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Protective effect of exogenous hydrogen sulfide on pulmonary artery endothelial cells by suppressing endoplasmic reticulum stress in a rat model of chronic obstructive pulmonary disease

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ABSTRACT

Background: Chronic obstructive pulmonary disease (COPD) is a multicomponent disorder characterized by inflammation, representing a significant leading cause of chronic morbidity and mortality. Reports have implicated hydrogen sulfide (H_2S) in both the pathology and treatment of COPD. The present study aimed to explore the effects involved with exogenous H_2S on endoplasmic reticulum stress (ERS) and pulmonary artery endothelial cells (PAECs) in a rat model of COPD.

Methods: Rat models of COPD were successfully established by means of passive smoke exposure and intratracheal injection with lipopolysaccharide (LPS). Pulmonary function tests were performed and histopathological changes were observed. The expression of ERS markers, glucose-regulated protein-78 (GRP78), and C/EBP homologous protein (CHOP) and caspase-12, associated with ERS-induced apoptosis, were determined by western blot and immunohistochemistry methods. TUNEL assay was applied to determine the apoptosis index (AI) in PAECs.

Results: Treatment with NaHS was followed by the exhibition of markedly increased forced expiratory volume over 0.3 s (FEV0.3)/forced vital capacity (FVC) and dynamic lung compliance as well as integral optical density (IOD), with decreased RI among COPD rats. Western blot analysis, immunohistochemistry and TUNEL assay results revealed there to be reduced expressions of GRP78, CHOP and caspase-12 in the lung tissues and AI of PAECs, post NaHS treatment.

Conclusion: The key findings of the current study highlight ERS in COPD rats, as well as well as reduced apoptosis in PAECs in connection with exogenous H_2S by suppressing ERS.

1. Introduction

Chronic obstructive pulmonary disease (COPD) can be defined as a disease state characterized by exposure to noxious agents, consequently resulting in irreversible airflow limitation and shortness of breath [1]. COPD is widely considered to lead to chronic inflammatory injury to the lung, predominately accompanied by intrathoracic airways disease as well as emphysematous destruction [2]. A strong correlation exists between COPD and systemic inflammation accompanied by its comorbidities, such as cardiovascular disease, osteoporosis, diabetes and anxiety/depression [3,4]. Reports have predicted that COPD will eventually become the fourth leading cause of morbidity and mortality worldwide by the year 2030, as well as projections indicating that it may become the third leading cause of chronic disability by the year 2020 [5]. Medial hypertrophy with variable adventitial changes dominates early pulmonary artery hypertension (PAH) and purely hypoxic pulmonary hypertension, with intimal proliferation representing a prominent factor in the progression of COPD [6]. Pulmonary arterial hypertension (PAH) is illustrated by an abnormal growth rate and enhanced glycolysis of the pulmonary artery endothelial cells (PAECs) [7].

The endoplasmic reticulum (ER) represents the cellular central organelle responsible for protein folding and trafficking, calcium storage, lipid synthesis as well as a host of other cellular functions [8]. Pathological irritants can disrupt ER homeostasis, resulting in its subsequent failure to adaptive accordingly, resulting in an accumulation of newly synthesized unfolded proteins [9]. In the event of an unfolded protein eliciting a response, a number of inflammatory and stress signaling pathways are blocked, which act to further induce diseases such as diabetes and neurodegeneration [10]. Studies have indicated that

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protracted and excessive ER stress (ERS) can trigger cellular apoptotic programs [11]. Stefania et al. asserted that HLA-B*35 activate ERS, which induces the dysfunction and death of endothelial cells [12]. Hydrogen sulfide (H_2S), as a toxic gas, is an endogenous signaling gas transmitter [13]. H_2S treatment has been shown to ameliorate heart failure, while application of the agent has also been used in cases of high fat diet-induced cardiac dysfunction, ischemic heart failure and pressure overload-induced heart failure [8]. More recently, numerous studies have indicated that the inhibition of ERS could contribute to the advantage of H_2S [14–16]. Furthermore, H_2S supplement therapy has been demonstrated to block the expression of ERS-associated proteins, such as C/EBP homologous protein (CHOP), as well as antagonizing cellular injury in rat models of hyperhomocysteinemia [17]. Thus, considering the potential functions of H_2S, the present study set out to explore the effect of exogenous H_2S on ERS as well as the apoptosis of PAECs in a rat model of COPD.

2. Materials and methods

2.1. Ethnics statement

All experiment protocols were conducted with the approval of the Animal Ethics Committee of Fujian Medical University as well as strict adherence in accordance with ethical principles.

2.2. Experimental animals

Forty-eight male Sprague-Dawley (SD) rats (12 weeks old, 250 ~ 290 g) in specific pathogen free (SPF) conditions were purchased from Hunan SJA Laboratory Animal Co., Ltd. (Changsha, China) housed, and placed on an adaptive feeding program for one week prior to experiment. The rats were provided with free access to food and water.

