Biochemical and Biophysical Research Communications 433 (2013) 519–525

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Sulfur dioxide upregulates the inhibited endogenous hydrogen sulfide pathway in rats with pulmonary hypertension induced by high pulmonary blood flow

Liman Luo [a,1], Die Liu [a,1], Chaoshu Tang [b,c], Junbao Du [a,c], Angie Dong Liu [d], Lukas Holmberg [d], Hongfang Jin [a,*]

[a] Department of Pediatrics, Peking University First Hospital, Beijing 100034, China
[b] Department of Physiology and Pathophysiology, Peking University Health Science Center, Beijing 100191, China
[c] Key Laboratory of Molecular Cardiology, Ministry of Education, Beijing 100191, China
[d] Department of Medical and Health Sciences, Linköping University, Linköping 58183, Sweden

ARTICLE INFO

*Article history:*
Received 19 February 2013
Available online 21 March 2013

*Keywords:*
Pulmonary hypertension
Pulmonary vascular remodeling
Sulfur dioxide
Hydrogen sulfide

ABSTRACT

Pulmonary hypertension (PH) is an important pathophysiological process in the development of many diseases. However, the mechanism responsible for the development of PH remains unknown. The objective of the study was to explore the possible impact of sulfur dioxide ($SO_2$) on the endogenous hydrogen sulfide ($H_2S$) pathway in rats with PH induced by high pulmonary blood flow. Compared with sham group, the systolic pulmonary artery pressure (SPAP) in the shunt group was significantly increased, along with the increased percentage of muscularized arteries and partially muscularized arteries of small pulmonary arteries. Compared with the shunt group, SPAP in the shunt + $SO_2$ group was significantly decreased, and the percentage of muscularized pulmonary arteries was also decreased. Additionally, rats that developed PH had significantly lower levels of $SO_2$ concentration, aspartate aminotransferase (AAT) activity, protein and mRNA expressions of AAT2 in pulmonary tissues. Administration of an $SO_2$ donor could alleviate the elevated pulmonary arterial pressure and decrease the muscularization of pulmonary arteries. At the same time, it increased the $H_2S$ production, protein expression of cystathionine-γ-lyase (CSE), mRNA expression of CSE, mercaptopyruvate transsulphurase (MPST) and cystathionine-β-synthase (CBS) in the pulmonary tissue of the rats. The results suggested that endogenous $SO_2$/AAT2 pathway and the endogenous $H_2S$ production were downregulated in rats with PH induced by high pulmonary blood flow. However, $SO_2$ could reduce pulmonary arterial pressure and improve the pulmonary vascular pathological changes in association with upregulating endogenous $H_2S$ pathway.



## 1. Introduction

Pulmonary hypertension (PH) is an important pathophysiological process in the development of a variety of clinical cardiac and pulmonary diseases, and has critical influence on the progress and prognosis of the diseases [1]. High pulmonary blood flow-induced PH is a serious complication in patients with congenital left-to-right shunt. Previous studies indicate that enhanced vasomotor response and vascular remodeling are part of the pathogenesis in high pulmonary blood flow-induced PH [2,3]. However, the exact mechanisms remain unclear. Gaseous signaling molecules, with its properties of quick generation, rapid diffusion, extensive action and short half-life, play an important role in vascular regulation. The lung is an organ where gas exchange takes place, and therefore, gasotransmitters play a prominent role in the regulation of pulmonary circulation. Previous research demonstrated that both nitric oxide (NO) [4–6] and hydrogen sulfide ($H_2S$) [7,8] exerted significant mitigation effect on high pulmonary blood flow-induced pulmonary hypertension. They reduced the mean pulmonary arterial pressure, decreased deposition of collagen I and collagen III in the pulmonary arterial walls, and induced apoptosis of pulmonary artery smooth muscle cells through activation of the Fas signaling pathway, thus alleviating pulmonary vascular structural remodeling [4–8]. Recently, our research group demonstrated that sulfur dioxide ($SO_2$) could be endogenously generated in the cardiovascular system [9]. Also, the sulfur dioxide/aspartate amino transferase pathway was present in a variety of organs and tissues of rats [10]. $SO_2$ could significantly inhibit pulmonary vascular structural remodeling in monocrotaline-induced PH and hypoxic PH in rats [11,12]. However, its role in the pathophysiological process of increased pulmonary blood flow-induced PH remains unknown.

* Corresponding author. Address: Department of Pediatrics, Peking University First Hospital, No. 1, Xi'anmen Street, Xicheng District, Beijing 100034, China. Fax: +86 10 66530532.
*E-mail address:* jinhongfang51@126.com (H. Jin).
[1] They contributed equally to this work.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.03.014

Our research group discovered that $H_2S$ was involved in the regulation of high pulmonary blood flow-induced PH [13,14]. The endogenous $H_2S$/CSE, MPST and CBS pathways participated in the pathogenesis of high blood flow-induced PH [15,16]. $SO_2$ and $H_2S$ are generated from methionine metabolic pathway. However, whether $SO_2$ has any impact on the endogenous $H_2S$ generating pathway in pulmonary arteries in the pathogenesis of high blood flow-induced PH is still unclear. Therefore, the present study was undertaken to investigate the possible role of $SO_2$ in the development of high blood flow-induced PH and the impact of $SO_2$ on the endogenous $H_2S$/CSE, MPST and CBS pathways in PH induced by high pulmonary blood flow.

