

Full-length article

Hydrogen sulfide regulates lung tissue-oxidized glutathione and total antioxidant capacity in hypoxic pulmonary hypertensive rats¹

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Key words

hydrogen sulfide; oxidative stress; hypoxia; pulmonary hypertension; superoxide dismutase

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Abstract

Aim: To investigate the modulatory effect of sodium hydrosulfide on lung tissue-oxidized glutathione and total antioxidant capacity in the development of hypoxic pulmonary hypertension (HPH). **Methods:** After 21 d of hypoxia, the mean pulmonary artery pressure was measured by cardiac catheterization. The plasma H₂S level and production of H₂S in the lung tissues were determined by using a spectrophotometer. The lung homogenates were assayed for total antioxidant capacity (T-AOC), superoxide dismutase (SOD), oxidized glutathione (GSSG), reduced glutathione and malonaldehyde by colorimetry. The mRNA level of SOD was analyzed by real-time PCR, and the SOD expression was detected by Western blotting. **Results:** In the hypoxia group, the plasma H₂S concentration and H₂S production in the lung was significantly decreased compared with the control group (187.2±13.1 vs 299.6±12.4 μmol/L; 0.138±0.013 vs 0.289±0.036 nmol·mg⁻¹·min⁻¹, *P*<0.01). The administration of sodium hydrosulfide could reduce the mean pulmonary artery pressure by 31.2% compared with the hypoxia group (*P*<0.01). Treatment with sodium hydrosulfide decreased GSSG, and the T-AOC level of the lung tissues was enhanced compared with the hypoxia group (*P*<0.05). There were no significant changes in the lung tissue SOD mRNA level, protein level, and its activity among the 3 groups. **Conclusion:** Oxidative stress occurred in the development of HPH and was accompanied by a decrease in the endogenous production of H₂S in the lung tissues. H₂S acted as an antioxidant during the oxidative stress of HPH partly as a result of the attenuated GSSG content.

Introduction

Hypoxic pulmonary hypertension (HPH), an important pathophysiological process in the development of a variety of clinical cardiac and pulmonary diseases^[1], has critical influence on the proceeding and prognosis of the diseases. Until recently, the mechanism of HPH has not yet been fully understood. Recent studies have shown that oxidative stress plays a role in the development of pulmonary hypertension^[2,3]. Long-term hypoxic animal models have been known to exhibit the overproduction of reactive oxygen species (ROS)^[2]. An excess of ROS has been suggested to trigger pulmonary arterial vasoconstriction through the increment of cy-

tosolic calcium in the pulmonary arterial myocyte^[4,5]. Hiromitsu *et al* showed that adrenomedullin, which is not only a potent vasodilator but also an antioxidant, could protect against pulmonary vascular remodeling induced by hypoxia^[6]. However, the oxidative/antioxidative state and its mechanism have not been clearly elucidated.

Hydrogen sulfide (H₂S) is a newly-found gasotransmitter recently demonstrated to play similar roles as nitric oxide and carbon monoxide in the body, especially in the cardiovascular system^[7-11]. Our previous works revealed that the endogenous cystathionine-γ-lyase (CSE)/H₂S pathway existed in the vessels^[12,13]. As well as this, we found that the endogenous CSE/H₂S pathway was down-regulated in

hypoxia pulmonary hypertension; exogenously-administered sodium hydrosulfide (NaHS) could reduce pulmonary hypertension and inhibit pulmonary arterial remodeling^[14]. Recently, H₂S was shown to protect primary rat cortical neurons from oxidative injury by stimulating the synthesis of antioxidant glutathione^[15]. Whiteman *et al* indicated that H₂S could significantly inhibit hypochlorous acid-mediated damage to biomolecules and to cultured human neuroblastoma SH-SY 5Y cells^[16]. Furthermore Geng *et al* drew the conclusion that H₂S protected the heart from isoproterenol-induced ischemic injury, at least in part, through scavenging oxygen-free radicals and attenuating lipid peroxidation^[17]. Thus endogenous H₂S might be a new antioxidant in our organism. However, it is unclear whether the endogenous H₂S contributes to the pathophysiology of oxidative stress in HPH. We hypothesized that H₂S might participate in the regulation of oxidative stress and act as an antioxidant in HPH. To investigate the possible modulating effect of H₂S on oxidative stress during HPH and its possible molecular mechanisms, we put rats in a hypoxic environment to observe changes in the lung tissue CSE activity and plasma H₂S concentration, oxidative stress indexes, and the protective effects after the exogenous administration of H₂S.

