

# Effects of sulfur dioxide on the expressions of MUC5AC and ICAM-1 in airway of asthmatic rats

Ruijin Li, Ziqiang Meng \*, Jingfang Xie

*Institute of Environmental Medicine and Toxicology, Shanxi University, Taiyuan 030006, China*

Received 15 January 2007

Available online 21 May 2007

## Abstract

Asthma is a chronic inflammatory disease characterized by infiltration and activation of various inflammatory cells and mucus secretion. To investigate the effects of SO<sub>2</sub> on the expressions of asthma related-genes, male Wistar rats were challenged by ovalbumin (OVA) or SO<sub>2</sub> (2 ppm) inhalation alone or together. Bronchoalveolar lavage and histopathologic examination were performed 24 h after the last treatment. The mRNA and protein levels of MUC5AC and ICAM-1 were analyzed in lungs and tracheas using real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) assay, immunohistochemistry method and Western blot analysis, respectively. Exposure to OVA or to OVA plus inhaled SO<sub>2</sub> significantly caused increases of the mRNA and protein levels of MUC5AC and ICAM-1 in lungs and tracheas of rats compared with the control ( $P < 0.05$  or  $P < 0.01$ ), but the increases of mRNA and protein levels after SO<sub>2</sub> inhalation were not statistically significant. Exposure to OVA plus inhaled SO<sub>2</sub> significantly not only induced the mRNA and protein expressions of these genes, but also induced the infiltration of inflammatory cells in lungs and tracheas and the increase of the numbers of inflammatory cells in bronchoalveolar lavage fluids (BALF), versus exposure to OVA alone. Meanwhile, a synergistic effect on the pathological changes between SO<sub>2</sub> and OVA was observed in lungs after SO<sub>2</sub> and OVA exposure. These results suggested that SO<sub>2</sub> could increase the expressions of MUC5AC and ICAM-1 on the transcription and translation levels in the lungs and tracheas from asthmatic rats, which might be one of the possible mechanisms that SO<sub>2</sub> pollution aggravates asthma disease.

© 2007 Published by Elsevier Inc.

**Keywords:** Sulfur dioxide; Asthma; Rats; MUC5AC; ICAM-1

## 1. Introduction

It is estimated that about 155 million people worldwide are affected by asthma, which is associated with airway hyperresponsiveness, chronic inflammation, and mucus overproduction (Wills-Karp, 2000; Duncan, 2004; Wegmann et al., 2005). Recent study has shown that the development of asthma involves many genes and environmental factors (Blumenthal, 2005), of which mucin 5, subtypes A and C (MUC5AC) and intercellular adhesion molecule 1 (ICAM-1) play an important role in the pathogenesis of

asthma among plenty of asthma related-genes (Wegner et al., 1990; Morcillo and Cortijo, 2006).

Asthma is recognized to a chronic airway inflammatory disease characterized by infiltration and activation of various inflammatory cells (Hamid et al., 2003), including eosinophils, neutrophils, lymphocytes, macrophages, and so on. Accumulation of these cells within the airways can be influenced by expression of adhesion molecules on airway epithelium (Hogg et al., 1991). ICAM-1 is an adhesion molecule expressed on multiple cell types throughout the body, and functions to regulate infiltration of inflammatory cells within the airway. It plays an important role in establishing and maintaining inflammation in bronchial asthma (Wegner et al., 1990). MUC5AC is one of the mucin genes, which is expressed mostly in the respiratory tract epithelium and related with mucus secretion (Alimam

\* Corresponding author. Fax: +86 351 7011895.

E-mail addresses: [lirj@sxu.edu.cn](mailto:lirj@sxu.edu.cn) (R. Li), [zqmeng@sxu.edu.cn](mailto:zqmeng@sxu.edu.cn) (Z. Meng).

et al., 2000). Although mucins mainly encoded by MUC5AC are the necessary components of host defense, they represent an important cause of airway obstruction when secreted in excess (Knowles and Boucher, 2002).

Sulfur dioxide (SO<sub>2</sub>) is one of the most important air pollutants. The previous studies showed that SO<sub>2</sub> inhalation could affect the respiratory system in children and elderly people (Tseng and Li, 1990; Derya, 2003). Low dose exposure of SO<sub>2</sub> might aggravate asthma symptoms in asthmatic subjects (Sheppard et al., 1980; Linn et al., 1987; Grella et al., 2002). Recent studies showed that SO<sub>2</sub> exposure could change the mRNA expressions of apoptosis-related genes (p53, bax and bcl-2), proto-oncogenes (c-fos, and c-jun) and metabolizing enzymes (CYP1A1 and 1A2) in rats (Bai and Meng, 2005; Qin and Meng, 2005, 2006). However, few studies have focused on the effects of SO<sub>2</sub> on the expressions of asthma related-genes.

The asthmatic rat model challenged by ovalbumin (OVA) had been applied in the experiments and therapies of asthma (Wang and Li, 2003; Pilegaard and Madsen, 2004; Wu et al., 2006) for the advantages of genetic homogeneity of the study subjects as well as ready availability (Hylkema et al., 2002). In this study, we analyzed the expressions of MUC5AC and ICAM-1 in lungs and tracheas of rats exposed to OVA plus inhaled SO<sub>2</sub> using real-time RT-PCR assay and immunohistochemistry method and Western blot analysis, respectively.

## 2. Materials and methods

### 2.1. Animals and treatment protocols

Male Wistar rats were used as described previously (Qin and Meng, 2006). All animal procedures were approved by the Shanxi University Animal Investigational Committee and performed in accordance with the Guide for the Care and Use of Laboratory Animals published by Ministry of Health People's Republic of China. The treatment protocol was shown in Table 1.

