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Hydrogen sulfide reduces skeletal muscle atrophy in pulmonary hypertension rats through Notch signaling pathway

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Abstract

Background: To explore whether hydrogen sulfide can atrophy the skeletal muscles of rats with hypoxic pulmonary hypertension, and to explore the preliminary mechanism of action.

Methods: 60 SD rats, male, were divided in a random manner into control group (C), hypoxia group (Sa), hydrogen sulfide group (S), hydrogen sulfide + hypoxia group (S/Sa). The pure passive hypoxic stimulation method was used twice a day, and the hypoxic treatment was continued for 4 months. The control group did not intervene. Intervention with hydrogen sulfide after 3 months of experiment, the intervention ended.After that, the rats in each group were weighed. A small animal ventilator was used to detect lung function, while samples of whole blood, lung tissue, and skeletal muscle were collected for enzyme-linked immunoassay (ELISA) to detect IL-6, IL-8, IL-10, and ROS levels. RT-PCR detects the mRNA content of Notch-1 and Notch-3 in skeletal muscle; Western blot detects the protein content of Notch-1 and Notch-3 in skeletal muscle.

Results: Hydrogen sulfide can cause skeletal muscle dysfunction in hypoxic pulmonary artery rats through oxidative stress-inflammatory mechanisms. Hydrogen sulfide can reduce the level of ROS in lung tissue, serum and skeletal muscle of hypoxic pulmonary hypertension rats and inhibit the expression of IL-6, IL-8, IL-10, and improve hypoxia through suppressed oxidative stress-inflammatory mechanisms The functional status of skeletal muscle in rats with pulmonary hypertension.

Conclusion: Hydrogen sulfide promotes skeletal muscle atrophy in rats with hypoxic pulmonary hypertension through Notch signaling pathway.

Keywords: hypoxia; pulmonary hypertension; hydrogen sulfide; Notch

Introduction

Pulmonary hypertension is a preventable and treatable lung disease, which is characterized by continuous and progressive pulmonary hypertension, which may be accompanied by chronic inflammatory reactions of the airways. More and more people believe that pulmonary hypertension no longer only affects the lungs, and can induce complications such as hypoxemia, heart disease, osteoporosis, musculoskeletal diseases and lung cancer by causing systemic inflammation. It severely affects the labor ability and quality of life ofpatients. At the same time, the disability rate and fatality rate of pulmonary hypertension are also high, which brings continuous and unbearable economic burden to patients, families and society [1-3].

Risk factors concerned with the occurrence and development of pulmonary hypertension involve chronic hypoxia, genetic individual susceptibility, air pollution, inhalation of harmful gases and particulate matter, lung tissue infection by bacteria or viruses, and bronchial hyperresponsiveness. Among them, the long-term Chronic hypoxia is one of the most important factors leading to the occurrence of pulmonary hypertension. Therefore, the role of hypoxic pulmonary hypertension has long attracted people's attention [4-9].

Pulmonary hypertension not only causes airflow limitation, but also affects the dysfunction of tissues and organs outside the respiratory system,

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such as skeletal muscle atrophy, cardiovascular and renal dysfunction, and skeletal muscle atrophy is particularly prominent in patients with advanced pulmonary hypertension. The damage of these organs and systems outside the lungs is closely related to the chronic inflammation of the lungs caused by pulmonary hypertension [10-13]. Peripheral skeletal muscle dysfunction, as an important extrapulmonary manifestation of pulmonary hypertension, is one of the main factors leading to patient activity limitation. Skeletal muscle dysfunction often damages the structure and function of respiratory muscles, decreases respiratory muscle reserve and endurance, decreases muscle mass and muscle fiber atrophy. Therefore, in patients with pulmonary hypertension, skeletal muscle atrophy can be detected clinically, and the muscle strength of the lower limbs is more obvious than that of the upper limbs, and the endurance of the quadriceps is also significantly reduced [14-16].

In this study, a pulmonary hypertension rat model was established by hypoxic stimulation, and hydrogen sulfide intervention was used to observe whether it can effectively reduce the oxidative stress and inflammation of the internal and external lung tissues of pulmonary hypertension, and whether it can treat skeletal muscle atrophy in hypoxic pulmonary hypertension rats. It has a protective effect and is used for the treatment of patients with hypoxic pulmonary hypertension by hydrogen sulfide and improve skeletal muscle dysfunction to provide animal experimental evidence.