Materials and methods

Materials and reagents NaHS was purchased from Sigma (St Louis, MO, USA). TRIzol reagent was bought from Invitrogen (Carlsbad, CA, USA). Oligo d(T)₁₅ primer, dNTP, and M-MLV reverse transcriptase were bought from Promega (San Luis Obispo, CA, USA). All the other chemicals in the study were purchased from Beijing Chemical Reagents (Beijing, China).

Hypoxic pulmonary hypertensive rat model The study was approved by the Animal Research Committee of Peking University (Beijing, China). The rats were exposed to hypoxia according to the methods of Zhang *et al*^[14]. Twenty male Wistar rats (180–220 g) were randomly divided into the control group ($n=6$), hypoxia group ($n=6$), and hypoxia+NaHS group ($n=8$). The rats were exposed to normobaric hypoxia (10% O₂) in a transparent plastic hypoxic chamber (for rats in the hypoxia group and the hypoxia+NaHS group) for 3 weeks and 6 h every day. The rats in the control group were housed in identical cages adjacent to the hypoxia chamber and breathed room air. Hypoxia was generated by infusing N₂ into the chamber. The degree of hypoxia was maintained by the balance between infusing nitrogen and the inward leak of air through holes in the chamber. For rats in the hypoxia+NaHS group, NaHS, dissolved in physiological saline at a dosage of 14 $\mu\text{mol/kg}$ body weight, was intraperito-

neally injected every day before hypoxia. The same volume of physiological saline was injected for the rats in the other 2 groups.

Measurement of hemodynamic parameters and sample preparation Three weeks after hypoxic exposure, the rats were anesthetized with urethane (1 g/kg body weight) intraperitoneally. A silicone catheter (0.9 mm in outer diameter) was introduced into the right jugular vein via a venotomy and passed across the tricuspid valve and right ventricle (RV) into the pulmonary artery. The mean pulmonary artery pressure (mPAP) was simultaneously recorded (90308-11-17-38; SpaceLabs, Issaquah, WA, USA). The mean aortic pressure was also recorded by carotid artery catheterization (90308-11-17-38, SpaceLabs, USA). After the chest was opened, the left lower part of the lung tissues was rapidly removed to liquid nitrogen for quick freezing and then stored at $-70\text{ }^{\circ}\text{C}$. Then the heart was excised and the atria were removed. The free wall of the RV was separated from the heart. These tissues were blotted and weighed using an electronic scale. The wet weight ratio of the RV to left ventricle (LV) plus septum, RV/(LV+septum [SP]), was calculated as an indicator of right ventricular hypertrophy. The blood plasma was also prepared for the measurement of the plasma H₂S concentration.

Measurement of H₂S concentration in the plasma In a test tube containing 0.5 mL of 1% zinc acetate and 2.5 mL distilled water, 0.1 mL plasma was added. Then 0.5 mL of 20 $\mu\text{mol/L}$ *N,N*-dimethyl-*p*-phenylenediamine sulfate in 7.2 mol/L HCl and 0.4 mL of 30 mmol/L FeCl₃ in 1.2 mol/L HCl were also added to the test tube for 20 min incubation at room temperature. The protein in the plasma was removed by adding 1 mL of 10% trichloroacetic acid to the solution and centrifuged. The optical absorbance of the resulting solution at 670 nm was measured with a spectrometer (Shimadzu UV 2100, Kyoto, Japan). The H₂S concentration in the solution was calculated against the calibration curve of the standard H₂S solution.

Measurement of H₂S production According to Stipanuk and Beck^[18], the lung tissues were homogenized in 50 mmol/L ice-cold potassium phosphate buffer (pH 6.8). Reactions were performed in 25 mL Erlenmeyer flasks. The reaction mixture contained (in mmol/L): 10 *L*-cysteine 2 pyridoxal 5'-phosphate, 100 potassium phosphate buffer (pH 7.4), and 10% (w/v) homogenates. The total volume of the reaction mixture was 1 mL. A small piece of filter paper was put into the central well of the flask and 0.5 mL of 1% zinc acetate was also added in the central well for trapping evolved H₂S in the mixture. The flasks were then flushed with N₂ before being sealed with a double layer of parafilm.