### 2.2. Cell counts, hematoxylin and eosin (HE) staining, and immunohistochemistry

Rats were killed by anesthesia (sodium pentobarbital, 90 mg/kg, i.p.) 24 h after the last treatment. Then, the right lung was ligated, and the left lung was immediately lavaged with phosphate-buffered saline (PBS pH 7.4) solution. Such process was repeated three times with 5 ml PBS as total volume. The counts of total cells in bronchoalveolar lavage fluids (BALF) were determined with a hemacytometer, and inflammatory cells were counted with Wright-Gemsa staining.

After the collection of BALF, the right lung was frozen in liquid nitrogen for RNA and protein extraction, and fixed in 4% paraformaldehyde in PBS for the HE staining and immunohistochemical analysis, respectively.

### 2.3. Real time quantitative RT-PCR

RNA isolation, cDNA synthesis and PCR analysis were performed as described previously (Qin and Meng, 2006). Sequences of primers and probes are listed in Table 2. Each treatment had six samples and each PCR was carried out in duplicate. The conditions of PCR were as follows: 3 min at 95 °C, 45 cycles of 20 s at 94 °C, 20 s at 65 °C (MUC5AC) or 60 °C (ICAM-1), and 20 s at 72 °C. Fluorescence data were acquired at the 72 °C step. The relative quantification of the expressions of the target genes was measured using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA as an internal control.

### 2.4. Western blot analysis

Western blot analysis was performed as described previously (Qin and Meng, 2006). Briefly, proteins were separated by SDS–polyacrylamide gel electrophoresis (PAGE; 12% separating, 4% stacking) and transferred to NC membranes (Amersham Pharmacia Biotech, Inc., Piscataway, NJ). After the membranes were blocked in 5% nonfat dry milk in PBS buffer containing 0.1% Tween 20, immunostaining was performed with goat polyclonal antibody specific for rat ICAM-1 (SC-1511, Santa Cruz, CA, USA) and mouse monoclonal antibody for actin (Oncogene, Boston, MA) at a concentration of 1:300 (for ICAM-1) or 1:5000 (for actin) overnight at 4 °C. Anti-goat or anti-mouse secondary antibody (Amersham Pharmacia Biotech, Inc.) at a concentration of 1:5000 was added to membranes and incubated for 1.5 h at room temperature. The protein signal was amplified and visualized via chemiluminescence using the ECL Western blotting detection system and Hyperfilm ECL autoradiography film (Amersham Pharmacia Biotech, Inc.). Images were quantified using the Labworks v3.0.2 image scanning and analysis software (Ultra-Violet Products Lds., UVP).

Table 1  
Treatment protocols of rats for gene expression measures

| Group                                        | Treatment protocols                                                                        |                                                                                           |                                                                                                                       |
|----------------------------------------------|--------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------|
|                                              | Day 1st                                                                                    | Day 8th                                                                                   | Days 15–21                                                                                                            |
| Exposure to SO <sub>2</sub>                  | Injected by 1 ml saline/each rat                                                           | Non-treatment                                                                             | Exposed to SO <sub>2</sub> (about 2 ppm, 1 h/day)                                                                     |
| Exposure to OVA (Sigma, grade II)            | Sensitized by 100 mg OVA in 1 ml saline mixed with 100 mg of Al(OH) <sub>3</sub> /each rat | Sensitized by 10 mg OVA in 1 ml saline mixed with 100 mg of Al(OH) <sub>3</sub> /each rat | Challenged with aerosolized 1% OVA (0.5 h/day)                                                                        |
| Exposure to OVA plus inhaled SO <sub>2</sub> | Sensitized by 100 mg OVA in 1 ml saline mixed with 100 mg of Al(OH) <sub>3</sub> /each rat | Sensitized by 10 mg OVA in 1 ml saline mixed with 100 mg of Al(OH) <sub>3</sub> /each rat | After SO <sub>2</sub> inhalation (about 2 ppm, 1 h/day), the rats were challenged with aerosolized 1% OVA (0.5 h/day) |
| The control                                  | Injected by 1 ml saline/each rat                                                           | Non-treatment                                                                             | Fresh air inhalation                                                                                                  |

The rats were divided randomly into four equal groups of six animals each. They were treated by an intraperitoneal (i.p.) injection, and 1 ml of Bordetella Pertussis vaccine containing  $6 \times 10^9$  heat-killed organisms was also given intraperitoneally as adjuvant. The rats exposed to SO<sub>2</sub> alone or to OVA plus inhaled SO<sub>2</sub> were performed using the technique of SO<sub>2</sub> dynamic inhalation as described previously (Qin and Meng, 2006). The mean concentration of SO<sub>2</sub> was  $5.60 \pm 1.12$  (2 ppm).

Table 2  
Primers and probes sequences used in real-time RT-PCR

| Gene   | Accession Number | Sequences                                  |
|--------|------------------|--------------------------------------------|
| GAPDH  | NM_017008        |                                            |
|        | Forward primer   | 5'-ATGTATCCGTTGTGGATCTGAC-3'               |
|        | Reverse primer   | 5'-CCTGCTTCACCACCTTCTTG-3'                 |
|        | TaqMan probe     | 5'-FAM-TGCCGCTGGAGAAACCTGCCA-TAMRA-3'      |
| MUC5AC | AB042530         |                                            |
|        | Forward primer   | 5'-AACTCTGCCACCACAAGC-3'                   |
|        | Reverse primer   | 5'-TGCCATCTATCCAATCAGTCCAAT-3'             |
|        | TaqMan probe     | 5'-FAM-TGCCAGAGCCAACCTCAGCCACATC-TAMRA-3'  |
| ICAM-1 | NM_012967        |                                            |
|        | Forward primer   | 5'-GAACTGGACCTGGCAAGAG-3'                  |
|        | Reverse primer   | 5'-GTACCATTTCATCTCCCGTTTG-3'               |
|        | TaqMan probe     | 5'-FAM-AGACTTCACCATCCCGATAGGCAGCG-TAMRA-3' |