Materials and Method

Animal

Choose 60 healthy SPF male SD rats, weighing 184.05±13.61g, 8 weeks old, with uniform hair, flexible movements, and no unfavorable phenomena such as hair loss and tail docking. The experimental rats were fed in a special breeding room with a temperature of about 22°C, a humidity of 60% to 70%, and free drinking and eating. The animals were purchased from Changsha Tianqin Biotech Technology Co. Ltd, qualified by the Hunan Animal Quarantine Station, license number:SCXK (Hunan) 2019-0012.

Establishment of rat model of hypoxic pulmonary hypertension

60 SD rats, male, were divided in a random manner into control group (C), hypoxia group (Sa), hydrogen sulfide group (S), hydrogen sulfide + hypoxia group (S/Sa). The rats are placed in a self-made airtight box every day, and a mixture of nitrogen and air is used to establish a hypoxic environment to establish a hypoxic pulmonary hypertension rat model.

Rat lung tissue specimen collection

After taking the alveolar lavage fluid, look for the left main bronchus to be clamped, open the right main bronchus, and clamp the right middle lobe bronchus at the same time. Use a syringe to inject an appropriate amount of 4% paraformaldehyde (containing 1‰ DEPC water). When the upper lobe expands Then use a thin thread to ligate the right upper lobe bronchus, cut the right upper lung tissue, and place it in 4% paraformaldehyde for fixation

After hours, it was embedded in paraffin, the thickness of the section was about 5μm, and it was stained with HE and observed under a light microscope. Then cut the middle lobe of the lung and store it in a refrigerator at -80°C for use in ELSIA detection.

IL-6, IL-8, IL-10, ROS indicator detection

Take lung tissue as an example, cut about 1g of lung tissue into a mortar, immediately add liquid nitrogen to freeze, grind and homogenize in an ice bath for about 5-10 minutes to make 10% homogenate, centrifuge at 4℃, 3500r/min 、 Centrifuge for 10-15min.

Take the supernatant for later use, proceed according to the operation steps of the TNF-α, IL-6, MDA, ROS kit instructions, and determine the corresponding concentration.

Skeletal muscle immunohistochemical staining

Prepare skeletal muscle (approximately 5μm) according to the immunohistochemical preparation requirements, follow the SABC kit test instructions, dilute the primary antibody with PBS solution, and incubate the primary antibody overnight at 4°C. After incubating the primary antibody, rinse it with PBS solution, repeat 3 times, add the secondary antibody dropwise, and incubate at 37°C for about 10-30 minutes. Rinse it with PBS solution again and circulate 3 times. Add proper amount of horseradish enzyme working solution dropwise and incubate for 10-30 minutes. Rinse 3 times with PBS solution. Perform DAB color development on it, fully rinse after color development, counter-stain, dehydrate, transparent, and mount the film.

Wesron-bloting detects protein levels

Cut 0.25g of tissue, wash the tissue with ice-cold PBS, add 300ul of RIPA lysate and grind the tissue

repeatedly in a homogenizer until it is homogenized; on ice, protein lysis for 30 minutes; 4℃, 12000rpm for 15min. 10% separation glue, shake immediately after adding TEMED, and then fill the glue. After pouring the glue, seal the glue with isopropanol. According to the results of protein quantification, load 10ul of denatured protein every empty sample and start electrophoresis. Concentrated gel electrophoresis voltage 80V, the separation gel electrophoresis voltage is 120V. Prepare 6 pieces of filter paper of the same size as the glue and 1 piece of PVDF membrane. The PVDF membrane is first soaked in methanol, and then put into the transfer buffer solution together with the filter paper until it is completely saturated. Transfer the protein to the polyvinylidene fluoride blotting membrane on a trihydrochloric acid polyacrylamide gel with a voltage of 120V. Specific polyclonal antibodies are used as probes on the membrane. Obtain the same amount of protein from the protein lysate for electrophoresis analysis. β-actin is used as an internal reference.

Statistical analysis

GraphPad Prism8 statistical software was used to analyze the data statistically. Measurement data are presented as mean±standard deviation (x±s) and analyzed by t-test of group design data; Sperman correlation is used for correlation analysis, and the statistical difference was considered significant if $p<0.05$.

Results

Pathological changes of lung tissue injury caused by hypoxia in rats

After hypoxia rats were treated with hypoxia, the lung tissues of the rats were stained with HE and the lung tissue morphology changes were observed under an optical microscope. The results showed that the lung tissue morphology of rats in the experimental group showed typical pathological changes of pulmonary hypertension: a lot of Alveolar fusion, alveolar wall thinning and rupture, inflammatory cell infiltration. In the control group, the lung tissue structure was observed to be normal, and there was no infiltration of inflammatory cells. The experimental results show that the establishment of hypoxic pulmonary hypertension rat model can be used for follow-up experiments. The results are shown in Figure 1.

