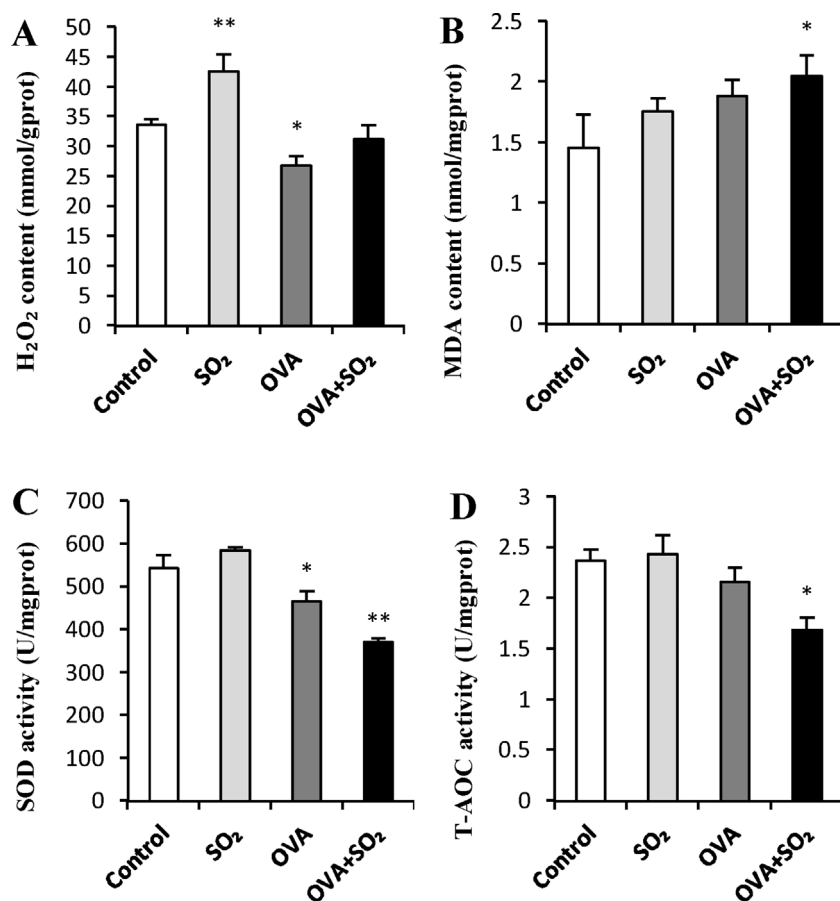


Fig. 7. Effects of SO₂, OVA, OVA + SO₂ exposure on H₂O₂ content (A), MDA content (B), SOD (C) and T-AOC (D) activities in the lung from mice. The results were expressed as mean $\pm$ SE (n = 6); * P < 0.05, ** P < 0.01 versus control.