### 2.5. Immunohistochemistry

Immunohistochemistry analysis for MUC5AC protein expression was performed as described previously (Bai and Meng, 2005). The anti-MUC5AC mouse monoclonal antibody (45M1, Neomarkers, Fremont, CA) was incubated at a dilution of 1:200 overnight at 4 °C. A horseradish peroxidase-conjugate polyclonal rabbit anti-mouse antibody was used as the secondary antibody reagent. The reaction product was visualized using diaminobenzidine (DAB) as a substrate. The negative control was accomplished by substituting MUC5AC antibody with PBS. MUC5AC positive staining was shown at 400× magnification.

### 2.6. Statistical analysis

All values are expressed as means ± SD. Statistical analysis was performed using a one-way ANOVA test. A level of  $P < 0.05$  was accepted as statistically significant.

## 3. Results

In order to study the effects of SO<sub>2</sub> and OVA on the body weights of rats, we quantified the body weights of rats during the experiment. Although the results showed that the final weights of both control group and other groups were significantly increased relative to the beginning values (data not shown), no significant differences were found in the mean body weight gain of rats exposed to SO<sub>2</sub> and/or OVA compared with control. Moreover, at the end of treatment the rats in the control were in condition, and the rats exposed to SO<sub>2</sub> approximately in good order. However, other rats exposed to OVA alone or to OVA plus inhaled SO<sub>2</sub> showed abnormal symptoms such as quick breathing and sluggish movement, etc.

### 3.1. Inflammatory cell counts in BALF

The numbers of total cells, eosinophils, lymphocytes, macrophages, and neutrophils in BALF exposed to SO<sub>2</sub> alone were increased, but no significant difference was found compared with the control (Fig. 1). The counts of both total cells and inflammatory cells were significantly increased exposed to OVA ( $P < 0.05$  or  $P < 0.01$ ) or to OVA plus inhaled SO<sub>2</sub> ( $P < 0.05$  or  $P < 0.01$ ), versus the control. Furthermore, exposure to OVA plus inhaled SO<sub>2</sub> significantly

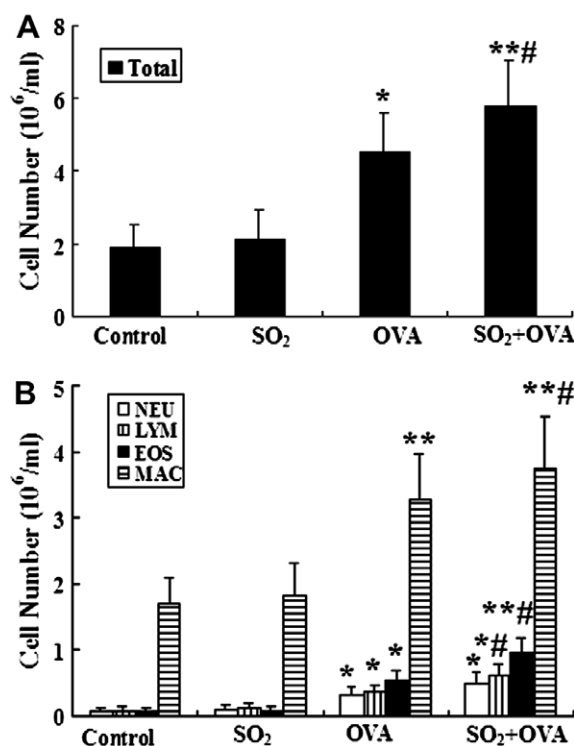


Fig. 1. Cell counts in BALF of rats in the different groups. (A) The number of total cells. (B) The numbers of inflammatory cells. Key to abbreviations: Total, total cells; EOS, eosinophils; LYM, lymphocytes; MAC, macrophages; NEU, neutrophils. Data are expressed as means ± SD of six individual samples. \* $P < 0.05$ , \*\* $P < 0.01$  versus corresponding control; # $P < 0.05$  versus corresponding rats exposed to OVA.

increased the numbers of total cells, eosinophils, lymphocytes, and macrophages except for neutrophils compared with exposure to OVA alone (Fig. 1A and B) ( $P < 0.05$ ).

### 3.2. Histopathological observation and immunohistochemistry analysis

In histopathologic examination, there was no abnormality in lungs and tracheas of rats in the control (Figs. 2A and 3A). The pathological changes, such as the influx of inflammatory cells, the thickened alveolar walls, the diminished