The effect of hydrogen sulfide on the body weight of rats with pulmonary hypertension

On the 90th day of continuous hypoxic stimulation, hydrogen sulfide was used for intervention, and hydrogen sulfide was given by intragastric administration once every 2 days.

After days, the weights of rats in each group were measured, and the results showed that the weight of the experimental group increased significantly compared with the control group, indicating a statistically significant difference ($P<0.05$). The results are shown in Figure 2.

Effect of hydrogen sulfide on cytokines in rats with pulmonary hypertension

By measuring the changes of IL-6, IL-8, IL-10, and ROS content in rat lung tissue, serum and skeletal muscle, it can be seen that the content of the control group is lower than that of the experimental group, indicating a statistically significant difference ($P<0.05$). The results are shown in Figure 3 and Figure 4.

Expression of Notch-1 and Notch-3 in rat skeletal muscle

After using hydrogen sulfide to treat hypoxic pulmonary hypertension rats, the mRNA and protein expression of Notch-1 and Notch-3 in rat skeletal muscle were detected by applying RT-PCR and Western blot. The results show, regardless of whether it is mRNA expression or protein, the experimental group has a significant increase compared with the control group, and the statistical difference is considered significant ($P<0.05$). The results are shown in Figure 5 and Figure 6.

Discussion

The results drawn from this study indicated that the levels of IL-6, IL-8, and IL-10 in the lung tissue, serum and skeletal muscle of rats after hypoxic stimulation were significantly higher than those in the control group, and the statistical difference was considered significant in comparison with the control group. Further experiments used hydrogen sulfide to intervene in hypoxic pulmonary hypertension rats. It was observed that hydrogen sulfide can significantly reduce the expression of IL-6, IL-8, and IL-10 in lung tissue, serum and skeletal muscle, compared with the control group. The statistical difference is considered significant, indicating that hydrogen sulfide has a certain inhibitory effect on related cytokines in the lung tissue of rats with hypoxic pulmonary hypertension [17,18].

The mechanism of action needs to be further studied and elucidated. ROS is a by-product of

biological aerobic metabolism, including superoxide anion free radicals, hydroxyl free radicals and hydrogen peroxide. ROS is mainly produced by inflammatory cells activated by neutrophils, eosinophils, macrophages, lymphocytes, etc. When ROS is excessively produced, it can lead to lipid, protein and DNA peroxidation. ROS has a certain lytic effect on alveolar type Ⅱ epithelial cells, which can weaken the ability of epithelial cells to participate in repair after injury and affect the reconstruction of extracellular matrix. The increase of ROS such as superoxide anion free radicals and hydrogen peroxide can be detected in the alveolar lavage fluid and lung tissue of patients with pulmonary hypertension [19-21]. The increase of ROS is related to the activation of alveolar neutrophils and macrophages. The study results suggested that the levels of ROS in lung tissue, serum and skeletal muscle of rats after hypoxic stimulation were significantly higher than those in the control group, and the statistical difference was considered significant in comparison with the control group. Further experiments used hydrogen sulfide to intervene in hypoxic pulmonary hypertension rats. It was observed that salidroside can significantly reduce the ROS content in lung tissue, serum and skeletal muscle, which is statistically different from the control group. It is suggested that hydrogen sulfide can reduce the content of ROS in serum and skeletal muscle of hypoxic pulmonary hypertension rats' lungs and extrapulmonary tissues through antioxidant effects. This study determined the expression of Notch-1 and Notch-3 in the skeletal muscle of a rat model of hypoxic pulmonary hypertension. By giving hydrogen sulfide to intervene in hypoxic pulmonary hypertension rats, it was observed that hydrogen sulfide can significantly reduce the protein and mRNA content of Notch-1 and Notch-3 in the skeletal muscle tissue of the experimental group rat model, compared with the control group. The difference between the comparisons is statistically significant, indicating that hydrogen sulfide has a certain effect against muscle atrophy. In previous animal models and patients with pulmonary hypertension, it has been confirmed that the expression of Notch-1 and Notch-3 proteins in skeletal muscle cells that can cause atrophy due to the large release of inflammatory factors and cytokines, accumulation of ROS, and so on.

