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

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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.

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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