## 2. Materials and methods

### 2.1. Preparation of the animal model

Male Wistar rats weighing from 130 to 190 g were provided by the Animal Research Committee of the First Hospital, Peking University. Twenty-four rats were randomly divided into three groups: the sham group ($n = 8$), the shunt group ($n = 8$) and the shunt + $SO_2$ group ($n = 8$). Rats in the shunt group and the shunt + $SO_2$ group were subjected to an abdominal aorta-inferior vena cava shunting to create an animal model of high pulmonary blood flow. All groups of rats were raised under the same diet and drinking conditions. Starting from the second day of the shunting operation, rats from the shunt + $SO_2$ group were given intraperitoneal injection of an $SO_2$ donor, $Na_2SO_3$/$NaHSO_3$, which was dissolved in physiological (0.9%) saline (0.54 mmol/kg per 0.18 mmol/kg body weight) before injection. $Na_2SO_3$/$NaHSO_3$ was injected once per day (85 mg/kg body weight) during eight weeks. The shunt group was given the same amount of physiological saline.

### 2.2. Measurement of systolic pulmonary artery pressure

Eight weeks after the administration of the $SO_2$ donor, rats were anesthetized with urethane (1 g/kg body weight). A silicone catheter (outer diameter, 0.9 mm) was inserted into the right jugular vein through venotomy, and then passed through the tricuspid valve and right ventricle until it reached the pulmonary artery. The other end of the catheter was connected to a Multi-Lead Physiological Monitor (BL-420F, Chengdu TME Technology, Chengdu, China) through a P50 pressure transducer. The curves of systolic pulmonary artery pressure (SPAP) were traced, and SPAP was measured.

### 2.3. Pulmonary vascular morphological changes

The lung lobes were fixated by 10% (W/V) formaldehyde buffer, and the paraffin sections were done. Subsequently, HE staining was applied. Pulmonary vascular morphology was observed and the percentage of muscularized pulmonary arteries of the total amount of small pulmonary arteries was calculated.

### 2.4. Determination of $SO_2$ content in lung tissue

$SO_2$ concentrations in the lung tissue samples were detected by high-performance liquid chromatography (HPLC, Agilent 1100 series, Agilent Technologies, Palo Alto, CA, USA). Samples of the lung tissues (100 ml) were mixed with 70 ml of 0.212 Mm sodium borohyderide in 0.05 M Tris–HCl (pH 8.5) and incubated at room temperature for 30 min. They were then mixed with 10 ml of 70 Mm monobromobimane in acetonitrile. Fifty milliliters of 1.5 M perchloric acid was added to the mixture after incubation for 10 min at 42 °C. Protein precipitates were removed by centrifugation at 12400 g for 10 min. By adding 20 ml of 2.0 M Tris, the supernatant was neutralized, and then gently mixed and centrifuged again at 12400 g for 10 min at room temperature. Then, the neutralized supernatant (100 ml) was transferred and stored at 4 °C in foil wrapped tubes, and 5 ml of the sample was injected into the HPLC column. The column was equilibrated with methanol, acetic acid, and water in the ratio of 5.00:0.25:94.75 (by volume, pH 3.4). Sulfite-bimane adduct was detected by excitation at 392 nm and emission at 479 nm.

### 2.5. Determination of AAT activity in lung tissue

AAT Determination Kit (Nanjing Jiancheng Biological Engineer Academy, Nanjing, China) was used to detect AAT activity in the lung tissue samples. The samples were homogenated with 0.01 mol/L phosphate-buffered saline (PBS; weight: volume, 1:10). Protein precipitates were removed by centrifugation at 2500 g for 10 min at room temperature. The tissue protein was measured by the Coomassie method and the supernatant was used to detect AAT content by using the Reitman method.

### 2.6. Assay of endogenous $H_2S$ concentration

The lung tissue samples were firstly suspended in 50 mmol/L ice-cold potassium phosphate buffer (pH 6.8). The mixture contained (in mmol/L): 100 potassium phosphate buffer (pH 7.4), 10 L-cysteine, 2-pyridoxal-5′-phosphate, and 10% (w/v) tissue homogenate. Cryovial test tubes were used as the center wells containing 0.5 mL of 1% zinc acetate as trapping solution and a filter paper. The reaction was performed in a 25 mL of Erlenmeyer flask (Pyrex). The reaction started by transferring the flasks from ice to a 37 °C shaking water bath. After incubation for 90 min, 0.5 mL of 50% trichloroacetic acid was added to stop the reaction. The flasks were sealed again and incubated at 37 °C for another 60 min. The contents of the center wells were then transferred to test tubes, each containing 3.5 mL of water. Afterwards 0.5 mL of 20 mmol/L *N*,*N*-dimethyl-p-phenylenediamine sulfate in 7.2 mol/L HCl was added, followed by the addition of 0.4 mL of 30 mmol/L $FeCl_3$ in 1.2 mol/L HCl. The absorbance at 670 nm was measured later with a spectrophotometer (Shimadzu UV 2100, Japan). The $H_2S$ concentration was calculated against the calibration curve of the standard $H_2S$ solutions.

### 2.7. Expression of CSE, MPST and CBS in pulmonary blood vessels determined by immunohistochemical analysis

The paraffin sections were dewaxed and hydrated, and then processed by 3% $H_2O_2$ for 10 min at room temperature, followed by antigen repairing for 10 min (microwave heating method). The slides were washed twice with phosphate-buffered saline (PBS), then blocked with goat serum at 37 °C for 30 min, and incubated overnight at 4 °C with CSE, MPST and CBS antibodies (diluted at 1:200, 1:200, and 1:200, respectively). The slides were then rinsed in PBS twice. Biotinylated anti-rabbit or anti-mouse IgG was incubated for 1 h at 37 °C. After rinsed in PBS twice, slides were stained with 3,30-diaminobenzidine to develop color. The slides were then dehydrated through a graded ethanol series and dimethylbenzene. Positive signals were defined as brown granules in the tissue samples under optical microscope.