It is observed from the results of this experiment that salidroside can reduce the content of ROS in skeletal muscle and the expression of IL-6, IL-8 and IL-10 inflammatory cytokines in skeletal muscle, suggesting the antioxidant and inflammation of hydrogen sulfide Effect on The hypoxic pulmonary hypertension rat model of skeletal muscle atrophy has a certain protective effect. The specific pathway through which hydrogen sulfide exerts its antioxidant effect to reduce the accumulation of ROS, thereby reducing the expression of Notch-1, Notch-3 and protein in skeletal muscle tissue, remains to be clarified by further experimental studies.

References

[1] Howden EJ, Ruiz-Carmona S, Claeys M, et al. Oxygen Pathway Limitations in Patients with Chronic Thromboembolic Pulmonary Hypertension. Circulation. 2021. 143(21): 2061-2073.

[2] Nguyen T, Bowen TS, Augstein A, et al. Expression of MuRF1 or MuRF2 is essential for the induction of skeletal muscle atrophy and dysfunction in a murine pulmonary hypertension model. Skelet Muscle. 2020. 10(1): 12.

[3] McDowell RE, Aulak KS, Almoushref A, et al. Platelet glycolytic metabolism correlates with hemodynamic severity in pulmonary arterial hypertension. Am J Physiol Lung Cell Mol Physiol. 2020. 318(3): L562-L569.

[4] Vinke P, Bowen TS, Boekschoten MV, Witkamp RF, Adams V, van Norren K. Anti-inflammatory nutrition with high protein attenuates cardiac and skeletal muscle alterations in a pulmonary arterial hypertension model. Sci Rep. 2019. 9(1): 10160.

[5] Garfield BE, Crosby A, Shao D, et al. Growth/differentiation factor 15 causes TGFβ-activated kinase 1-dependent muscle atrophy in pulmonary arterial hypertension. Thorax. 2019. 74(2): 164-176.

[6] Sithamparanathan S, Rocha MC, Parikh JD, et al. Skeletal muscle mitochondrial oxidative phosphorylation function in idiopathic pulmonary arterial hypertension: in vivo and in vitro study. Pulm Circ. 2018. 8(2): 2045894018768290.

[7] Himori K, Abe M, Tatebayashi D, et al. Superoxide dismutase/catalase mimetic EUK-134 prevents diaphragm muscle weakness in monocrotalin-induced pulmonary hypertension. PLoS One. 2017. 12(2): e0169146.

[8] Suman AC, ÉAPND C, SGZ B, et al. Evaluating respiratory musculature, quality of life, anxiety, and depression among patients with indeterminate chronic Chagas disease and

symptoms of pulmonary hypertension. Rev Soc Bras Med Trop. 2017. 50(2): 194-198.
[9] Boucherat O, Potus F, Bonnet S. microRNA and Pulmonary Hypertension. Adv Exp Med Biol. 2015. 888: 237-52.
[10] Malenfant S, Potus F, Mainguy V, et al. Impaired Skeletal Muscle Oxygenation and Exercise Tolerance in Pulmonary Hypertension. Med Sci Sports Exerc. 2015. 47(11): 2273-82.
[11] Brown MB, Chingombe TJ, Zinn AB, et al. Novel assessment of haemodynamic kinetics with acute exercise in a rat model of pulmonary arterial hypertension. Exp Physiol. 2015. 100(6): 742-54.
[12] Malenfant S, Potus F, Fournier F, et al. Skeletal muscle proteomic signature and metabolic impairment in pulmonary hypertension. J Mol Med (Berl). 2015. 93(5): 573-84.
[13] Gurtu V, Michelakis ED. Emerging therapies and future directions in pulmonary arterial hypertension. Can J Cardiol. 2015. 31(4): 489-501.
[14] Paulin R, Michelakis ED. The metabolic theory of pulmonary arterial hypertension. Circ Res. 2014. 115(1): 148-64.
[15] Kušíková K, Feichtinger RG, Csillag B, et al. Case Report and Review of the Literature: A New and a Recurrent Variant in the VARS2 Gene Are Associated with Isolated Lethal Hypertrophic Cardiomyopathy, Hyperlactatemia, and Pulmonary Hypertension in Early Infancy. Front Pediatr. 2021. 9: 660076.
[16] Vieira JS, Cunha TF, Paixão NA, et al. Exercise intolerance establishment in pulmonary hypertension: Preventive effect of aerobic exercise training. Life Sci. 2020. 261: 118298.
[17] Tran D, Munoz P, EMT L, et al. Inspiratory Muscle Training Improves Inspiratory Muscle Strength and Functional Exercise Capacity in Pulmonary Arterial Hypertension and Chronic Thromboembolic Pulmonary Hypertension: A Pilot Randomised Controlled Study. Heart Lung Circ. 2021. 30(3): 388-395.
[18] Zhou N, Tang L, Jiang Y, et al. Whole-exome sequencing reveals a novel mutation of MT-ND5 gene in a mitochondrial cardiomyopathy pedigree: Patients who show biventricular hypertrophy, hyperlactacidemia, pulmonary hypertension, and decreased exercise tolerance. Anatol J Cardiol. 2019. 21(1): 18-24.
[19] Spiesshoefer J, Herkenrath S, Mohr M, et al. Diaphragm function does not independently predict exercise intolerance in patients with precapillary pulmonary hypertension after adjustment for right ventricular function. Biosci Rep. 2019. 39(9).
[20] Keusch S, Turk A, Saxer S, et al. Rehabilitation in patients with pulmonary arterial hypertension. Swiss Med Wkly. 2017. 147: w14462.
[21] Panagiotou M, Johnson MK, Louvaris Z, et al. A study of clinical and physiological relations of daily physical activity in precapillary pulmonary hypertension. J Appl Physiol (1985). 2017. 123(4): 851-859.

