Sodium hydrosulfide alleviates pulmonary artery collagen remodeling in rats with high pulmonary blood flow

Abstract This study aimed to explore the effect of sodium hydrosulfide (NaHS) on pulmonary artery collagen remodeling in rats with high pulmonary blood flow. Thirty-two Sprague-Dawley rats were randomly divided into a sham group, shunt group, sham + NaHS (an H₂S donor) group, and shunt + NaHS group. After 11 weeks of shunting, mean pulmonary artery pressure (MPAP), relative median area (RMA) of pulmonary arteries, H₂S concentration in lung tissues, plasma endothelin-1 (ET-1) levels, and ET-1 mRNA in lung tissues were investigated. Collagen I and collagen III were evaluated by immunohistochemistry. Hydroxyproline assay and Sirius-red staining were performed. Matrix metalloproteinase-13 (MMP-13), tissue inhibitor of metalloproteinase-1 (TIMP-1), and connective tissue growth factor (CTGF) were evaluated by immunohistochemistry. After 11 weeks of shunting, rats showed a significant pulmonary hypertension and pulmonary artery collagen remodeling in association with a decrease in lung tissue H₂S content. After NaHS treatment for 11 weeks, lung tissue H₂S content was increased, whereas MPAP was attenuated and RMA was reduced. Meanwhile, pulmonary artery collagen I and collagen III protein expressions of intra-acinar pulmonary arteries were inhibited, but MMP-13/TIMP-1 ratio was augmented with a decreased plasma ET-1 content and lung tissue ET-1 mRNA and CTGF expressions. The downregulation of H₂S is involved in the development of pulmonary artery collagen remodeling induced by high pulmonary blood flow.

Key words Hydrogen sulfide · Collagen · Pulmonary artery · Tissue inhibitor of metalloproteinase-1 · Matrix metalloproteinase-13

Introduction

Pulmonary hypertension (PH) is a progressive complication in congenital heart disease (CHD) with a left-to-right shunt characterized by high pulmonary blood flow. The studies on the mechanisms responsible for the development of pulmonary hypertension attract great attention to this field, and remarkable progress has been made in understanding the pathophysiologic and molecular mechanisms responsible for pulmonary hypertension secondary to CHD with left-to-right shunt. It was found that a variety of vasoactive factors including gasotransmitters nitric oxide (NO) and carbon monoxide (CO) played an important role in the regulation of high pulmonary blood flow-induced pulmonary hypertension. However, the mechanisms responsible for the development of pulmonary hypertension induced by high pulmonary blood flow are still not fully understood. Hydrogen sulfide (H₂S), which was recognized as a toxic gas in the past, was recently discovered to play important roles in regulating relaxation and antiproliferation of vascular smooth muscle cells in pulmonary hypertensive rats. We recently found that there was a downregulation of H₂S production in rats with chronic pulmonary hypertension. However, whether the decreased production of H₂S in the development of pulmonary hypertension has any pathophysiological significance has not been understood. Therefore, the present study was designed to investigate the possible role of the changes in H₂S production in the development of pulmonary hypertension and pulmonary vascular structural remodeling induced by high pulmonary blood flow and its possible mechanisms.
Materials and methods

Rat model of high pulmonary blood flow

The experiment was conducted in accordance with the Guide to The Care and Use of Experimental Animals issued by the Ministry of Health of the People's Republic of China. Male Sprague-Dawley rats were provided by the Animal Research Centre of Peking University First Hospital. The rats were kept in a temperature-controlled room with a 06:00 to 18:00 h light/dark cycle. Tap water and rat chow were provided ad libitum. The animal model was established by an abdominal aorta-inferior vena cava shunting operation according to the method described by Garcia and Diebold with some modifications. Briefly, 32 male Sprague-Dawley rats, weighing 120–140 g, were randomly divided into a shunt group (n = 8), shunt + sodium hydrosulfide (NaHS, H₂S donor) group (n = 8), sham group (n = 8), and sham + NaHS group (n = 8). Rats in the shunt and shunt + NaHS groups were anesthetized with 0.25% pentobarbital sodium (40 mg/kg, intraperitoneal injection). The abdominal aorta and inferior vena cava were exposed, then a bulldog vascular clamp was placed across the aorta cauda to the left renal artery. The aorta was punctured at the union of the segment two-thirds caudal to the renal artery and one-third cephalic to the aortic bifurcation with an 18-gauge disposable needle. Then the needle was slowly withdrawn and a 9-0 silk thread was used to stitch the puncture of the abdominal wall. In the sham and sham + NaHS groups, rats underwent the same experimental protocol as mentioned above except for the shunt procedure. Rats in the shunt + NaHS and sham + NaHS groups were intraperitoneally injected with NaHS at 56 μmol/kg per day for 11 weeks as described, and at the same time rats in the shunt and sham groups were injected with the same volume of physiological saline.