### 2.8. Determination of AAT2, CSE, MPST and CBS protein expression in lung tissue by using Western blot analysis

The lung tissue samples were homogenized and lysed. Equal amounts of protein were boiled and separated by SDS–PAGE and were transferred to nitrocellulose membranes. The primary

**Table 1**
Primers and TaqMan probes used in quantitative real-time PCR for the measurement of AAT2, CSE, MPST, CBS and β-actin cDNAs in rats.

| cDNA | Oligonucleotide | Sequence | Product size (bp) |
|---|---|---|---|
| AAT2 | Forward primer | 5′-GAGGGTCGGAGCCAGCTT-3′ | 121 |
| | Reverse primer | 5′-GTTTCCCCAGGATGGTTTGG-3′ | |
| | TaqMan probe | 5′-TTTAAGTTCAGCCGAGATGTCTTTC-3′ | |
| CSE | Forward primer | 5′-GCTGAGAGCCTGGGAGGATA-3′ | 101 |
| | Reverse primer | 5′-TCACTGATCCCGAGGGTAGCT-3′ | |
| | TaqMan probe | 5′-CTGAGCTTCCAGCAATCATGACCCATG-3′ | |
| MPST | Forward primer | 5′-CGGCGCTTCCAGGTAGTG-3′ | 131 |
| | Reverse primer | 5′-CTGGTCAGGAATTCAGTGAATGG-3′ | |
| | TaqMan probe | 5′-CGCAGCTGGCCGTTTCCA-3′ | |
| CBS | Forward primer | 5′-CTCCGGGAGAAGGGTTTTGA-3′ | 81 |
| | Reverse primer | 5′-CATGTTCCCGAGAGTCACCAT-3′ | |
| | TaqMan probe | 5′-AGGCACCTGTGGTCAACGAGTCTGG-3′ | |
| β-Actin | Forward primer | 5′-ACCCGCGAGTACAACCTTCTT-3′ | 80 |
| | Reverse primer | 5′-TATCGTCATCCATGGCGAACT-3′ | |
| | TaqMan probe | 5′-CCTCCGTCGCCGGTCCACAC-3′ | |

TaqMan probe labeled with FAM at the 5′end and TAMRA at the 3′ end. AAT: aspartate aminotransferase; CSE: cystathionine-γ-lyase; MPST: mercaptopyruvate transsulphurase; CBS: cystathionine-β-synthase.

antibody dilutions were 1:1000 for AAT2 and CSE, 1:5000 for MPST and 1:1000 for CBS. Secondary antibodies were incubated for 1 h at room temperature. Immunoreaction was visualized, exposed to X-ray film (Kodak Scientific), and then quantified by use of AlphaImager (San Leandro, CA, USA).

### 2.9. Expressions of AAT2, CSE, MPST and CBS mRNA in lung tissue by using quantitative real-time polymerase chain reaction (PCR)

RNA in the lung tissue samples was extracted using the Trizol reagent and reverse-transcribed by oligo(dT)15 primer and M-MLV reverse transcriptase. The sequences of the primers and the probes are shown in Table 1. In each sample, β-actin was used to calibrate the sample amount used for the determination.

### 2.10. Data analysis

For comparison of the means between more than two groups, one-Way ANOVA was used. Mean comparisons between groups were used when homogeneity of variance. The least significant difference method (LSD) was used, and in heterogeneity of variance the Tambane's T2 was used. For mean comparisons between two groups, t test was used. All the data were analysed by SPSS 16.0 (Chicago, IL, USA). A value of $P < 0.05$ was considered statistically significant. Results are expressed as mean ± standard error.

## 3. Results

### 3.1. Systolic pulmonary artery pressure

Compared with that of sham group, SPAP in shunt group was significantly increased ($P < 0.01$), whereas it was significantly decreased in shunt + $SO_2$ group compared with that of the shunt group ($P < 0.01$) (Fig. 1).

### 3.2. Pulmonary vascular morphological changes by HE staining

Compared with that of the sham group, the ratio of the number of muscularized arteries (MA) to the number of small pulmonary arteries in shunt group was significantly increased, but the ratio of non-muscularized arteries (NMA) was decreased ($P < 0.01$) (Fig. 1). However, compared with that of shunt group, the ratio of MA to small pulmonary arteries in shunt + $SO_2$ group was significantly decreased, but the percentage of NMA was increased ($P < 0.01$) (Fig. 1).

### 3.3. Determination of $SO_2$ content in lung tissue

The $SO_2$ content in the lung tissue samples of shunt group was significantly decreased compared with sham group ($P < 0.05$) (Fig. 2).

### 3.4. Measurement of AAT activity

The AAT activity in the lung tissue samples of shunt group was significantly decreased compared with the sham group ($P < 0.05$) (Fig. 2).

### 3.5. $H_2S$ concentration in lung tissue

The $H_2S$ concentration in the lung tissue samples of shunt group was significantly decreased compared with that of sham group ($P < 0.05$), whereas lung tissue $H_2S$ concentration in shunt + $SO_2$ group was significantly increased compared with that of the shunt group ($P < 0.01$) (Fig. 1).

### 3.6. Expression of CSE, MPST and CBS in small pulmonary arteries determined by immunohistochemical analysis

The proteins of CSE, MPST and CBS were expressed in both endothelial cells and smooth muscle cells of small pulmonary arteries. Compared with those of sham group, the immunostaining signals of CSE in small pulmonary arteries in shunt group were significantly decreased ($P < 0.01$). The $SO_2$ donor significantly increased the immunostaining signals of CSE in small pulmonary arteries in the rats of shunt group ($P < 0.01$) (Fig. 3). Compared with those of sham group, the immunostaining signals of MPST in small pulmonary arteries in shunt group were significantly decreased ($P < 0.05$). The $SO_2$ donor significantly increased its expression in small pulmonary arteries of shunt group ($P < 0.05$) (Fig. 3). Compared with those of the sham group, the immunostaining signals of CBS in small pulmonary arteries in shunt group were significantly decreased ($P < 0.01$). However, CBS expressions in small pulmonary arteries in the rats of shunt + $SO_2$ group were significantly increased as compared with those of shunt group ($P < 0.01$) (Fig. 3).