Figure legends

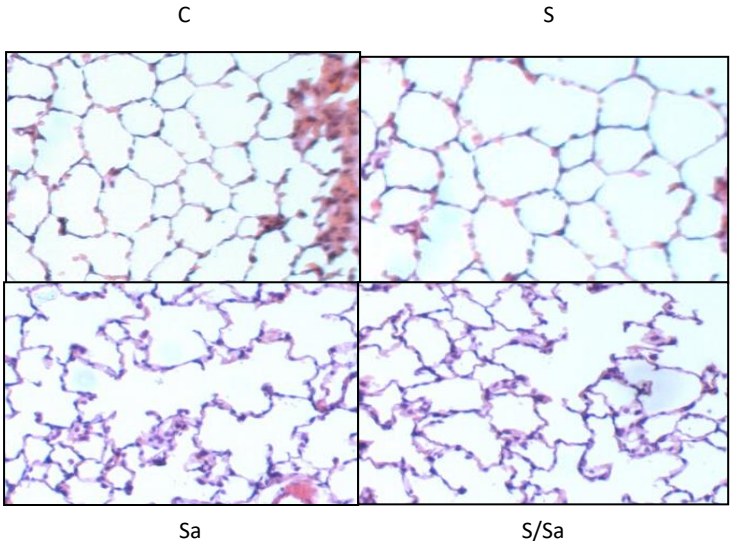

Figure 1. The pathological changes of hypoxia on lung tissue damage in rats. C: control group, Sa: hypoxia group, S: hydrogen sulfide group, S/Sa: hydrogen sulfide + hypoxia group. In comparison with the control group, the statistical difference was significant (* P<0.05).

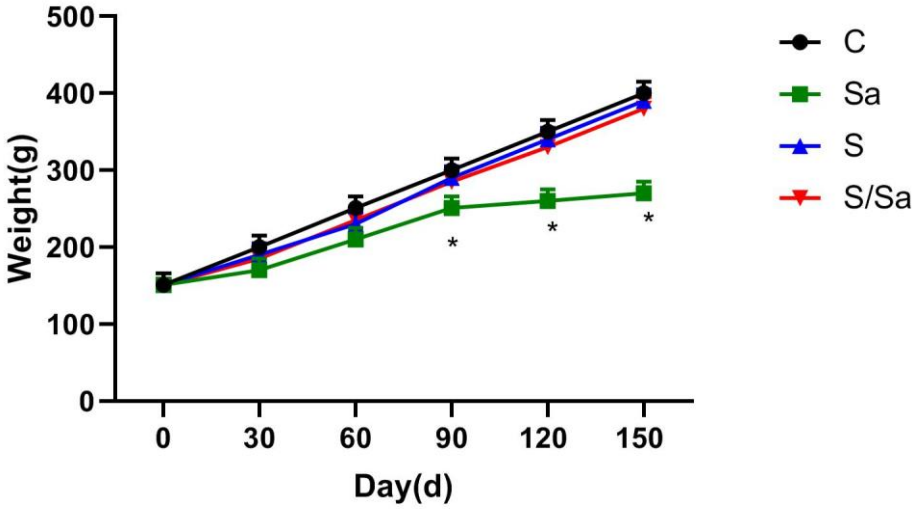

Figure 2. The effect of hydrogen sulfide on the body weight of rats with pulmonary hypertension. C: control group, Sa: hypoxia group, S: hydrogen sulfide group, S/Sa: hydrogen sulfide+hypoxia group. In comparison with the control group, the statistical difference was significant (* P<0.05).