Goenka and Kaplan, 2011). To determine whether the amplified Th2 inflammatory responses in asthmatic mice were due to Th2 differentiation mediated by IL-4/JAK/STAT6 pathway, we further investigated the mRNA expression of JAK1, JAK3 and STAT6 in the lung. Our results showed that the transcriptional levels of JAK1 and STAT6 were significantly up-regulated in mice exposed to OVA or OVA + SO₂, suggesting that JAK/STAT6 pathway contributed to OVA and/or SO₂-induced Th2 responses. STAT6 is canonically responsive to IL-4 and/or IL-13 (Goenka and Kaplan, 2011; Park et al., 2012). No significant increases of IL-4 and IL-13 expression were observed in mice exposed to SO₂ alone. Unexpectedly, SO₂ exposure significantly elevated STAT6 mRNA levels, versus the control ($P < 0.01$). This paradox could be partially explained by the discoveries that reactive oxygen species (ROS), such as H₂O₂, are known as important regulators of physiological cell signaling (Rhee, 2006; Finkel, 2011). It is reported that ROS could reversibly oxidize PTP1B, which is considered an important regulator of STAT6 in IL-4/STAT6 pathway (Sharma et al., 2008). *In vitro*, IL-4 combined with exogenous H₂O₂ further increased the level of STAT6 phosphorylation, relative to IL-4 treatment alone (Dwivedi et al., 2015). Another report showed that brief exposure to H₂O₂ rapidly induced STAT6 activation in astrocytes (Park et al., 2012). These discoveries implied that STAT6 might function as a ROS sensor in cell signaling. SO₂ is a major gaseous pollutant, and airway is the primary target of its toxic effects. In airway epithelium, inhaled SO₂ easily forms sulfurous acid which directly causes ROS production (Meng et al., 2003; Yun et al., 2011). Exposure to SO₂ significantly raised STAT6 mRNA levels, which might be due to the role of ROS produced by SO₂ in the lung. Therefore, we determined oxidative damage indexes. Our results showed that exposure to SO₂ alone significantly increased H₂O₂ content but did not change MDA content and T-SOD and T-AOC activities in the lung of mice. These findings implied that SO₂ exposure alone induced STAT6 expression probably by producing ROS, such as H₂O₂. Excess ROS generated by SO₂ can elicit oxidative damage and participate in the pathogenesis of airway inflammation (Yun et al., 2011). OVA + SO₂ exposure induced lipid peroxidation and changed anti-oxidative status in the lung, which was demonstrated by the increased MDA content and decreased activities of T-SOD and T-AOC. The oxidative damage caused by OVA plus SO₂ might be another important reason for the SO₂ amplified inflammatory response in OVA-induced asthmatic mice.

The molecular mechanism of Th2 differentiation induced by allergen can be described as follows: binding of IL-4 to its receptor (IL-4R α subunit) on T cells initiates phosphorylation of JAKs and subsequent STAT6 molecules. The phosphorylated STAT6 molecules dimerized and translocated to the nucleus where they interact with promoters of Th2 cytokines, resulting in differentiation of Th2 cells (Walford and Doherty, 2013; Ashino et al., 2014; Pernis and Rothman, 2002). In this study, OVA exposure induced the expression of JAK1, STAT6, and downstream Th2 cytokines, suggesting IL-4/JAK/STAT6 pathway was activated in the current mouse asthmatic model. The STAT6 expression induced by SO₂ exposure, as well as high levels of Th2 cytokine expression enhanced by SO₂ in OVA-induced asthmatic mice, implied that exposure to SO₂ alone might activate STAT6 pathway. The induced Th2 cytokines could further activate IL-4/JAK/STAT6 pathway, thus forming a positive auto-regulatory loop that can promote the Th2 inflammatory responses and worsen asthma.

Our results suggest that for people with no pre-existing asthma SO₂ exposure could increase the likelihood for them to develop allergic diseases, because SO₂ exposure could damage airway epithelium and thus enhance the access of inhaled allergens. For asthmatic patients, SO₂ exposure might aggravate asthmatic symptoms by inducing excess mucus production, amplifying Th2 inflammatory responses, and inducing oxidative damage in the airway. Although the mechanisms of SO₂ induced and/or aggravated asthma are very complex and more work is needed, the Th2-dependent JAK/STAT6 activation pathway represents a potential target for preventing airway injuries in polluted areas.

5. Conclusion

In this study, we showed that SO₂ exposure alone caused slight airway injury, increased H₂O₂ content, and induced STAT6 expression in the lung of mice. SO₂ also augmented the degree of pulmonary pathological injury, significantly increased in the inflammatory cell counts, and markedly induced the expression of the mucin gene MUC5AC and the pro-inflammatory cytokine TNF- α in OVA-induced asthmatic mice. Besides, up-regulated expression of Th2 cytokines and JAK/STAT6 pathway components (JAK1 and STAT6) were observed after OVA exposure and OVA + SO₂ exposure. These results provide evidence that SO₂ enhances Th2 inflammatory responses in lungs of OVA-induced asthmatic mice by activating STAT6 pathway. These findings suggest a STAT6-mediated mechanism for the aggravation of asthma after SO₂ exposure, and underscore the importance for assessing the biological safety of SO₂ exposure and its health effects.

Conflict of interest

The authors declare that there are no conflict of interest.

Acknowledgments

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