The catalytic reaction was initiated by transferring the flasks from an ice bath to a 37 °C shaking water bath. After 90 min at 37 °C, the reactions were stopped by injecting 0.5 mL of 50% trichloroacetic acid. The flasks were incubated in the shaking water bath for an additional hour at 37 °C to complete the trapping of H₂S. The content of the central well was transferred to test tubes and mixed with 3.5 mL distilled water and 0.5 mL of 20 μmol/L *N,N*-dimethyl-*p*-phenylenediamine sulfate in 7.2 mol/L HCl. To each tube, 0.4 mL of 30 mmol/L FeCl₃ in 1.2 mol/L HCl was added immediately. After 20 min incubation at room temperature, the optical absorbance of the resulting solution at 670 nm was measured with a spectrometer (Shimadzu UV 2100, Japan). The H₂S concentration in the solution was calculated against the calibration curve of the standard H₂S solution. For each sample, the measurement was done in duplicate. The H₂S production was expressed as nmol·mg⁻¹·min⁻¹.

Measurement of oxidative stress indicators The lower part of the left lung tissues were homogenized in 50 mmol/L ice-cold potassium phosphate buffer (pH 6.8) at the rate of 10% (w/v). The lung homogenates were assayed for total antioxidant capacity (T-AOC), superoxide dismutase (SOD), oxidized glutathione (GSSG), reduced glutathione (GSH), and malondialdehyde (MDA) by using colorimetry.

Measurement of SOD mRNA in lung tissues by quantitative RT-PCR Total RNA was extracted from the rat lung tissues using TRIzol reagent. The cDNA was synthesized using oligo d(T)₁₅ primer and M-MLV reverse transcriptase. The PCR primers were used to amplify the cDNA. The primers of SOD were:

SOD₁ (rat-forward): 5'-AAAGGACGGTGTGGCCAAT-3'

SOD₁ (rat-reverse): 5'-TCCACCTTTGCCAAGTCAT-3'

SOD₂ (rat-forward): 5'-CGTCACCGAGGAGAAGTACCA-3'

SOD₂ (rat-reverse): 5'-GGCTCAGGTTTGTCCA-GAAAAAT-3'.

The primers were β-actin were:

β-actin (rat-forward): 5'-ATCTGGCACCACACCTTC-3'

β-actin (rat-reverse): 5'-AGCCAGGTCCAGACGCA-3'

*Taq*man probe was synthesized with 6-carboxyfluorescein (FAM) reporter dye at the 5'-end and 6-carboxytetramethylrhodamine (TAMRA) quencher at the 3'-end.

The reaction mixture for the real-time PCR (final volume of 50 μL) contained 5 μL 10× PCR buffer, 2 μL of 7.5 μmol/L each primer, 2 μL of 2.5 mmol/L each dNTP mixture, 0.5 μL of *Taq* DNA polymerase, and 5 μL of rat lung cDNA. Amplification was performed using an ABI 7300 real-time PCR system (Applied Biosystems, Foster, CA, USA) under the following conditions: 95°C for 5 min,

followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. Ten-fold dilution series (1×10⁻¹–1×10⁻⁶) were prepared from an extract of the passaged viruses of all genotypes available and analyzed by both PCR methods. Slopes and linear ranges of the standard curves for each genotype were assessed after real-time PCR. Real-time PCR amplified all genotypes with excellent efficiency with correlation coefficients above 0.98.

Measurement of SOD expression in lung tissues by Western blotting Rat lung tissues were homogenized and lysed. Equal amounts of protein were boiled and separated by SDS-PAGE and electrophoretically transferred to a nitrocellulose membrane according to experiment protocols. The primary antibody dilution was 1:8000 for the SOD₁ (Stressgen, Ann Arbor, MI, USA) and SOD₂ antibodies (Stressgen, USA) and 1:10000 for β-actin (Labvision, Fremont, CA, USA). Horseradish peroxidase-conjugated secondary antibody (Santa Cruz, CA, USA) was used at 1:12000. The immunoreactions were visualized by electrochemiluminescence (ECL) and exposed to X-ray film (Kodak Scientific Imaging Film, Rochester, NY, USA).