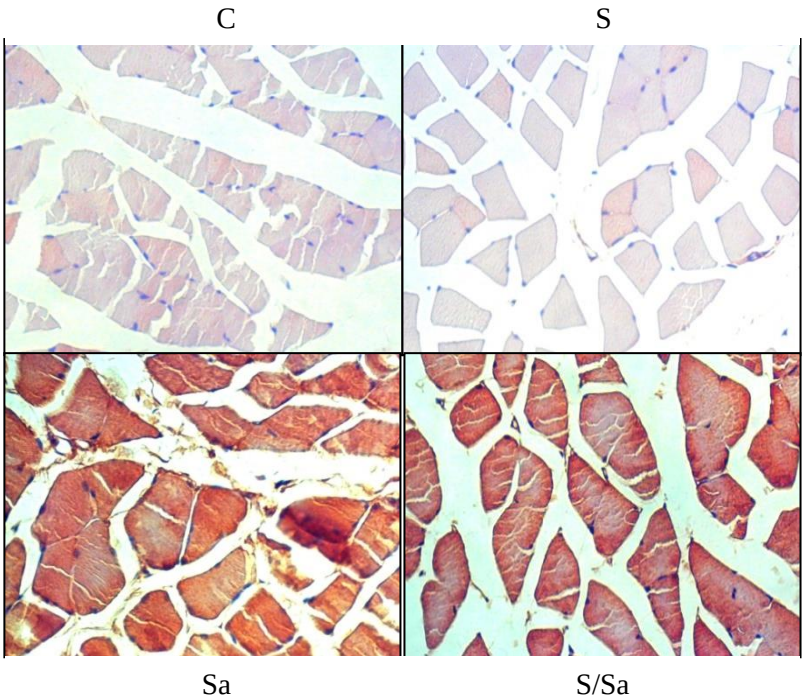

Figure 3. The effect of hydrogen sulfide on the skeletal muscle of rats with pulmonary hypertension. In the control group, the skeletal muscle muscle mass decreased and the muscle fiber atrophy.