Measurement of mean pulmonary artery pressure (MPAP) and sample preparation

At the end of the experiment, the animals were weighed and anesthetized with pentobarbital sodium (40 mg/kg, intraperitoneal injection). A silicone catheter (0.9 mm in outer diameter) was introduced under fluoroscopy into the right jugular vein via a venotomy and passed across the tricuspid valve and right ventricle into the pulmonary artery. The other end of the catheter was connected to a transducer (YZ-05-1, Beijing, China). The mean pulmonary artery pressure (MPAP) was simultaneously recorded.

The right side of the lung tissue was removed and kept in liquid nitrogen for a quick freezing and then stored at −80°C for homogenate. The left lower part of lung tissue was removed to 10% (w/v) paraformaldehyde for fixation.

Evaluation of Qp/Qs

The animals were weighed and anesthetized with pentobarbital sodium (40 mg/kg, intraperitoneal injection). Blood sample (0.5 ml) was obtained from the pulmonary artery, external carotid artery, and jugular vein, respectively, for blood gas analysis using a Blood Gas Analysis Apparatus (GASTAT-3, Techno Medica, Tokyo, Japan) at 11 weeks of the experiment. Qp/Qs was calculated by the formula: Qp/Qs = [oxygen saturation of aorta (%) – oxygen saturation of inferior vena cava (%)]/oxygen saturation of pulmonary vein (%) – oxygen saturation of pulmonary artery (%). When the oxygen saturation of the aorta was >95%, the oxygen saturation of pulmonary vein was regarded as 100%. When the oxygen saturation of the aorta was <95%, the oxygen saturation of pulmonary vein was regarded as 95%. The ratio Qp/Qs was calculated as an indicator to evaluate the pulmonary flow and body flow.

Measurement of H₂S in lung tissue

Lung tissue was homogenized in a 10-fold volume (w/v) of an ice-cold potassium phosphate buffer (pH 6.8). The reaction was performed in 25 ml in an Erlenmeyer Pyrex flask. Cryovial test tubes (2 ml) were used as the center wells, each containing 1 M NaOH of 0.5 ml. The reaction mixture contained lung tissue homogenate and 1 M HCl in a ratio of 1:5. The flasks containing reaction mixture and central wells were flushed with N₂ 30 s before being sealed with a double layer of parafilm. The reaction was initiated by transferring the flasks from ice to a shaking water bath at 37°C. After incubation at 37°C for 4 h, the contents of the central wells were then transferred to 10-m1 beakers, each containing 0.5 ml of antioxidant solution. Subsequently, the solution was measured with a sulfide-sensitive electrode (PXS-270, Shanghai, China), and the lung tissue H₂S content was calculated against the calibration curve of the standard H₂S solution according to Zhao et al.

Morphological analysis of rat small pulmonary arteries

Lung tissue fixed in 10% paraformaldehyde was dehydrated, embedded in paraffin, and sectioned at a thickness of 4 μm. The elastic fiber in lung tissues was stained according to the modified Weigert method and counterstained with Van Gieson solution. Morphological analysis was performed using a video-linked microscope digitizing board system (Leica Q550CW, Wetzlar, Germany). Only vessels showing clearly defined external and internal elastic lamina were used in analysis. The relative medial area (RMA) of rat small pulmonary arteries was calculated according to the method of Barth et al.