Data analysis All data were expressed as mean±SD. For the comparison of differences among the 3 groups, one-way ANOVA followed by a *post-hoc* analysis (listed significant difference test, LSD) was conducted using SPSS 13.0 statistic analysis software (SPSS, Chicago, IL, USA). To compare the changes of the 2 groups, we selected the independent-samples *t*-test method. A value of *P*<0.05 was considered statistically significant.

Results

Hemodynamics After 3 weeks of hypoxia, the mPAP was increased by 45.3% compared with rats in normoxic environment (23.7±2.2 vs 16.3±3.7 mmHg, *P*<0.01) in the present study. The ratio of RV/(LV+SP) was also increased by 41.4% in the rats of the hypoxia group compared with that of the control group (*P*<0.01). These results showed that the HPH rat model was reproduced successfully. To examine if the exogenous H₂S impacts on hypoxic pulmonary hypertension, we administered 14 μmol·kg⁻¹·d⁻¹ NaHS, a donor of H₂S. The results showed that the mPAP of the hypoxia group was markedly decreased, and the RV/(LV+SP) was also reduced (all *P*<0.01), as shown in Table 1 and Figure 1.

Plasma H₂S concentration and H₂S production of lung tissues The plasma level of H₂S and its production in the lung tissues decreased significantly in the hypoxia group compared with the control group (187.2±13.1 vs 299.6±12.4 μmol/L, 0.138±0.013 vs 0.289±0.036, respectively, all *P*<0.01). After treatment with NaHS, the plasma level of H₂S and H₂S

Table 1. Comparison of mPAP and RV/(LV+SP) among 3 groups of rats. Mean±SD. ^c*P*<0.01 vs control group. ^f*P*<0.01 vs hypoxia group.

Groups	<i>n</i>	mPAP (mmHg)	RV/(LV+SP) (mg/mg)
Control group	6	16.325±3.673	0.286±0.0191
Hypoxia group	6	23.712±2.191 ^c	0.405±0.0283 ^c
Hypoxia+NaHS group	8	16.293±2.784 ^f	0.311±0.0194 ^f

mPAP, mean pulmonary arterial pressure; RV/(LV+SP), wet weight of right ventricle/(left ventricle+septum);

Table 2. Comparison of plasma H₂S concentration and H₂S production in lung tissues among 3 groups of rats. Mean±SD. ^c*P*<0.01 vs control group. ^f*P*<0.01 vs hypoxia group.

Groups	<i>n</i>	Plasma H ₂ S (μmol/L)	H ₂ S production in lung tissues (nmol·mg ⁻¹ ·min ⁻¹)
Control group	6	299.6±12.4	0.289±0.036
Hypoxia group	6	187.2±13.1 ^c	0.138±0.013 ^c
Hypoxia+NaHS group	8	309.2±13.6 ^f	0.312±0.017 ^f

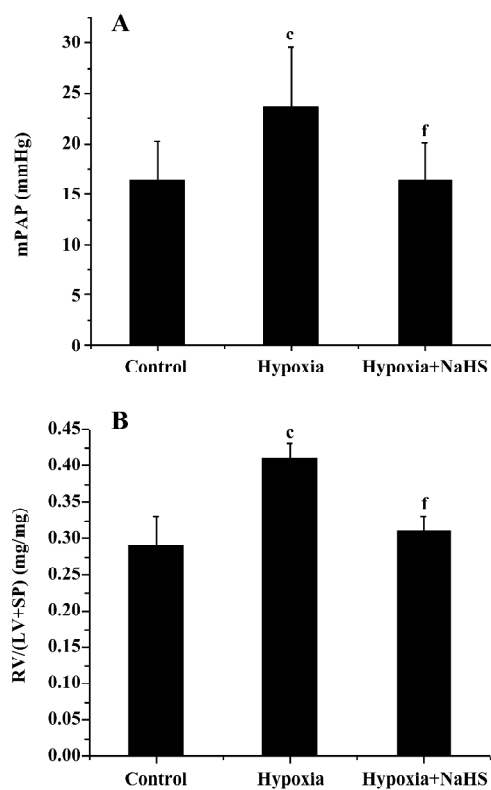


Figure 1. Comparison of mPAP and RV/(LV+SP) between the 3 groups of rats. (A) mPAP, (B) RV/(LV+SP). ^c*P*<0.01 vs control group. ^f*P*<0.01 vs hypoxia group.

production in the lung tissues increased to 1.7- and 2.3-fold, respectively, in the hypoxia+NaHS group compared with the hypoxia group, as shown in Table 2 and Figure 2.