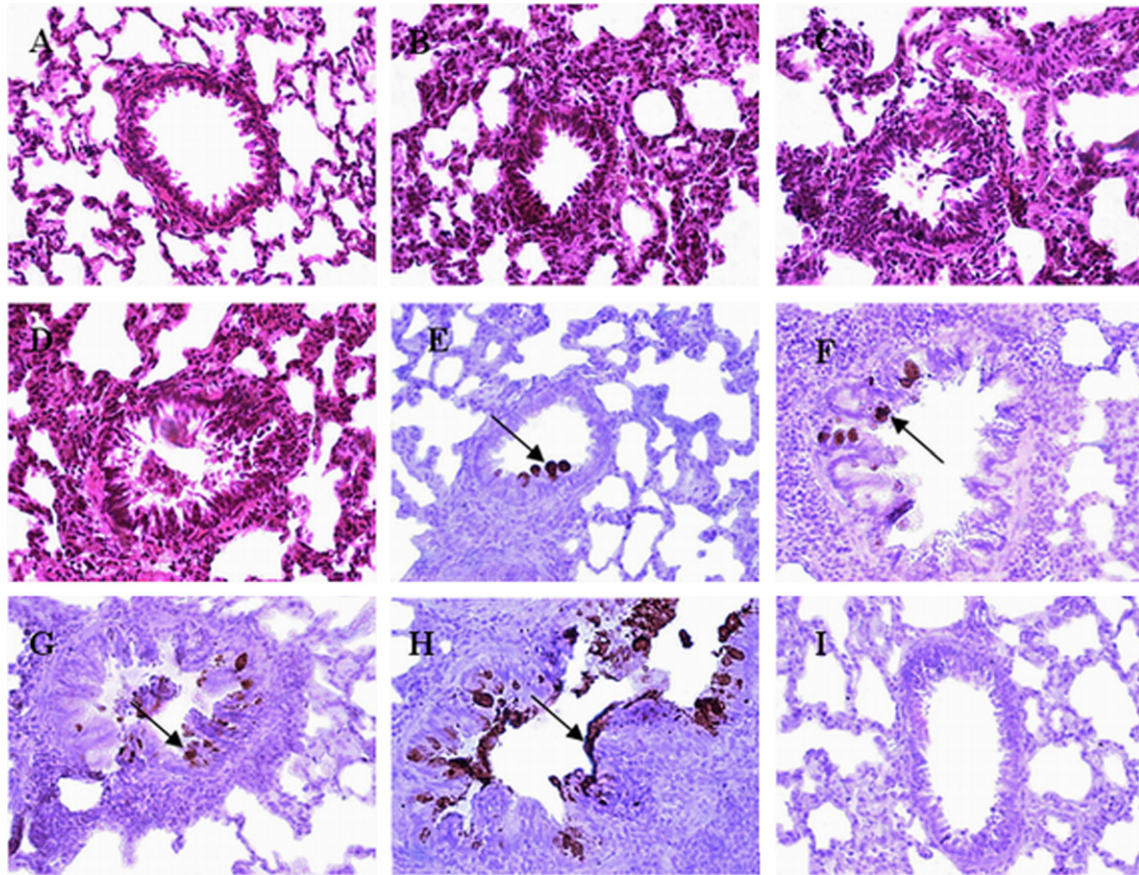


Fig. 2. HE staining and immunohistochemical staining using antibody to MUC5AC in lungs of rats exposed to SO<sub>2</sub> or/and OVA. (A–D) HE staining in lungs of rats exposed to the control, exposed to SO<sub>2</sub>, exposed to OVA and exposed to OVA plus inhaled SO<sub>2</sub>, respectively; (E–H) immunohistochemical staining for MUC5AC in lungs of rats exposed to the control, exposed to SO<sub>2</sub>, exposed to OVA and exposed to OVA plus inhaled SO<sub>2</sub>, respectively; and (I) negative control without primary antibody. At 400× magnification. →, Positive staining

alveolar spaces and hyperplasia of basement membrane and smooth muscle layer in the lungs and tracheas, were observed in rats exposed to OVA or SO<sub>2</sub> inhalation alone or together (Figs. 2B–D and 3B–D) compared with the control. Meanwhile, exposure to OVA plus inhaled SO<sub>2</sub> induced more pathological changes compared with exposure to OVA alone (Figs. 2C, D and 3C, D).

MUC5AC positive expression was mainly observed in the surface of both bronchial and tracheal epithelial cells, and partly expressed in the tracheal submucosal gland cells of rats (Figs. 2E–H and 3E–H, arrows). MUC5AC expression was induced by exposure to SO<sub>2</sub> alone (Figs. 2F and 3F), while its expression was significantly increased in bronchi and tracheas exposure to OVA alone or to OVA plus inhaled SO<sub>2</sub> (Figs. 2G, H and 3G, H) compared with the control (Figs. 2E and 3E). After treatment with the SO<sub>2</sub> plus OVA, MUC5AC was highly expressed compared with exposure to OVA (Figs. 2G, H and 3G, H). Weak background staining appeared in negative control slides not treated with MUC5AC antibody (Figs. 2I and 3I).

### 3.3. Real time RT-PCR analysis

The mRNA levels of MUC5AC and ICAM-1 were assessed in lungs and tracheas of rats exposed to SO<sub>2</sub>

and/or OVA (Fig. 4). The mRNA expressions of MUC5AC and ICAM-1 were significantly induced in lungs and tracheas of rats treated with OVA compared with the control (for lungs: 1.23- and 1.40-fold,  $P < 0.01$  or  $P < 0.05$ ; for tracheas: 1.49- and 2.60-fold,  $P < 0.05$  or  $P < 0.01$ ). Significant increases in the mRNA levels of MUC5AC and ICAM-1 were observed in rats treated with OVA plus inhaled SO<sub>2</sub> (for lungs: 1.41- and 2.01-fold,  $P < 0.01$ ; for tracheas: 1.76- and 3.12-fold,  $P < 0.01$  or  $P < 0.001$ ), relative to the control (Fig. 4). No changes of mRNA levels were observed in rats exposed to SO<sub>2</sub> alone. Furthermore, the MUC5AC and ICAM-1 mRNA levels exposed to OVA and SO<sub>2</sub> were increased more strongly than those of lungs and tracheas of rats exposed to OVA alone ( $P < 0.05$  or  $P < 0.01$ ).