### 3.7. Expression of AAT2, CSE, MPST and CBS in lung tissue by using Western blot analysis

Compared with that of sham group, the protein expression of AAT2 in the lung tissue samples of the rats in shunt group was significantly decreased ($P < 0.01$) (Fig. 1) and so was the protein

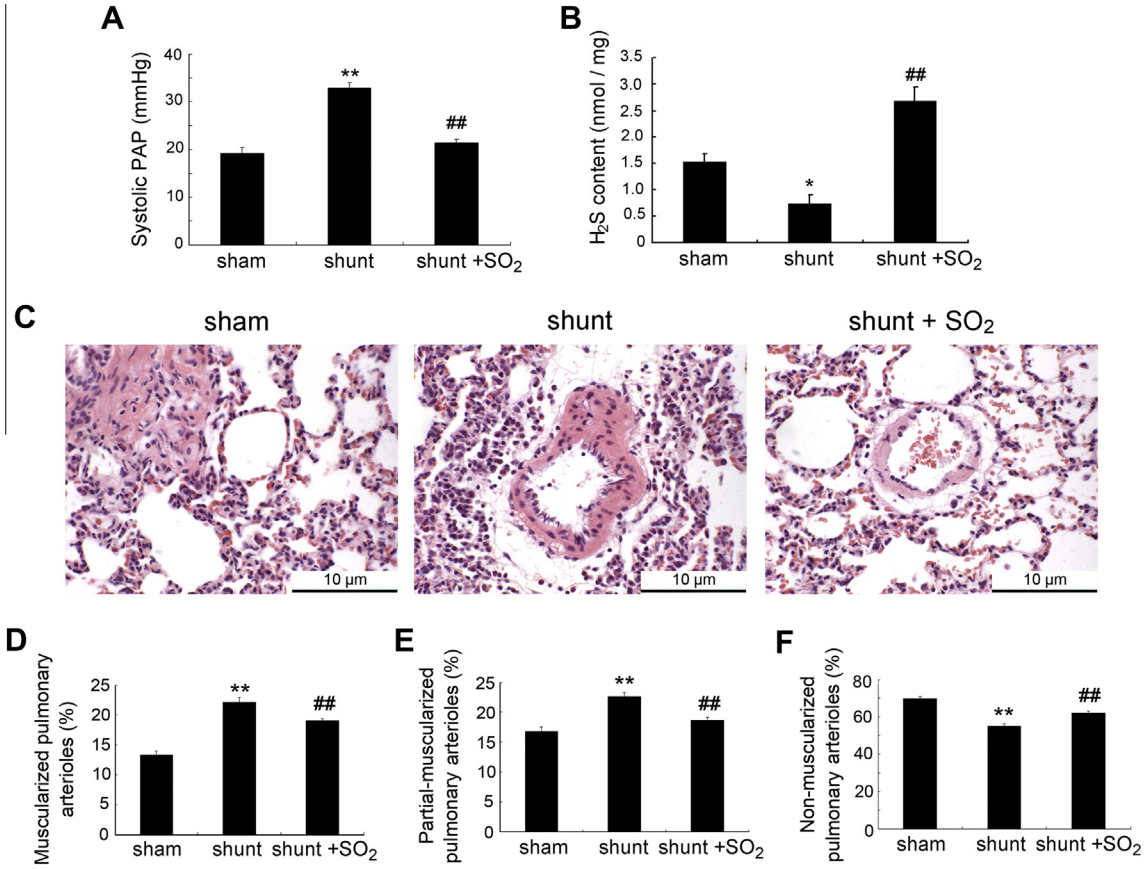


**Fig. 1.** (A) Systolic pulmonary artery pressure between the sham group, the shunt group and the shunt + $SO_2$ group; (B) The $H_2S$ concentration in lung tissue between the sham group, the shunt group and the shunt + $SO_2$ group ($^{*}P < 0.05$ vs. sham, $^{**}P < 0.01$ vs. shunt, $^{\#\#}P < 0.01$ vs. shunt); (C) Small pulmonary artery morphology detected by HE staining; (D) The percentage of MA in small pulmonary artery; (E) The percentage of PMA in small pulmonary artery; (F) The percentage of NMA in small pulmonary artery ($^{**}P < 0.01$ vs. shunt, $^{\#\#}P < 0.01$ vs. shunt).