Hydroxyproline assay of lung tissue

Lung tissue homogenate was added with 0.2 ml of 6 nmol/l HCl and then was dehydrated for 14 h. pH value was
adjusted by 6 nmol/l NaOH and then the tissue was clarified by centrifugation at 2000 rpm for 10 min. Hydroxyproline assay was performed with an experimental kit (Jiancheng, Nanjing, China).

Sirius-red staining analysis of collagen I and collagen III

The paraffin sections were dewaxed and hydrated, and then processed by Sirius-red staining for 1 min. Type I and type III collagen fibers were distinguished under polarizing microscope.

Immunohistochemical analysis of collagen I, collagen III, tissue inhibitor of metalloproteinase-1, matrix metalloproteinase-13 and connective tissue growth factor

The paraffin sections for immunohistochemistry were dewaxed and hydrated, then processed by 3% H2O2 for 15 min, followed by antigen repairing (digestion with gastric enzyme for 30 min at 37°C for collagen I and collagen III; heating in a microwave for 15 min at 99°C for TIMP-1, MMP-13, and CTGF). The slides were blocked with normal bovine serum albumin (BSA) for 30 min at room temperature. Collagen I and collagen III (Boster Bioengineering, Wuhan, China), TIMP-1 and MMP-13 (Neomarkers, Fremont, CA, USA), and CTGF (Boster) antibodies were then added at 37°C for 2 h, respectively. In sequence, the biotinylated anti-rabbit IgG and horseradish peroxidase streptavidin (Santa Cruz, Santa Cruz, CA, USA) were added at 37°C for 30 min, respectively. Then DAB was added for 1-10 min and Mayer's hematoxylin for 1 min. The expression of antibody by smooth muscle cells in intrapulmonary arteries was observed under microscope. The yellow-brown cytoplasm represented positive signals of antibody expression. The mean value of percentage of pulmonary artery smooth muscle cells expressing different abundances of antibody (0%, ~50%, and ~100%) was determined.

Measurement of plasma endothelin-1 and endothelin-1 mRNA in lung tissue by competitive quantitative reverse transcription–polymerase chain reaction

Plasma ET-1 concentration was detected by radioimmunoactivity kit (Institute of Radioimmunoactivity, PLA General Hospital, Beijing, China).

Total RNA was extracted from rat lung tissue using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The cDNA was synthesized using oligo (dT)20 primer and M-MLV reverse transcriptase (Promega, Madison, WI, USA). The polymerase chain reaction (PCR) primers used amplifying the fragment of ET-1 cDNA were:

ET-1-S: 5’-GAGCTGAGAGGAACTGCGAGA
ET-1-A: 5’-GGCTGTGACTGCTTGCAGA
Competitive internal primer: 5’-CTGTTGCTGACAGCTTT
GGGACAGGTTT

The polymerase chain reaction (PCR) using these two primers above yielded a 446 bp fragment of wild-type rat ET-1 cDNA. The competitive internal standard for the measurement of ET-1 cDNA had the same sequences as the 446 bp fragment of wild-type rat ET-1 cDNA, except that a fragment of 61 bp at the downstream site of ET-1-S primer was deleted. Competitive quantitative PCR was performed in a 20 μl PCR tube containing 3 μl of rat lung cDNA, 1 μl of 3.9 nmol/l competitive internal standard, 1 μl of 5 pmol/each ET-S and ET-A mixture, 1 μl of 2.5 mmol/each dNTP mixture, 2.5 μl of 10x PCR buffer (MgCl2 15 mmol/l), and 1.25 U Taq DNA polymerase, in a total volume of 25 μl. Polymerase chain reaction products were separated in a 2% agarose gel and stained by ethidium bromide. The ratio of optical density to the two DNA bands was measured by a Gel Image Analysis System (AlphaImager, Alpha Innotech, San Leandro, CA, USA) under UV light. A standard curve of the ratio was drawn using the same condition as described above except that rat lung cDNA was changed to a series of dilutions of the plasmid containing the 446 bp wild-type ET-1 cDNA fragment. The relative amount of ET-1 cDNA in samples was then obtained from the standard curve. To calibrate the amount of sample loaded in PCR mixture, β-actin cDNA was measured using the same method after the quantitative PCR. Three microliters of PCR product was amplified again using the two rat β-actin primers (β-actin-S: 5’-ATCTGG GCACCA ACACCC GTC, β-actin-A: 5’-AGGCA GGTCC AGACGCA). Relative amount of β-actin cDNA in loaded sample was then obtained from a pre-made standard curve of β-actin cDNA measurement. Standardized relative amount of ET-1 cDNA was used for further analysis.