Oxidative stress indicators In the present study, we chose several oxidative injurants and antioxidants to evaluate the redox state *in vivo*. Our data showed that the GSSG of lungs in the rats of the hypoxia group was higher than those of the control group (0.026±0.005 vs 0.016±0.003 g/g, *P*<0.01). After we treated the hypoxia group with the donor of H₂S,

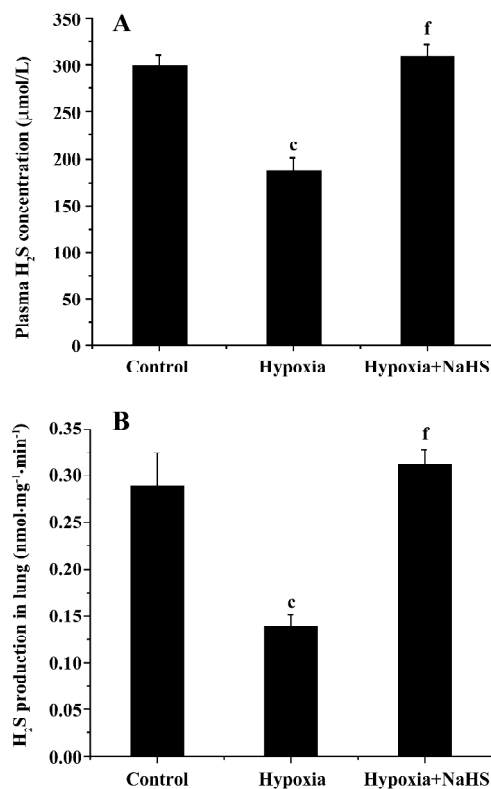


Figure 2. Comparison of the H₂S plasma level and H₂S production in lung tissues among the 3 groups of rats. Alterations of H₂S in plasma (A) and lung tissues (B). ^c*P*<0.01 vs control group. ^f*P*<0.01 vs hypoxia group.

the GSSG level in the hypoxia+NaHS group was decreased by 23% compared with the hypoxia group (*P*<0.05). The MDA level increased obviously in the hypoxia group as compared with the control group (0.0054±0.0005 vs 0.0032±0.007 mmol/g, *P*<0.01). However, treatment with NaHS could not reverse the elevation of MDA under hypoxic challenge. The T-AOC of rat lung tissues in the hypoxia group was significantly lower than that of the control group (15361.2±221.6 vs 19578.6±2994.7 U/g, *P*<0.01). After administration with NaHS,

Table 3. Oxidative stress indicators in pulmonary tissue homogenates of rats. Mean±SD. ^c*P*<0.01 vs control group. ^f*P*<0.01 vs hypoxia group.

Groups	<i>n</i>	T-AOC (U/g)	SOD (U/g)	GSH (g/g)	GSSG (g/g)	MDA (mmol/g)
Control group	6	19578.6±2994.7	62545.2±13956.4	0.012±0.004	0.016±0.003	0.0032±0.0007
Hypoxia group	6	15361.2±2216.1 ^c	49094.5±30591.8	0.080±0.003	0.026±0.005 ^c	0.0054±0.0005 ^c
Hypoxia+NaHS group	8	18337±1847.8 ^f	57014.9±7091.1	0.011±0.004	0.020±0.004 ^f	0.0046±0.001

T-AOC: total antioxidant capacity; SOD: superoxide dismutase; GSSG: oxidized glutathione; GSH: reduced glutathione; MDA: malondialdehyde.

however, the T-AOC was enhanced by 19% as compared with of the hypoxia group (*P*<0.05). For the other 2 antioxidants, our data showed that there were no significant differences among the 3 groups in lung SOD and GSH levels, as shown in Table 3.

SOD mRNA level To explore the mechanism responsible for the possible role of H₂S in the regulation of antioxidative response, we chose the most important antioxidant, SOD, in our further study. There were no significant differences, however, in the lung SOD₁ and SOD₂ mRNA levels among the rats of the 3 groups, as shown in Table 4.

Table 4. SOD₁ and SOD₂ mRNA level among 3 groups. Mean±SD.