### 3.4. Effects of chemicals on ICAM-1 protein expressions in lungs

In Fig. 5, exposure to OVA or to OVA plus inhaled SO<sub>2</sub> significantly caused increase of ICAM-1 protein levels in lungs of rats compared with the control ( $P < 0.05$  or  $P < 0.01$ ), but the increase of protein levels after SO<sub>2</sub> inhalation was not statistically significant. Moreover, exposure to OVA plus inhaled SO<sub>2</sub> significantly induced ICAM-1

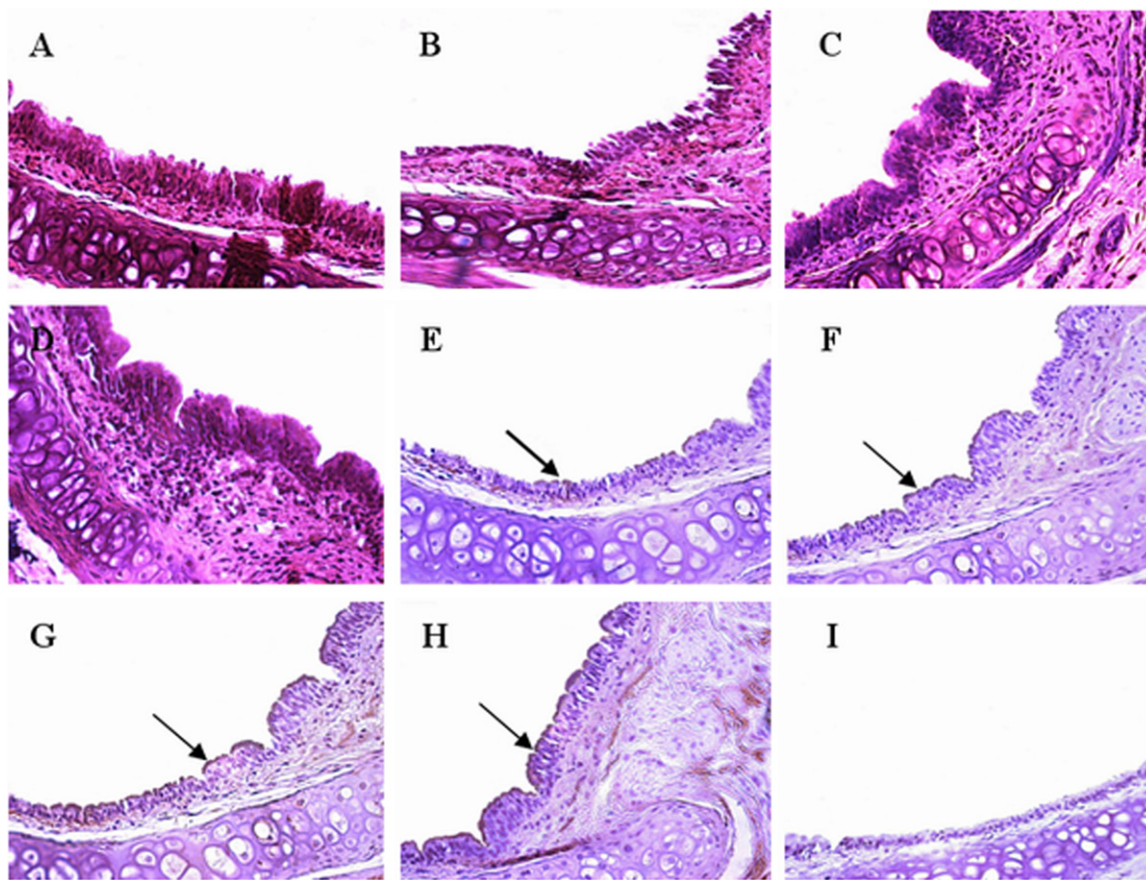


Fig. 3. HE staining and immunohistochemical staining using antibody to MUC5AC in tracheas of rats exposed to SO<sub>2</sub> or/and OVA. (A–D) HE staining in tracheas of rats exposed to the control, exposed to SO<sub>2</sub>, exposed to OVA and exposed to OVA plus inhaled SO<sub>2</sub>, respectively; (E–H) immunohistochemical staining for MUC5AC in tracheas of rats exposed to the control, exposed to SO<sub>2</sub>, exposed to OVA and exposed to OVA plus inhaled SO<sub>2</sub>, respectively; and (I) negative control without primary antibody. At 400× magnification. →, Positive staining

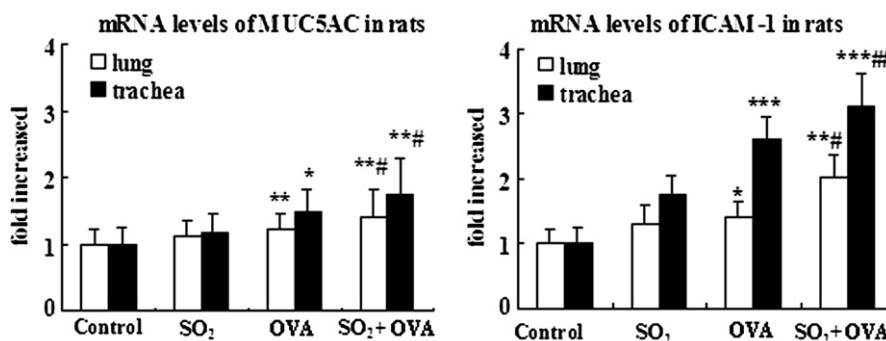


Fig. 4. mRNA levels of MUC5AC and ICAM-1 in lungs and tracheas of rats in the different groups. Mean expression in each treated group is shown as a fold increase compared to mean expression in the control that has been ascribed an arbitrary value of 1. Each column and bar represents means  $\pm$  SD of six individual samples. Statistical significances were analyzed using one-way ANOVA compared with the control, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . Exposure to OVA plus inhaled SO<sub>2</sub> increased MUC5AC and ICAM-1 mRNA expressions versus exposure to OVA alone (# $P < 0.05$ , ## $P < 0.01$ ).

protein expression compared with exposure to OVA alone ( $P < 0.05$ ).