Statistical analysis

All data are expressed as mean ± SD. One-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls multiple comparison test was used by SPSS 10.0 statistical software. A value of P < 0.05 was considered statistically significant.

Results

Changes in Qp/Qs and lung tissue H2S

In the rat model of abdominal aorta-inferior vena cava shunt, Qp/Qs in the shunt group and shunt + NaHS group increased significantly compared with that of the sham group (P < 0.01). The Qp/Qs in shunt and shunt + NaHS groups did not differ significantly. Lung tissue H2S content decreased significantly in the shunt group compared with that of the sham group (P < 0.01). After administration of NaHS for 11 weeks, lung tissue H2S content increased significantly, in contrast to that of the shunt group (P < 0.01). The sham and sham + NaHS groups, however, did not differ significantly in lung tissue H2S content (Table 1).
Table 1. Changes of parameters in rats with high pulmonary blood flow (n = 8) (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>Qp/Qs</th>
<th>Lung tissue H2S (μmol/mg)</th>
<th>MPAP (mmHg)</th>
<th>RMA (%)</th>
<th>Hydroxyproline (mg/gPro)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shunt</td>
<td>2.123 ± 0.535*</td>
<td>20.177 ± 2.235*</td>
<td>27.582 ± 2.967*</td>
<td>37.392 ± 3.046*</td>
<td>1.346 ± 0.121*</td>
</tr>
<tr>
<td>Shunt + NaHS</td>
<td>1.979 ± 0.408</td>
<td>43.342 ± 1.575**</td>
<td>24.173 ± 3.027**</td>
<td>27.165 ± 2.956**</td>
<td>1.228 ± 0.061**</td>
</tr>
<tr>
<td>Sham</td>
<td>0.781 ± 0.116</td>
<td>40.157 ± 2.554</td>
<td>17.208 ± 1.318</td>
<td>21.316 ± 3.123</td>
<td>0.946 ± 0.104</td>
</tr>
<tr>
<td>Sham + NaHS</td>
<td>0.746 ± 0.221</td>
<td>39.338 ± 1.871*</td>
<td>16.883 ± 1.665</td>
<td>23.476 ± 2.621</td>
<td>1.055 ± 0.108</td>
</tr>
</tbody>
</table>

MPAP, mean pulmonary artery pressure; RMA, relative median area
* P < 0.01 vs sham group; ** P < 0.01 vs shunt group

Table 2. Changes in integral scores of collagen I, collagen III, MMP-13, TIMP-1, and CTGF expression in pulmonary arteries of different groups of rats (n = 8) (x ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>Collagen I</th>
<th>Collagen III</th>
<th>MMP-13</th>
<th>TIMP-1</th>
<th>MMP-13/TIMP-1</th>
<th>CTGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shunt</td>
<td>0.834 ± 0.093*</td>
<td>0.878 ± 0.067*</td>
<td>0.690 ± 0.078*</td>
<td>0.628 ± 0.035*</td>
<td>1.069 ± 0.105*</td>
<td>0.768 ± 0.054*</td>
</tr>
<tr>
<td>Shunt + NaHS</td>
<td>0.668 ± 0.038**</td>
<td>0.659 ± 0.047**</td>
<td>0.519 ± 0.033**</td>
<td>0.716 ± 0.051**</td>
<td>1.289 ± 0.113**</td>
<td>0.585 ± 0.073**</td>
</tr>
<tr>
<td>Sham</td>
<td>0.336 ± 0.061</td>
<td>0.359 ± 0.050</td>
<td>0.320 ± 0.0460</td>
<td>0.353 ± 0.043</td>
<td>0.941 ± 0.115</td>
<td>0.360 ± 0.067</td>
</tr>
<tr>
<td>Sham + NaHS</td>
<td>0.361 ± 0.068</td>
<td>0.366 ± 0.054</td>
<td>0.324 ± 0.057</td>
<td>0.361 ± 0.055</td>
<td>0.916 ± 0.214</td>
<td>0.374 ± 0.059</td>
</tr>
</tbody>
</table>