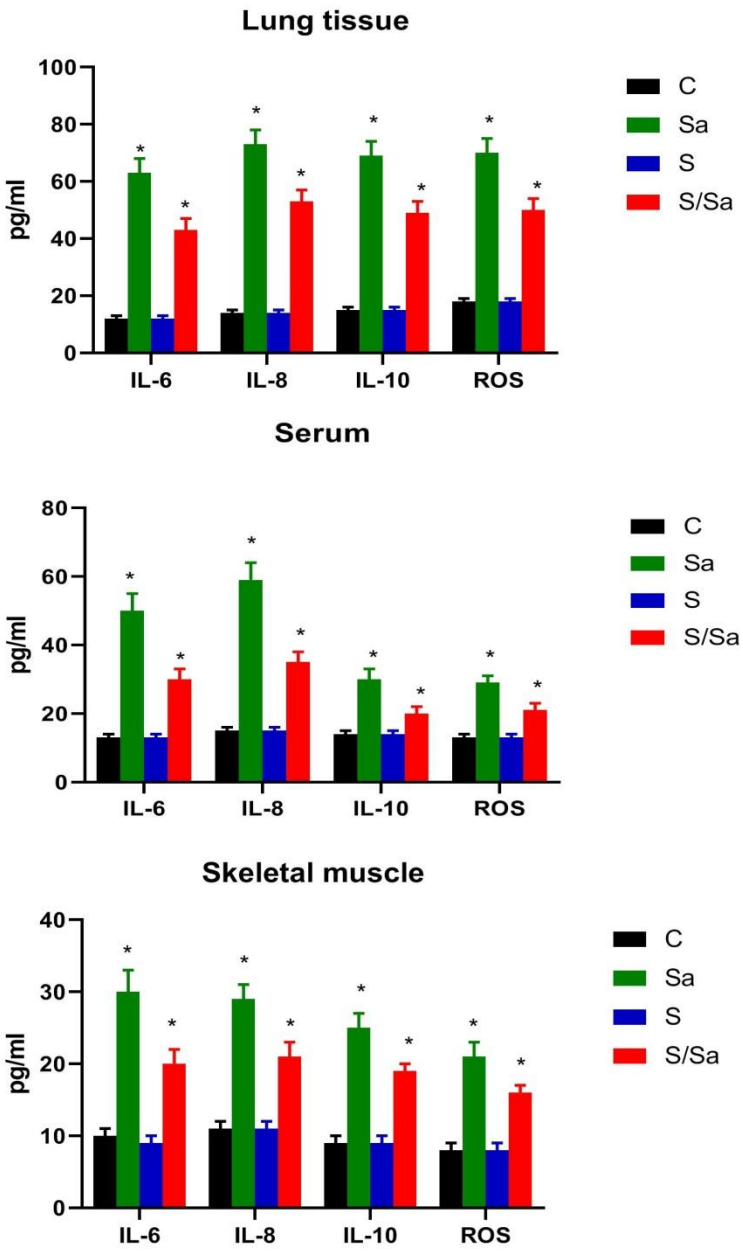

Figure 4. The effect of hydrogen sulfide on the contents of IL-6, IL-8, IL-10 and ROS in lung tissue, serum and skeletal muscle of rats with pulmonary hypertension. C: control group, Sa: hypoxia group, S: hydrogen sulfide group, S/Sa: hydrogen sulfide+hypoxia group. In comparison with the control group, the statistical difference was significant (* P<0.05).