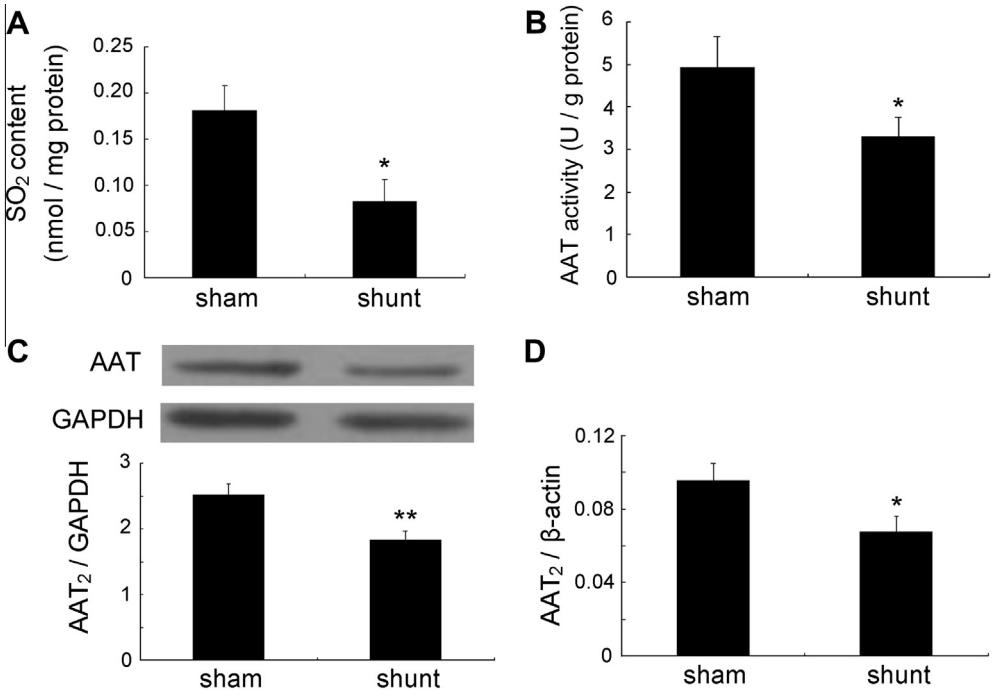


**Fig. 2.** (A) The content of $SO_2$ in lung tissue between the sham group and the shunt group; (B) The AAT activity in lung tissue between the sham group and the shunt group; (C) The protein expression of AAT2 in lung tissue in rats between the sham group and the shunt group determined by Western blot; (D) The expression of AAT2 mRNA in lung tissue in rats between the sham group and the shunt group determined by real-time PCR ($^{*}P < 0.05$ vs. sham, $^{**}P < 0.01$ vs. shunt).

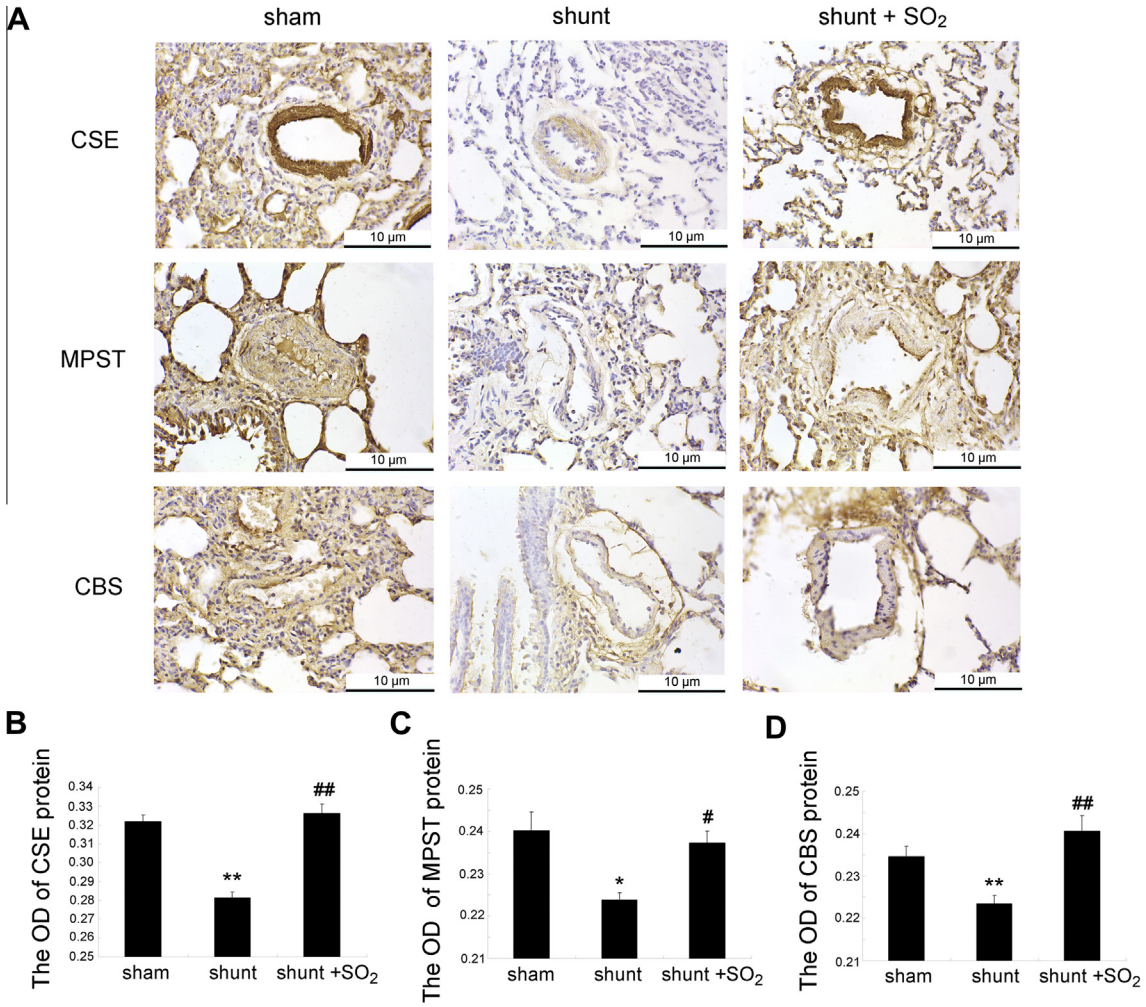


**Fig. 3.** (A) The expression of CSE, MPST and CBS in small pulmonary arteries of lung tissue in rats between the sham group, the shunt group and the shunt + $SO_2$ group determined by immunohistochemistry (DAB dye); (B–D) Mean optical density analysis of protein expression of CSE, MPST and CBS in small pulmonary arteries in lung tissue in rats between the sham group, the shunt group and the shunt + $SO_2$ group ($^{*}P < 0.05$ vs. sham, $^{**}P < 0.01$ vs. shunt, $^{\#}P < 0.05$ vs. shunt, $^{\#\#}P < 0.01$ vs. shunt).