MMP-13: matrix metalloproteinase-13; TIMP-1, tissue inhibitor of metalloproteinase-1; CTGF, connective tissue growth factor
* P < 0.05 vs sham group; ** P < 0.05 vs shunt group

Sodium hydrosulfide attenuated MPAP and reduced RMA of pulmonary arteries

After 11 weeks of shunting, the MPAP increased by 48.63% compared with that of the sham group (P < 0.01). After administration with NaHS for 11 weeks, MPAP decreased significantly compared with that of the shunt group (P < 0.01). There were no significant differences in MPAP between the sham and sham + NaHS groups (P > 0.05) (Table 1).

As compared with that of the sham group, RMA of intra-acinar pulmonary arteries in the shunt group increased by 39.91% (P < 0.01). After administration with NaHS for 11 weeks, RMA decreased significantly compared with that of the shunt group (P < 0.01). There were no significant differences in RMA between the sham and sham + NaHS groups (P > 0.05) (Table 1).

Sodium hydrosulfide alleviated pulmonary artery extracellular matrix collagen accumulation

In contrast to that of the sham group, hydroxyproline concentration of rat lung tissue in the shunt group increased significantly (P < 0.01). After administration of NaHS for 11 weeks, hydroxyproline concentration of rat lung tissue decreased significantly compared with that of the shunt group (P < 0.01). There were no significant differences, however, in hydroxyproline concentration of rat lung tissue between the sham and sham + NaHS groups (P > 0.05) (Table 1).

Also, Sirius-red staining showed that collagen I and collagen III in rat pulmonary artery spatial accumulation were more obvious in the shunt group than in the sham group. As compared with the shunt group, shunt + NaHS group collagen I and collagen III in rat pulmonary artery spatial accumulation were obviously decreased (Fig. 1).

In contrast to those of the sham group, collagen I and collagen III protein expressions of intra-acinar pulmonary arteries in the shunt group were upregulated significantly (P < 0.01). After administration with NaHS for 11 weeks, collagen I and collagen III protein expressions of intra-acinar pulmonary arteries were downregulated significantly compared with those of the shunt group (P < 0.01). There were no significant differences in collagen I and collagen III protein expressions of intra-acinar pulmonary arteries between the sham and sham + NaHS groups (P > 0.05) (Table 2).

Sodium hydrosulfide regulated pulmonary artery MMP-13 and TIMP-1 protein expressions

Matrix metalloproteinase-13 and TIMP-1 protein expressions of intra-acinar pulmonary arteries in the shunt group were upregulated significantly (P < 0.01), and the ratio of MMP-13/TIMP-1 protein expressions in the shunt group increased significantly (P < 0.01) compared with that of the sham group. After administration of NaHS for 11 weeks, TIMP-1 and MMP-13 protein expressions of intra-acinar pulmonary arteries were upregulated significantly, and the ratio of MMP-13/TIMP-1 increased significantly compared with that of the shunt group (P < 0.01). The shunt and shunt + NaHS groups did not differ significantly in TIMP-1 and MMP-13 protein expressions of intra-acinar pulmonary arteries, and the ratio of MMP-13/TIMP-1 (P > 0.05) (Table 2, Figs. 2 and 3).

Sodium hydrosulfide reduced ET-1 mRNA and CTGF expression in pulmonary arteries of shunted rats

In contrast to that of the sham group, the plasma ET-1 content in shunt group increased significantly (P < 0.01).
After administration with NaHS for 11 weeks, the plasma ET-1 content decreased significantly compared with that of the shunt group. However, the sham and sham + NaHS groups did not differ significantly in the plasma ET-1 content ($P > 0.05$). Lung tissue ET-1 mRNA expression was upregulated significantly in the shunt group compared with that of the sham group ($P < 0.01$). In contrast to that of the shunt group, ET-1 mRNA expression was significantly downregulated in lung tissue of rats in the shunt + NaHS group. However, there was no significant difference in lung tissue ET-1 mRNA expression between the sham and sham + NaHS groups (Figs. 4 and 5).