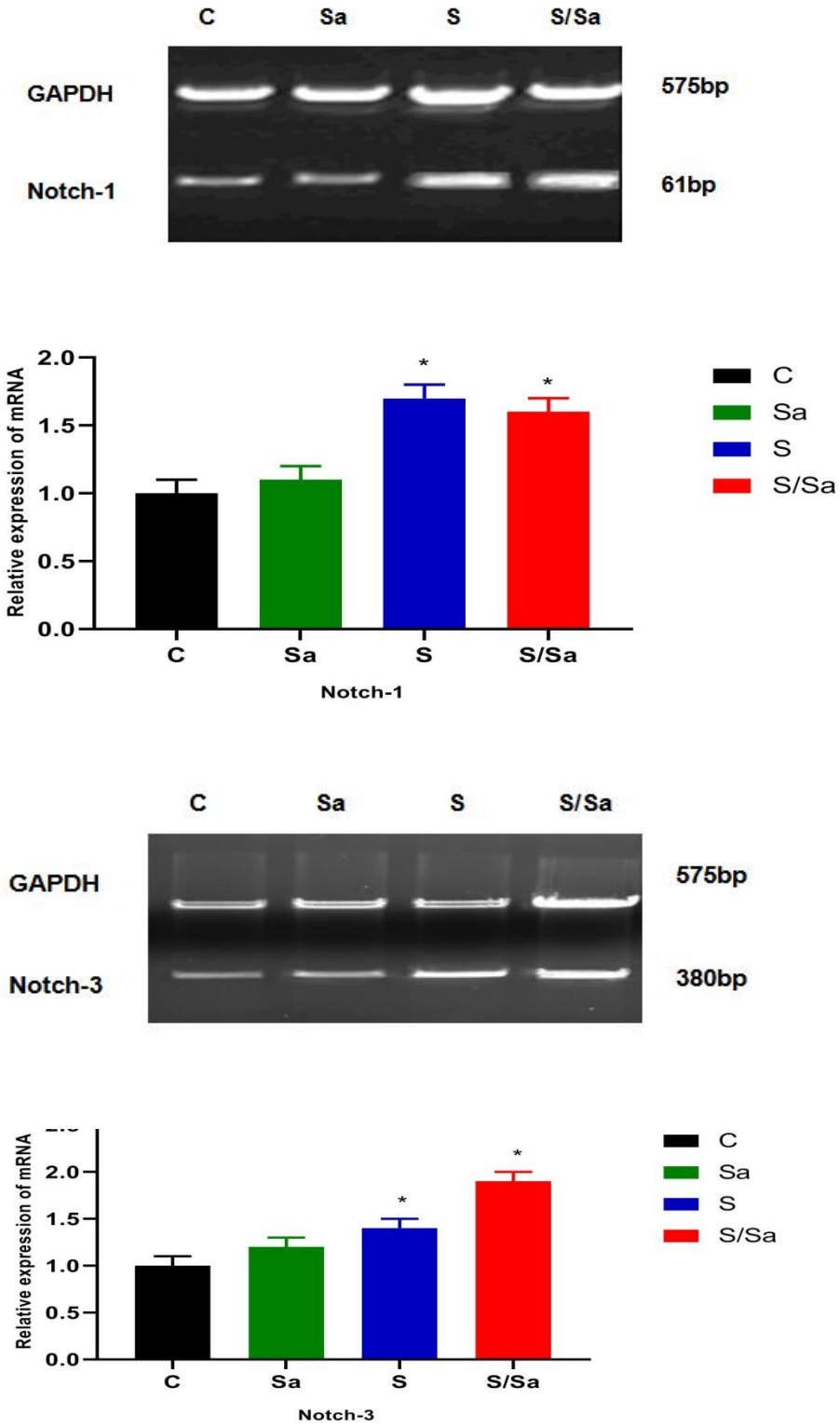

Figure 5. The effect of hydrogen sulfide on the expression of Notch-1 and Notch-3 mRNA in rat skeletal muscle. C: control group, Sa: hypoxia group, S: hydrogen sulfide group, S/Sa: hydrogen sulfide+hypoxia group. In comparison with the control group, the statistical difference was significant (* P<0.05).

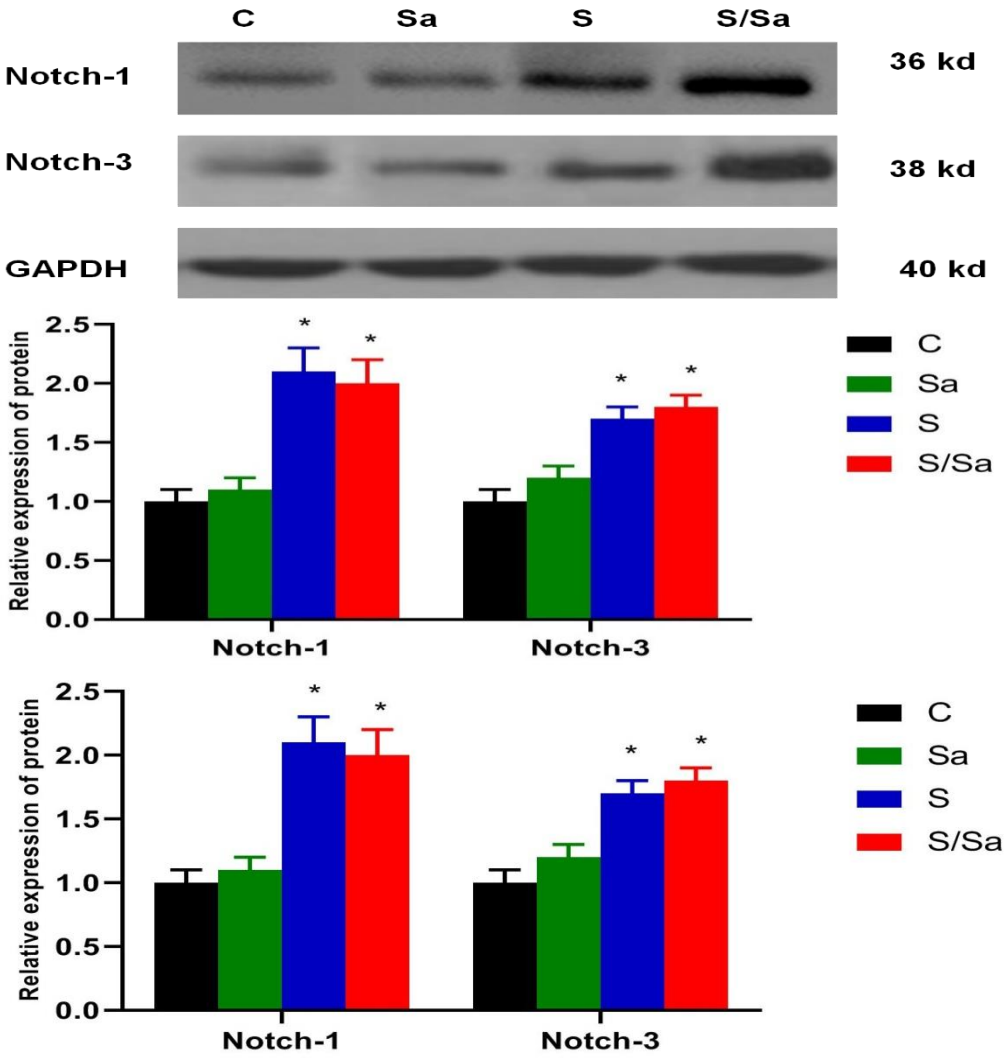

Figure 6. The effect of hydrogen sulfide on Notch-1 and Notch-3 protein expression in rat skeletal muscle. C: control group, Sa: hypoxia group, S: hydrogen sulfide group, S/Sa: hydrogen sulfide+hypoxia group. In comparison with the control group, the statistical difference was significant (* P<0.05).