Groups	<i>n</i>	SOD ₁ /β-actin	SOD ₂ /β-actin
Control group	6	0.168±0.131	0.105±0.102
Hypoxia group	6	0.113±0.051	0.069±0.020
Hypoxia+NaHS group	8	0.086±0.081	0.049±0.026

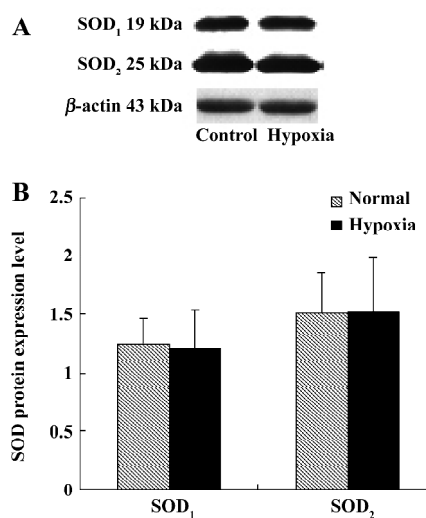
The data was obtained by quantitative real-time PCR. There were no significant differences in SOD₁ and SOD₂ mRNA expression among 3 groups.

SOD protein level We also examined the SOD₁ and SOD₂ protein expression levels in the lung tissues of rats in both the control group and hypoxia group. The results showed that there were no distinct differences between these 2 groups, as seen in Table 5 and Figure 3.

Table 5. SOD₁ and SOD₂ protein level between control group and hypoxia group. *n*=6. Mean±SD.

Groups	SOD ₁ /β-actin	SOD ₂ /β-actin
Control group	1.24±0.23	1.51±0.35
Hypoxia group	1.21±0.33	1.52±0.46

The data was obtained by Western blotting. There were no significant differences in SOD₁ and SOD₂ protein expression between two groups.

**Figure 3.** Comparison of SOD protein level in lung tissues between the control group and hypoxia group. (A) representative immunoblots of SOD₁ and SOD₂ in the lung tissues from the control group and the hypoxia group. (B) ratio of SOD expression to β-actin, with the expression of β-actin taken as 1. There were no significant differences between these 2 groups.

Discussion

Pulmonary hypertension is an important pathophysiological process of many hypoxic diseases, such as cardiopulmonary disease, mountain sickness, and sleep apnea syndrome^[19]. However, the pathogenesis of hypoxic pulmonary hypertension has not been fully understood^[20]. The overproduction of ROS can result in the progression of pulmonary vascular remodeling^[15]. Recently, endogenous H₂S has been suggested as a novel neuromodulator and signal transmitter^[21]. Our previous work revealed that the endogenous CSE/H₂S pathway was down-regulated in hypoxic pulmonary hypertension^[14]. H₂S could exert antioxidant effects *in vivo* and *in vitro*^[17,22]. Based on these observations, we hypothesized that H₂S might act as an antioxidant in HPH and protect the body from oxidative injury. The present study aimed to investigate the role of H₂S in the pathogenesis of

oxidative stress in HPH.

Pulmonary artery hypertension was defined as a mPAP of 25 mmHg or more and pulmonary capillary wedge pressure of 15 mmHg or less, both measured at rest by right heart catheterization according to WHO criteria^[23]. Our previous work demonstrated that after intermittent exposure to 10% oxygen for 6 h daily for 2–4 weeks, the rats could develop constant chronic pulmonary hypertension with right ventricular hypertrophy^[8,11,14,24]. In the present study, after 3 weeks of hypoxia, the mPAP increased significantly compared to the rats without hypoxia ($P < 0.01$). The ratio of RV/(LV+SP) in the hypoxia group was also higher than that in the control group ($P < 0.01$). These results showed that the rat model of HPH was reproduced successfully.