As compared with that of the sham group, CTGF protein expression of intra-acinar pulmonary arteries in the shunt group was upregulated significantly ($P < 0.01$). After administration of NaHS for 11 weeks, CTGF protein expressions of intra-acinar pulmonary arteries was downregulated significantly compared with that of the shunt group ($P < 0.01$). There were no significant differences in CTGF protein expression between the sham and sham + NaHS groups ($P > 0.05$) (Table 2 and Fig. 6).

**Discussion**

Congenital heart disease, in the presence of a significant left-to-right shunt, with increased pulmonary blood flow commonly leads to the development of pulmonary hypertension and its associated increased vascular reactivity. In many animal models, endothelial dysfunction is a precursor for smooth muscle dysfunction, and there is an apparent progression from endothelial dysfunction to smooth muscle dysfunction as vascular changes progress. In the present study, we established a chronic model of pulmonary hypertension with increased pulmonary blood flow in rats by an abdominal aorta–inferior vena cava shunting operation. In this model, we found that after 11 weeks of shunting, the MPAP increased by 48.63%, which suggested that rats with left-to-right shunt resulting in a high pulmonary blood flow developed PH. Furthermore, the RMA of the intra-acinar pulmonary arteries increased by 39.91%, which suggested that rats with high pulmonary blood flow developed pulmonary vascular structural remodeling.
Up to now the mechanisms responsible for the development of high pulmonary blood flow-induced pulmonary hypertension have not been fully understood. H$_2$S, a novel gasotransmitter, was found to be produced endogenously in mammalian tissues from L-cysteine metabolism mainly by three enzymes: cystathionine-β-synthetase (CBS), cystathionine-γ-lyase (CSE), and 3-mercaptosulfurtransferase (MST). The expression of these enzymes is tissue specific, and CSE mainly catalyzes H$_2$S metabolism in the cardiovascular system. Our previous studies revealed that H$_2$S played an important role in pathophysiological processes in cardiovascular systems such as spontaneous hypertension, nitric oxide (NO) synthase inhibitor-induced hypertension, hypoxia-induced pulmonary hypertension, septic and endotoxic shock, and isoproterenol-induced myocardial injury. As a result, H$_2$S is being regarded as a novel gasotransmitter in the cardiovascular system. More interestingly, we noticed that in an animal model of high pulmonary blood flow-induced pulmonary hypertension the production of endogenous H$_2$S as well as its CSE gene expression was downregulated. However, is there any possible pathophysiological relevance of the downregulation of H$_2$S production to the development of high pulmonary blood flow-induced pulmonary hypertension? Is it possible that the downregulation of H$_2$S production is involved in the pathogenesis of pulmonary hypertension induced by high pulmonary blood flow? To answer the above scientific questions, we applied NaHS, a H$_2$S donor, to a rat model of high pulmonary blood flow. It was found that after administration of NaHS for 11 weeks, lung tissue H$_2$S content increased significantly, but MPAP and RMA were markedly attenuated, which suggested that H$_2$S played a protective role in the development of PH and pulmonary vascular structural remodeling induced by high pulmonary blood flow, and the downregulation of H$_2$S production is probably involved in the development of pulmonary hypertension.

The possible mechanism by which H$_2$S regulated pulmonary artery pressure and alleviated pulmonary vascular structural remodeling induced by high pulmonary blood flow has not been revealed. Studies showed that the extracellular matrix (ECM) contributed a great deal to the formation of pulmonary vascular structural remodeling. Collagen I and collagen III are the main ingredients of ECM of blood vessels, and the excess accumulation in the absolute or relative content of collagen I and collagen III.
results in pulmonary vascular structural remodeling. Our previous studies showed that H$_2$S reduced the collagen expression of a hypoxia-induced pulmonary hypertensive rat. In the present study it was shown that, under a polarizing microscope, collagen I and collagen III accumulation in pulmonary artery wall developed with pulmonary vascular structural remodeling. Also, it was found that hydroxyproline concentration of rat lung tissue increased and the expression of collagen I and collagen III was upregulated in the pulmonary artery after 11 weeks in a left-to-right shunted rat model. However, when the rats were administered NaHS for 11 weeks, along with the increase in lung tissue H$_2$S hydroxyproline concentration of rat lung tissue decreased and the expression of collagen I and collagen III accumulation was obviously lessened, which suggested that H$_2$S probably regulated high pulmonary blood flow-induced pulmonary hypertension by downregulating the expression of collagens, important components of vascular ECM.