#### 4. Discussion

SO<sub>2</sub> is a common air pollutant in densely populated areas as well as in areas polluted by coal-fired power plants, smelters, and sulfuric acid factories. Exposure to SO<sub>2</sub> in

low levels may affect the respiratory system in human beings (Bethel et al., 1985; Myers et al., 1986), and the previous reports had demonstrated that SO<sub>2</sub> at dose levels of 1.5 and 2 ppm could induce the respiratory tract inflammation and increase of airway resistance in experimental animals (Mehiman 1983; Samukawa et al., 2003). SO<sub>2</sub> at 2 ppm was used in this study to examine the effects of its exposure. Although the experiment may be viewed as beyond the

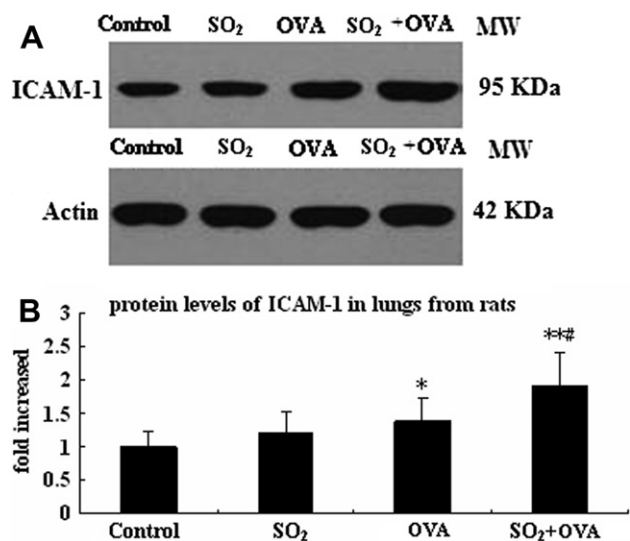


Fig. 5. ICAM-1 Protein levels of in lungs of rats in rats treated with OVA or SO<sub>2</sub> inhalation alone or associated. (A) Western blot analysis was performed with antibody against ICAM-1 and with actin. Each figure corresponds to a representative experiment out of six experiments. (B) Each column and bar represents means  $\pm$  SD of six individual samples. Mean expression in each treated group is shown as a fold increased compared to mean expression in the control that has been ascribed an arbitrary value of 1. Statistical significances were analyzed using one-way ANOVA compared with the control, \* $P < 0.05$ , \*\* $P < 0.01$ . Exposure to OVA plus inhaled SO<sub>2</sub> increased ICAM-1 protein expression versus exposure to OVA alone (\* $P < 0.05$ ).

normal atmosphere encountered in the human environment ( $<0.5$  ppm), there are three important considerations to be made. First, it was reported that asthma symptoms could be aggravated in the asthmatics at the concentrations of SO<sub>2</sub> (1 and 5 ppm) (Sheppard et al., 1980; Horstman et al., 1988). In animal experiment, lung function changes were observed in asthmatic guinea pigs challenged by OVA following exposure 5 ppm SO<sub>2</sub> on 5 consecutive days over 8 h per day (Riedel et al., 1992). Recently, Park et al. (2001) reported that repeated exposure to low levels of SO<sub>2</sub> ( $<1$  ppm) even could enhance the development of OVA-induced asthmatic reactions in guinea pigs. Second, Threshold Limit Value (TLV) in the US American Conference of Governmental Industrial Hygienists (ACGIH) for SO<sub>2</sub> in industrial environment is 2 ppm, occupational exposure limit values (OELs) for SO<sub>2</sub> with a respiratory critical effect in workplace air in Swedish is also 2 ppm (Hansson and Rudén, 2006). The dose of SO<sub>2</sub> (2 ppm) therefore used in this study may have the actual meaning in an occupational exposure. Third, the animals in this study were subjected to intermittent exposure with relief periods between protocols (1 h a day on 5 consecutive days, with 23 h between exposures). Accordingly, the actual concentrations of gas reaching the airways in rats might have been lower than that in the exposure chamber (2 ppm). And the effect level for SO<sub>2</sub> in animal model might have been lower than OELs (2 ppm) for longer periods of time. This level is perhaps close to doses found in most industrial environments.

The lungs and tracheas are the first structures in the human airways that encounter most environmental exposures. Bronchi, bronchioles and alveoli in respiratory tract are responsible for the exchange of gases. It was demonstrated that allergen challenge of sensitized animals including rats induced infiltration of inflammatory cells in lung tissue or increase of inflammatory cells in BALF (Zeng et al., 2001). In this study, the numbers of total cells and most inflammatory cells in BALF of rats treated with OVA alone or with OVA plus inhaled SO<sub>2</sub> were increased, relative to the control rats. This result is in accordance with above report.

OVA, used as an allergen of murine asthmatic model, could induce unique histological characteristic of allergic inflammation in lung. SO<sub>2</sub> has been proposed to cause severe respiratory tract symptoms in both humans and animals. The combination effect of SO<sub>2</sub> and OVA on lung was observed in guinea pigs after SO<sub>2</sub> and OVA exposure (Riedel et al., 1992). As we observed in lung pathological data, the significantly pathological changes after co-exposure of OVA and SO<sub>2</sub> were observed compared with the rats exposed to either OVA or SO<sub>2</sub> alone. It was suggested that exposure to OVA plus SO<sub>2</sub> inhalation resulted in the pathological changes, which were possibly caused by OVA acting with SO<sub>2</sub> synergistically.