expression of CSE in lung tissues of shunt group ($P < 0.05$). However, the protein expression of CSE in the lung tissue samples of shunt + $SO_2$ group was significantly increased compared with that of shunt group ($P < 0.05$) (Fig. 4). Compared with sham group, the protein expression of MPST in the lung tissue samples of shunt group was significantly decreased ($P < 0.05$), whereas the protein expression in lung tissues of shunt + $SO_2$ group was significantly increased compared with that of the shunt group ($P < 0.05$) (Fig. 4). Compared with sham group, the protein expression of CBS in lung tissues of the shunt group was significantly decreased ($P < 0.01$). However, there was no significant difference in lung tissue protein expression of CBS between shunt + $SO_2$ group and shunt group (Fig. 4).

### 3.8. Expression of AAT2, CSE, MPST and CBS mRNAs in lung tissues

Compared with sham group, expression of AAT2 mRNA in the lung tissue samples of shunt group was decreased ($P < 0.05$) (Fig. 1). Expression of CSE mRNA in the samples of shunt rats was decreased significantly in comparison with that of sham group ($P < 0.01$), whereas the expression of CSE mRNA in the samples of shunt + $SO_2$ group was significantly increased compared with that of shunt group ($P < 0.01$) (Fig. 4). Expression of MPST mRNA in the lung tissue samples of shunt rats was decreased significantly in comparison with that of sham group ($P < 0.05$), whereas the expression of lung tissue MPST mRNA in shunt + $SO_2$ group was significantly increased compared with that of shunt group ($P < 0.05$) (Fig. 4). Expression of CBS mRNA in the lung tissue samples of shunt rats was decreased significantly in comparison with that of sham group ($P < 0.05$), whereas lung tissue expression of CBS mRNA in shunt + $SO_2$ group was significantly increased compared with that of shunt group ($P < 0.05$) (Fig. 4).

## 4. Discussion

PH is an important pathophysiological process in a variety of cardiovascular and pulmonary diseases. In the present study, a high blood flow-induced PH was successfully produced. SPAP was increased significantly in rats with high pulmonary blood flow. At the same time, $H_2S$ concentration in the shunt group was significantly decreased, in consistence with previous studies [15,16]. However, the mechanism responsible for the development of high pulmonary blood flow-induced PH remains unknown.

$SO_2$ is a small gaseous molecule which is considered to be a harmful air pollutant. Our research group recently reported that endogenous $SO_2$ pathway was distributed broadly in cardiovascular system [9] and in other organs [10]. It plays an important role in the pathophysiological process of a variety of diseases [17–29]. Our previous study [12] demonstrated that by promoting collagen

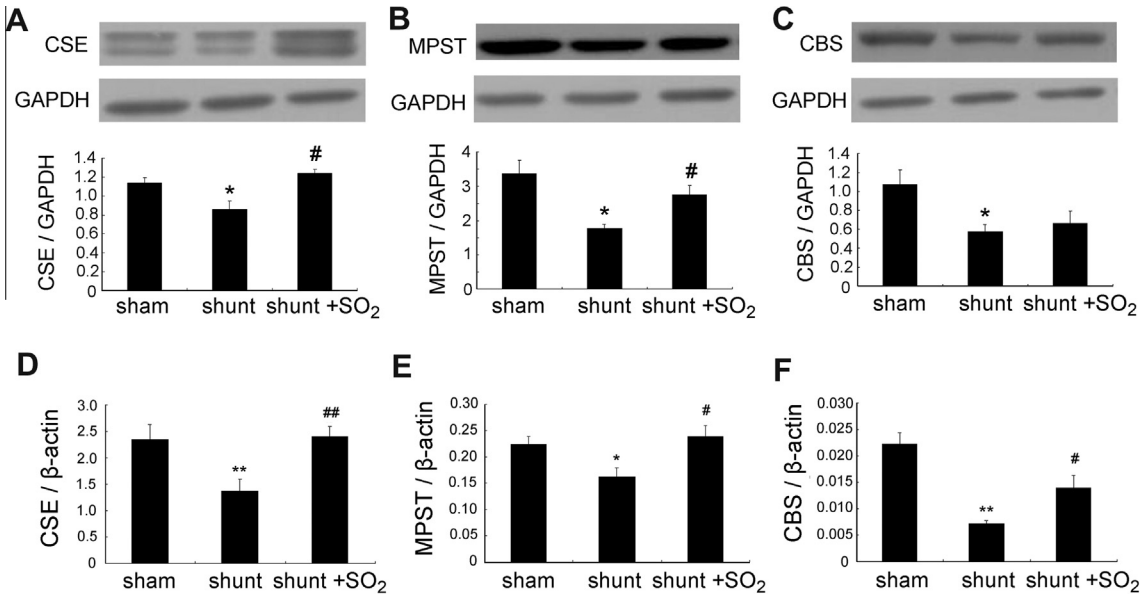


**Fig. 4.** (A–C) The protein expression of CSE, MPST and CBS in small pulmonary arteries in lung tissue in rats between the sham group, the shunt group and the shunt + $SO_2$ group determined by Western blot ($^{*}P < 0.05$ vs. sham, $^{\#}P < 0.05$ vs. shunt); (D–F) The expression of CSE, MPST and CBS mRNA in lung tissue in rats between the sham group, the shunt group and the shunt + $SO_2$ group determined by real-time PCR ($^{*}P < 0.05$ vs. sham, $^{**}P < 0.01$ vs. shunt, $^{\#}P < 0.05$ vs. shunt, $^{\#\#}P < 0.01$ vs. shunt).

degradation, $SO_2$ inhibited abnormal collagen accumulation in the pulmonary artery under hypoxic conditions. $SO_2$ could also inhibit pulmonary vascular structural remodeling [11] in PH induced by monocrotaline. In the present study, we discovered that endogenous $SO_2$/AAT2 pathway was downregulated in PH induced by high pulmonary blood flow, and in particular, $SO_2$ donor could markedly improve pulmonary arterial structural remodeling and alleviate PH, indicating that the downregulation of endogenous $SO_2$/AAT2 pathway plays an important role in the pathogenesis of high blood flow induced PH.