Long-term hypoxia can result in an increase in the rat plasma glutathione disulfide level *in vivo* and an increase in the formation of lipid peroxide products in cultured bovine pulmonary endothelial cells *in vitro*^[15,25]. The overproduction of ROS can induce the progression of pulmonary vascular remodeling. Thus several intrinsic substances to modulate pulmonary ROS levels may play protecting or aggravating roles in the progression of hypoxic pulmonary vascular remodeling. As we know, oxidative stress is an imbalance state of the oxidative and antioxidative systems^[26]. When the balance is disturbed, ROS, by promoting lipid peroxidation, may cause damage to DNA, proteins, lipid, and membranes in the cells^[27]. In the present study, MDA level of lung tissues in the hypoxia group increased after hypoxia exposure for 3 weeks (Table 3). Antioxidants are the primary defense against ROS. In the present study, we choose to detect several antioxidants in lung tissues, such as T-AOC and SOD. To further investigate the cellular redox status, we used GSH/GSSG as the intracellular redox buffers. Our data showed that T-AOC in the hypoxia group was significantly lower than that of the control group ($P < 0.01$), and GSSG in the hypoxia group was higher than that of the control group ($P < 0.01$). There were no significant differences in SOD and GSH between the hypoxia group and control group. It is worth noting that in our study, when the pulmonary hypertension formed, MDA and GSSG increased, whereas T-AOC which was representative of total antioxidant capacity, quenched obviously. Based on these results, we speculated that oxidative stress occurred and lasted in the development of HPH, which might facilitate the progression of pulmonary vascular remodeling.

H₂S is a strong reducing agent. It is a highly-reactive molecule and may easily react with other compounds, especially with ROS and nitrogen species^[16,17]. Kimura Y and

Kimura H found that H₂S increased the glutathione levels, which normally decrease during the cell death cascade, by enhancing the activity of γ -glutamylcysteine synthetase and up-regulating cysteine transport. Their observation revealed that H₂S protected neurons from oxytosis by increasing the production of antioxidant glutathione^[15]. The down-regulation of the endogenous H₂S pathway was involved in the pathogenesis of HPH in rats, and it was found that exogenously applied H₂S could exert protective effects on the pathogenesis of HPH^[14]. In present study, hypoxia-induced oxidative stress injury, accompanied by the H₂S plasma level and its production in lung tissues, were all quenched significantly. To explore the effects of H₂S on the regulation of oxidative stress, we administered the exogenous donor of H₂S by injecting NaHS intraperitoneally. We observed that after the treatment with exogenous H₂S, hypoxic pulmonary hypertension was attenuated, the T-AOC levels was increased by 19%, and GSSG content was decreased by 23%. These results suggested that H₂S might play an important role in regulating oxidative stress in hypoxia-induced pulmonary hypertension, at least by directly scavenging GSSG and increasing the T-AOC. In the present study we first characterized the antioxidative aspect of H₂S in the process of hypoxic pulmonary hypertension. An expanding body of experimental observations supports the concept that H₂S might scavenge excess oxidative stress production^[15,17,27]. However, the exact mechanism needs to be further investigated.

The SOD family is the key antioxidant enzyme during oxidative stress. Up to now, 3 SOD isoenzymes have been identified in mammals^[28]. The major intracellular SOD is a copper- and zinc-containing homodimer (Cu/Zn SOD or SOD₁) present throughout the cytoplasm and nucleus. The mitochondrial SOD (MnSOD or SOD₂) is synthesized in the cytoplasm and translocated to the inner matrix of mitochondria. The last SOD discovered is primarily extracellular (EC-SOD or SOD₃)^[27]. In order to detect whether SOD changes during the oxidative stress of HPH, we investigated the activity and transcriptional level of SOD₁ and SOD₂. Our study showed that there was no significant difference in SOD₁ and SOD₂ among the 3 groups. However, the SOD₃ level was not detected in our study. Whether extracellular SOD can exert important functions in HPH still remains to be answered.

In conclusion, oxidative stress was involved in hypoxic pulmonary hypertension. H₂S could enhance the T-AOC and attenuate GSSG in the lung tissues of rats with HPH. The present study also showed that H₂S had no effect on SOD mRNA or the protein level in the lung tissues of rats with chronic hypoxic pulmonary hypertension. These results suggest that H₂S not only ameliorates the pathological changes

of HPH, but also acts as a cardiovascular protective antioxidant in the development of HPH.

Author contribution

Hong-ling WEI, Chun-yu ZHANG, Hong-fang JIN, Chao-shu TANG designed research; Hong-ling WEI and Chun-yu ZHANG performed research; Jun-bao DU and Hong-fang JIN contributed new reagents or analytic tools; Hong-ling WEI, Jun-bao DU, Chun-yu ZHANG analyzed data; Hong-ling WEI and Jun-bao DU wrote the paper.

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