It was reported that ICAM-1 expression on airway epithelial cells had been implicated in the pathogenesis of inflammatory lung diseases such as asthma (Wegner et al., 1990). The elevated ICAM-1 levels were observed in the asthmatic patients (Yong et al., 1997), and the over-expression of ICAM-1 mRNA in bronchial tissue from asthmatic guinea pigs was demonstrated (Cao and Yang, 1997). The expression of ICAM-1 in epithelial cells causes the adhesion of neutrophils and eosinophils to epithelial cells (Jagels et al., 1999), and increased inflammation results in exacerbations characterized by dyspnea, wheezing, and coughing. In this study, the ICAM-1 levels in lungs and tracheas of rats exposed to OVA alone were higher than those of the control. After SO<sub>2</sub> and OVA exposure, furthermore, the mRNA and protein levels of ICAM-1 were significantly increased compared with exposure to OVA alone. In addition, the counts of total cells, eosinophils, macrophages and lymphocytes in BALF of rats treated with OVA plus inhaled SO<sub>2</sub> were significantly increased compared with exposure to OVA alone. This result was consistent with above report. It was implied that SO<sub>2</sub> increased ICAM-1 levels in airway and in turn ICAM-1 induced inflammation reactions and caused inflammatory cell infiltration, suggesting that SO<sub>2</sub> may enhance the airway injury in asthmatic rats.

MUC5AC is mainly expressed in the respiratory tract and related with mucus secretion (Alimam et al., 2000). In this study, OVA exposure induced the mRNA expression of MUC5AC compared with the control, while SO<sub>2</sub> plus OVA inhalation enhanced the mRNA levels of MUC5AC in lungs and tracheas of rats compared with exposure to OVA alone. In immunohistochemistry analysis, MUC5AC was highly expressed in bronchial and

tracheal epithelial cells of rats exposed to OVA plus inhaled SO<sub>2</sub>, versus exposure to OVA alone. It was implied that SO<sub>2</sub> could enhance MUC5AC gene over-expression in airway of asthmatic rats. Excess mucus production in the airways, with high viscoelasticity and adhesivity, is not easily transported by cilia beat or cough interactions. And accumulated mucus ultimately results in individual asthmatic symptoms such as sputum production, cough and dyspnea (Daviskas and Anderson, 2006).

In conclusion, the rat model induced by OVA in this study was used to investigate the molecular mechanism between SO<sub>2</sub> and pathogenesis of asthma and the expressions of MUC5AC and ICAM-1 in the lungs and tracheas from asthmatic rats exposed to SO<sub>2</sub>. The results indicated that the increases of MUC5AC and ICAM-1 mRNA and protein levels were not significant in lungs and tracheas of rats exposed to SO<sub>2</sub> alone compared with the control, but the elevated levels of these genes appeared in these tissues of rats exposed to SO<sub>2</sub> plus OVA compared with either the control or exposure to OVA, suggesting that SO<sub>2</sub> exposure could result in OVA-induced increases of transcription and translation levels of MUC5AC and ICAM-1 in lungs and tracheas of rats. At the same time, a synergistic effect on the pathological changes between SO<sub>2</sub> and OVA was observed in lungs after co-exposure of SO<sub>2</sub> and OVA. These results provide more useful evidences to understand the possible mechanism that SO<sub>2</sub> pollution aggravates asthma disease. Analysis of the molecular mechanisms of asthma related-genes has led to a better understanding of asthma pathogenesis.

## Acknowledgments

This research was supported by Grant 20677035 from the National Natural Science Foundation of China.