However, how $SO_2$ regulates PH induced by high pulmonary blood flow is still unclear. Both $SO_2$ and $H_2S$ are products of the methionine metabolism, but generated from the L-cysteine/CDO/AAT catalytic pathway and the L-cysteine/CSE/CBS catalytic pathway, respectively. Mitsuhaship [30,31] reported that $H_2S$ and $SO_2$ could convert into each other during oxidative stress in mammals. Therefore, it would be interesting to investigate whether there is any interaction between them *in vivo*. In order to better interpret the interaction between the metabolic family members $H_2S$ and $SO_2$ and their pathophysiological relevance, we designed this study to explore the possible impact of $SO_2$ on the endogenous $H_2S$ pathway in rats with PH induced by high pulmonary blood flow.

In pulmonary arteries of the shunt rats, the concentration of $H_2S$ in lung tissue, protein and mRNA expressions of CBS, CSE and MPST were significantly decreased. After giving $SO_2$ donor, pulmonary arterial pressure was decreased, meanwhile, $H_2S$ concentration, the gene expressions of CSE, MPST and CBS were increased, and the protein expressions of CSE and MPST were increased significantly, suggesting that $SO_2$ markedly upregulated the reduced endogenous $H_2S$ pathway.

Our study presented that $SO_2$ could upregulate $H_2S$ concentration and its generating enzymes, including CSE, MPST and CBS, suggesting that $SO_2$ plays a regulatory role in upregulating the endogenous $H_2S$ pathway in rats with PH induced by high pulmonary blood flow. To demonstrate the possible mechanism by which $SO_2$ upregulates the $H_2S$ pathway, our research discovered that the protein expression of CSE, MPST, and CBS and its mRNA expression in rat lungs were increased, suggesting that $SO_2$ could upregulate the protein expression of the key generating enzymes of $H_2S$ pathway likely through enhancing their gene transcription. Previous studies confirmed that $H_2S$ exerted strong vasorelaxing effect, and could inhibit pulmonary vascular structural remodeling [8]. Therefore, it is likely that $SO_2$ is involved in the mechanisms responsible for the development of high pulmonary blood flow-induced PH by upregulating the endogenous $H_2S$ pathway.

## Acknowledgments

This work is supported by Beijing Natural Science Foundation (7112130), Major Basic Research Development Program of People's Republic of China (2011CB503904, 2012CB517806), and the National Natural Science Foundation of China (81121061, 81070111, 31130030).

## References

[1] T. Forster, Current issues in pulmonary hypertension, Orv. Hetil. 153 (2012) 603–606.
[2] S.I. Said, Mediators and modulators of pulmonary arterial hypertension, Am. J. Physiol. Lung Cell Mol. Physiol. 291 (2006) L547–558.
[3] E.D. Michelakis, M.R. Wilkins, M. Rabinovitch, Emerging concepts and translational priorities in pulmonary arterial hypertension, Circulation 118 (2008) 1486–1495.
[4] B. Wei, J. Du, J. Li, J. Qi, C. Tang, The modulating effect of L-arginine on collagen metabolism of pulmonary artery in pulmonary hypertension induced by a left-to-right shunt, Zhonghua Yi Xue Za Zhi 82 (2002) 1273–1275.
[5] D. Junbao, Y. Hui, W. Bing, L. Jian, Q. Jianguang, T. Chaoshu, Effect of L-arginine on collagen of high flow-induced pulmonary arterial remodeling, Circ. J. 69 (2005) 603–608.
[6] W. Bing, D. Junbao, Q. Jianguang, L. Jian, T. Chaoshu, L-arginine impacts pulmonary vascular structure in rats with an aortocaval shunt, J. Surg. Res. 108 (2002) 20–31.
[7] X. Li, J. Du, H. Jin, B. Geng, C. Tang, Sodium hydrosulfide alleviates pulmonary artery collagen remodeling in rats with high pulmonary blood flow, Heart Vessels 23 (2008) 409–419.
[8] W. Li, H.F. Jin, H.F. Jin, D. Liu, J.H. Sun, P.J. Jian, X.H. Li, C.S. Tang, J.B. DU, Hydrogen sulfide induces apoptosis of pulmonary artery smooth muscle cell in rats with pulmonary hypertension induced by high pulmonary blood flow, Chin. Med. J. 122 (2009) 3032–3038.
[9] S.X. Du, H.F. Jin, D.F. Bu, X. Zhao, B. Geng, C.S. Tang, J.B. Du, Endogenously generated sulfur dioxide and its vasorelaxant effect in rats, Acta Pharmacol. Sin. 29 (2008) 923–930.
[10] Liman Luo, Stella Chen, Hongfang Jin, Chaoshu Tang, Du Junbao, Endogenous generation of sulfur dioxide in rat tissues, Biochem. Biophys. Res. Commun. 415 (2011) 61–67.