The stability of vascular collagens depends on the homeostasis of their synthesis and degradation. Matrix metalloproteinase-13 and TIMP-1 are important substances in maintaining specifically the homeostasis of ECM collagen. Extracellular matrix collagen will accumulate and pulmonary artery will become remodeled when the homeostasis of ECM collagen is demolished. It was found in our study that the expression of MMP-13 and TIMP-1 was upregulated in pulmonary artery after 11 weeks of left-to-right shunt in the rat model. At the same time, when the shunted rats were administered NaHS for 11 weeks, the expression of MMP-13 and TIMP-1 was upregulated further. Moreover, the increased extent of the MMP-13 was higher than that of TIMP-1. The above facts suggested that NaHS probably reduced ECM collagen accumulation through increasing its degradation, resulting in an imbalance of ECM metabolism in the model.

To examine the possible mechanisms responsible for the regulation of pulmonary vascular collagen remodeling by H$_2$S, we further studied the possible impact of H$_2$S on the metabolism of pulmonary vasoactive peptides. Endothelin-1 is a potent vasoconstrictor and smooth muscle mitogen.
Fig. 3A–D. Immunohistochemical analysis of TIMP-1 protein expression in pulmonary arteries of rats in different groups (DAB, ×200). A A pulmonary artery from rat of the sham group. B A pulmonary artery from a rat of the sham + NaHS group. C A pulmonary artery from a rat of the shunt group. D A pulmonary artery from a rat of the shunt + NaHS group.

Fig. 4. The changes in plasma concentration of ET-1 in rats of different groups.

That plays an important pathogenetic role in the development and progression of pulmonary hypertension by modulating both vasoconstriction and proliferation. Two endothelin receptor isoforms, endothelin-A (ET-A) and endothelin-B (ET-B), have been identified. ET-A receptors are found in the pulmonary vascular smooth muscle cells, and ET-B receptors are located both on the pulmonary vascular endothelial cells and smooth muscle cells. Bosentan, an endothelin receptor antagonist (ET-A and ET-B), has been used to decrease pulmonary resistance or reverse severe pulmonary hypertension in patients with pulmonary artery hypertension related to congenital heart disease. To explore whether NaHS has an effect on ET-1 content and ET-1mRNA expression, we applied NaHS for 11 weeks to rats with high pulmonary blood flow. It was found that plasma ET-1 concentration increased and ET-1mRNA expression was upregulated after 11 weeks of left-to-right shunt in the rat model, whereas when the rats were administered NaHS for 11 weeks, plasma ET-1 concentration decreased and lung tissue ET-1mRNA expression was downregulated. These results suggested that NaHS probably affected the transcription of lung tissue ET-1mRNA to regulate the plasma ET-1 concentration, which was probably involved in the mechanisms by which NaHS regulated.
The above findings provided evidence for the first time that the downregulation of H$_2$S is involved in the mechanisms responsible for the development of pulmonary artery collagen remodeling induced by high pulmonary blood flow. H$_2$S inhibited the production of some vasoactive peptides such as ET-1 and CTGF and, as a result, regulated homo-cysteine synthesis and degradation of ECM collagen metabolism, participating in the regulation of pulmonary artery collagen remodeling in rats with high pulmonary blood flow.

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References

Fig. 6A–D. Immunohistochemical analysis of connective tissue growth factor protein expression in pulmonary arteries of rats in different groups (DAB, x200). A A pulmonary artery from a rat of the sham group. B A pulmonary artery from a rat of the sham + NaHS group. C A pulmonary artery from a rat of the shunt group. D A pulmonary artery from a rat of the shunt + NaHS group.
binding protein-related protein-2 is a mediator in the induction of fibronectin by advanced glycosylation end-products in human dermal fibroblasts. Endocrinology 143:1260–1269