## References

- Alimam, M.Z., Piazza, F.M., Selby, D.M., Letwin, N., Huang, L., Rose, M.C., 2000. Muc5/Sac mucin messenger RNA and protein expression is a marker of goblet cell metaplasia in murine airways. *Am. J. Respir. Cell Mol. Biol.* 22 (3), 253–260.
- Bai, J., Meng, Z., 2005. Effects of sulfur dioxide on apoptosis-related gene expressions in lungs from rats. *Regul. Toxicol. Pharmacol.* 43 (3), 272–279.
- Bethel, R.A., Sheppard, D., Geffroy, B., Tam, E., Nadel, J.A., Boushey, H.A., 1985. Effects of 0.25 ppm sulfur dioxide on airway resistance in freely breathing, heavily exercising, asthmatic subjects. *Am. Rev. Respir. Dis.* 131, 659–661.
- Blumenthal, M.N., 2005. The role of genetics in the development of asthma and atopy. *Curr. Opin. Allergy Clin. Immunol.* 5 (2), 141–145.
- Cao, G., Yang, Z., 1997. ICAM-1 mRNA expression in the bronchial tissue of asthmatic guinea pigs. *Chin. J. Intern. Med.* 36, 805–807.
- Daviskas, E., Anderson, S.D.J., 2006. Hyperosmolar agents and clearance of mucus in the diseased airway. *Aerosol. Med. Spring* 19 (1), 100–109.
- Derya, K., 2003. The effects of ageing and sulfur dioxide inhalation exposure on visual-evoked potentials, antioxidant enzyme systems, and lipid-peroxidation levels of the brain and eye. *Neurotoxicol. Teratol.* 25, 587–598.
- Duncan, F.R., 2004. Airway mucus hypersecretion in asthma: an undervalued pathology? *Curr. Opin. Pharmacol.* 4 (3), 241–250.
- Grella, E., Paciocco, G., Caterino, U., Mazzarella, G., 2002. Respiratory function and atmospheric pollution. *Monaldi. Arch. Chest. Dis.* 57 (3–4), 196–199.
- Hamid, Q., Tulic, M.K., Liu, M.C., Moqbel, R., 2003. Inflammatory cells in asthma: mechanisms and implications for therapy. *J. Allergy Clin. Immunol.* 111 (1 Suppl), S5–S12.
- Hansson, S.O., Rudén, C., 2006. Evaluating the risk decision process. *Toxicology* 218, 100–111.
- Hogg, N., Bates, P.A., Harvey, J., 1991. Structure and function of intercellular adhesion molecule-1. *Chem. Immunol.* 50, 98–115.
- Horstman, D.H., Seal Jr., E., Folinsbee, L.J., Ives, P., Roger, L.J., 1988. The relationship between exposure duration and sulfur dioxide-induced bronchoconstriction in asthmatic subjects. *Am. Ind. Hyg. Assoc. J.* 49 (1), 38–47.
- Hylkema, M.N., Hoekstra, M.O., Luinge, M., Timens, W., 2002. The strength of the OVA-induced airway inflammation in rats is strain dependent. *Clin. Exp. Immunol.* 129 (3), 390–396.
- Jagels, M.A., Daffern, P.J., Zuraw, B.L., Hugli, T.E., 1999. Mechanisms and regulation of polymorphonuclear leukocyte and eosinophil adherence to human airway epithelial cells. *Am. J. Respir. Cell Mol. Biol.* 21 (3), 418–427.
- Pilegaard, K., Madsen, C., 2004. An oral Brown Norway rat model for food allergy: comparison of age, sex, dosing volume, and allergen preparation. *Toxicology* 196, 247–257.
- Knowles, M., Boucher, R., 2002. Mucus clearance as the primary innate defense mechanism for mammalian airways. *J. Clin. Invest.* 109, 571–577.
- Linn, W.S., Avol, E.L., Peng, R., Shamoo, D.A., Hackney, J.D., 1987. Replicated dose-response study of sulfur dioxide effects in normal, atopic and asthmatic volunteers. *Am. Rev. Respir. Dis.* 136, 1127–1134.
- Mehiman, M.A., 1983. Current toxicological information as the basis for sulfur oxide standards. *Environ. Health Perspect.* 52, 261–266.
- Morcillo, E.J., Cortijo, J., 2006. Mucus and MUC in asthma. *Curr. Opin. Pulm. Med* 12 (1), 1–6.
- Myers, D.J., Bigby, B.G., Boushey, H.A., 1986. The inhibition of sulfur dioxide-induced bronchoconstriction in asthmatic subjects by cromolyn is dose dependent. *Am. Rev. Respir. Dis.* 133, 1150–1153.
- Park, J.K., Kim, Y.K., Lee, S.R., Cho, S.H., Min, K.U., Kim, Y.Y., 2001. Repeated exposure to low levels of sulfur dioxide (SO<sub>2</sub>) enhances the development of ovalbumin-induced asthmatic reactions in guinea pigs. *Ann. Allergy Asthma Immunol.* 86 (1), 62–67.
- Qin, G., Meng, Z., 2005. Effect of sulfur dioxide inhalation on CYP1A1 and CYP1A2 in rat liver and lung. *Toxicol. Lett.* 160, 34–42.
- Qin, G., Meng, Z., 2006. The expressions of protooncogenes and CYP1A in lungs of rats exposed to sulfur dioxide and benzo(a)pyrene. *Regul. Toxicol. Pharmacol.* 45, 36–43.
- Riedel, F., Naujokat, S., Ruschoff, J., Petzoldt, S., Rieger, C.H., 1992. Sulfur dioxide-induced enhancement of inhalative allergic sensitization: Inhibition by anti-inflammatory treatment. *Int. Arch. Allergy Immunol.* 98, 386–391.
- Samukawa, T., Arasidani, K., Hori, H., Hirano, H., Arima, T., 2003. c-jun mRNA expression and profilin mRNA amplification in rat alveolar macrophages exposed to volcanic ash and sulfur dioxide. *Ind. Health* 41 (4), 313–319.
- Sheppard, D., Wong, W.S., Uehara, C.F., Nadel, J.A., Boushey, H.A., 1980. Lower threshold and greater bronchomotor responsiveness of asthmatic subjects to sulfur dioxide. *Am. Rev. Respir. Dis.* 122, 873–878.
- Tseng, R.Y., Li, C.K., 1990. Low level atmospheric sulfur dioxide pollution and childhood asthma. *Ann. Allergy* 65 (5), 379–383.
- Wang, W., Li, Y., 2003. Effect of dexamethasone on the expression of collagen and transforming growth factor-β1 in asthmatic rats. *Acta University Medicinalis Nanjing (Natural Science)* 23 (6), 595–597.
- Wegmann, M., Fehrenbach, H., Fehrenbach, A., Held, T., Schramm, C., Garn, H., Renz, H., 2005. Involvement of distal airways in a chronic model of experimental asthma. *Clin. Exp. Allergy* 35 (10), 1263–1271.

- Wegner, C.D., Gundel, R.H., Reilly, P., Haynes, N., Letts, L.G., Rothlein, R., 1990. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science* 247, 456–459.
- Wills-Karp, M., 2000. Murine models of asthma in understanding immune dysregulation in human asthma. *Immunopharmacology* 48 (3), 263–268.
- Wu, Y., Zhou, H., Xu, Y., Li, S., 2006. Enhanced expression of urocortin in lung tissues of rats with allergic asthma. *Biochem. Biophys. Res. Commun.* 341 (2), 532–540.
- Yong, C.L., Ki, T.C., Yang, K.R., 1997. Changes of soluble ICAM-1 levels in serum and bronchoalveolar lavage fluid from patients with atopic bronchial asthma after allergen challenge. *J. Asthma* 34 (5), 405–412.
- Zeng, Z., Cui, D., Liang, Y., Peng, R., Wang, D., 2001. A pathology study on the bronchioles and lung tissues in guinea pig asthma model. *Chin. J. Intern. Med.* 40 (3), 158–161.