[11] H.F. Jin, S.X. Du, X. Zhao, H.L. Wei, Y.F. Wang, Y.F. Liang, C.S. Tang, J.B. Du, Effects of endogenous sulfur dioxide on monocrotaline-induced pulmonary hypertension in rats, Acta Pharmacol. Sin. 29 (2008) 1157–1166.
[12] Y. Sun, Y. Tian, M. Prabha, D. Liu, S. Chen, R. Zhang, X. Liu, C. Tang, H. Jin, J. Du, Effects of sulfur dioxide on hypoxic pulmonary vascular structural remodeling, Lab. Invest. 90 (2010) 68–82.
[13] C. Tang, X. Li, J. Du, Hydrogen sulfide as a new endogenous gaseous transmitter in the cardiovascular system, Curr. Vasc. Pharmacol. 4 (2006) 17–22.
[14] Y. Wang, X. Zhao, H. Jin, H. Wei, W. Li, D. Bu, X. Tang, Y. Ren, C. Tang, J. Du, Role of hydrogen sulfide in the development of atherosclerotic lesions in apolipoprotein E knockout mice, Arterioscler. Thromb. Vasc. Biol. 29 (2009) 73–179.
[15] X.H. Li, D.F. Bu, H.F. Jin, Y.G. Ding, J.B. Du, J. Li, C.S. Tang, The dynamic changes in endogeous hydrogen sulfide pathway at the early stage of pulmonary hypertension induced by high pulmonary flow in rats, Zhongguo Ying Yong Sheng Li Xue Za Zhi 23 (2007) 75–79.
[16] X.H. Li, J.B. Du, C.S. Tang, Impact of hydrogen sulfide donor on pulmonary vascular structure and vasoactive peptides in rats with pulmonary hypertension induced by high pulmonary blood flow, Acta Acad. Med. Sin. 28 (2006) 159–163.
[17] S. Zhang, J. Du, H. Jin, W. Li, Y. Liang, B. Geng, S. Li, C. Zhang, C. Tang, Endogenous sulfur dioxide aggravates myocardial injury in isolated rat heart with ischemia and reperfusion, Transplantation 87 (2009) 517–524.
[18] R.Y. Zhang, J.B. Du, Y. Sun, S. Chen, H.J. Tsai, L. Yuan, L. Li, C.S. Tang, H.F. Jin, Sulfur dioxide derivatives depress L-type calcium channel in rat cardiomyocytes, Clin. Exp. Pharmacol. Physiol. 38 (2011) 416–422.
[19] W. Li, C. Tang, H. Jin, J. Du, Regulatory effects of sulfur dioxide on the development of atherosclerotic lesions and vascular hydrogen sulfide in atherosclerotic rats, Atherosclerosis 215 (2011) 323–330.
[20] X.B. Wang, X.M. Huang, T. Ochs, X.Y. Li, H.F. Jin, C.S. Tang, J.B. Du, Effect of sulfur dioxide preconditioning on rat myocardial ischemia/reperfusion injury by inducing endoplasmic reticulum stress, Basic Res. Cardiol. 106 (2011) 865–878.
[21] X.B. Wang, H.F. Jin, C.S. Tang, J.B. Du, Significance of endogenous sulfur-containing gases in the cardiovascular system, Clin. Exp. Pharmacol. Physiol. 37 (2010) 745–752.
[22] W. Lu, Y. Sun, C. Tang, T. Ochs, J. Qi, J. Du, H. Jin, Sulfur dioxide derivatives improve the vasorelaxation in the spontaneously hypertensive rat by enhancing the vasorelaxant response to nitric oxide, Exp. Biol. Med. (Maywood) 237 (2012) 867–872.
[23] Y. Liang, D. Liu, T. Ochs, C. Tang, S. Chen, S. Zhang, B. Geng, H. Jin, J. Du, Endogenous sulfur dioxide protects against isoproterenol-induced myocardial injury and increases myocardial antioxidant capacity in rats, Lab. Invest. 91 (2011) 12–23.
[24] S.S. Chen, C.S. Tang, H.F. Jin, J.B. Du, Sulfur dioxide acts as a novel endogenous gaseous signaling molecule in the cardiovascular system, Chin. Med. J. 124 (2011) 1901–1905.
[25] S. Chen, J. Du, Y. Liang, T. Ochs, D. Liu, L. Zhu, X. Tang, C. Tang, H. Jin, Sulfur dioxide inhibits excessively activated endoplasmic reticulum stress in rats with myocardial injury, Heart Vessels 27 (2012) 505–516.
[26] S. Chen, J. Du, Y. Liang, R. Zhang, C. Tang, H. Jin, Sulfur dioxide restores calcium homeostasis disturbance in rat with isopoterenol-induced myocardial injury, Histol. Histopathol. 27 (2012) 1219–1226.
[27] M. Collin, C. Thiemermann, Hydrogen sulfide and sulfite: novel mediators in the pathophysiology of shock and inflammation, Shock 24 (2005) 595–596.
[28] F.H. Pettit, D. Lyon, J.R. Brown, W. Shive, Evidence for sulfite as an essential metabolite for human peripheral lymphocytes, Biochem. Biophys. Res. Commun. 179 (1991) 611–614.
[29] X.H. Li, J.B. Du, D.F. Bu, X.Y. Tang, C.S. Tang, Sodium hydrosulfide alleviated pulmonary vascular structural remodeling induced by high pulmonary blood flow in rats, Acta Pharmacol. Sin. 27 (2006) 971–980.
[30] H. Mitsuhashi, S. Yamashita, H. Ikeuchi, T. Kuroiwa, Y. Kaneko, K. Hiromura, K. Ueki, Y. Nojima, Oxidative stress-dependent conversion of hydrogen sulfide to sulfite by activated neutrophils, Shock 24 (2005) 529–534.
[31] H. Mitsuhashi, Y. Nojima, T. Tanaka, K. Ueki, A. Maezawa, S. Yano, T. Naruse, Sulfite is released by human neutrophils in response to stimulation with lipopolysaccharide, J. Leukoc. Biol. 64 (1998) 595–599.