2.3. Animal grouping and model establishment

The SD rats were randomly allocated into four groups (12 per group) namely: the control group, the NaHS group, the COPD group and the COPD + NaHS group. The rat models were constructed by means of exposure to passive smoke as well as intra-tracheal instillation of lipopolysaccharide (LPS): (1) The COPD group: On the 1st and 31st day, were anesthetized *via* intraperitoneal injection of 1% pentobarbital sodium, with the skin of the neck disinfected followed by fixation on a board. Then, the neck skin was longitudinally cut open. The tissues and muscles were separated, and the trachea was exposed. After the trachea had been punctured using a number 4 needle, the rats were administered with LPS (200 μg/100 μL). From the 2nd to the 30th, and 32nd to the 60th days during the model establishment process, the rats were placed in a plexiglass box (72 μL, 30 cm × 40 cm × 60 cm) and exposed to smoke (the commercial cigarettes “BAISHA”, 13 mg tar and 13 mg nicotine per cigarette). Smoke was administered on a daily basis using twelve cigarettes each time for half an hour. Half an hour before instillation of LPS or smoke exposure, a dose of 0.5% sodium carboxymethyl cellulose suspension was given to the rats for intragastric administration (sodium carboxymethyl cellulose was used as a suspending agent which helped the drug form into a suspension and was helpful for intragastric administration). (2) The NaHS group: Rats were administered NaHS in accordance with the methods applied to the COPD group. From the 2nd to 30th, as well as the 32nd to the 60th days during the model establishment process, half an hour before instillation of LPS or smoke exposure a dose of 56 μmol/(kg/d) NaHS-0.5% sodium carboxymethyl cellulose suspension was given to the rats for intragastric administration purposes. (3) The control group: Rats were exposed to fresh air in the plexiglass box. On the 1st and 31st day during the model establishment process, the administration of LPS was replaced by saline, and half an hour before saline administration or inhalation of fresh air, a dose of 0.5% sodium carboxymethyl cellulose suspension was administered to the rats every day for intragastric administration. (4) The COPD + NaHS group: in accordance with the methods applied to the control group, half an hour before saline administration or inhaling fresh air, a dose of 56 μmol/(kg/d) NaHS-0.5% sodium carboxymethyl cellulose suspension was administered to the rats every day for intragastric administration. The behaviors of the rats were observed every day and the rats were weighed before and after experiments.

2.4. Pulmonary function test

Evaluations were conducted on the 60th day after modeling. The rats were anesthetized intraperitoneally with 1% pentobarbital sodium at a dose of 3 mL/mg, placed in the supine position on an operating table; with the neck skin cut approximately 2 cm followed by separation of the tissues. The trachea was exposed by means of an inverted T type incision and a Y type tracheal intubation was inserted. A pressure sensor and the flow velocity sensor were connected to the two ends of the tracheal tube, while the ventilator was connected with the flow velocity sensor. Tidal volume was set at 10 mL/kg and the respiratory rate as 60/min, with the MacLab data acquisition system connected. After a period of calm respiration, the rats were injected with air inflating (equal to deep inspiration) through three-limb tubes 5 times tidal volume at the end of exhalation and disconnected immediately, connected with negative pressure (− 25 cm H_2O) and deflated in order to elicit deep expiration synchronously. The ratio of forced expiratory volume at 0.3 s to forced vital capacity (FEV0.3/FVC), dynamic lung compliance (Cdyn) and airway resistance (RI) were calculated and recorded using a microcomputer processing program with volume alterations.

2.5. Hematoxylin and eosin (HE) staining

The lung tissue specimens were fixed with 4% paraformaldehyde for 24 h and embedded in paraffin, followed by the selection of 3 tissue sections from each rat cut into 4 μm serial slices. The slices were deparaffinized in xylene I and xylene II each for 20 min and dehydrated in absolute ethanol, 95% ethanol and 75% ethanol each for 5 min. The slices were then well rinsed under running water and immersed in double distilled water for 10 min. The slices were then stained with hematoxylin for 5 min and rinsed well under running water, followed by differentiation in 0.5% acid alcohol for 5 s until a clear nucleus and chromatin could be observed under a microscope. The slices were then rinsed under running water for 10 min, counterstained with eosin for 5 s, rinsed with running water again and immersed in double distilled water for 10 min. Finally, the slices underwent dehydration with absolute ethanol, 95% ethanol and twice with 75% ethanol each for 5 min. The sections were then sealed with the nail polish after drying, with the histopathological changes of the lung tissues observed under a microscope.

2.6. Pathological observation

After routine HE staining, each cross cut or beveled bronchus in each section observed with a diameter length of less than 0.3 were subsequently removed. The TD 2000 pathological image analysis system was used to measure the inner diameter, which was less than 2 mm. Subsequently, 2 small airways were taken in each section. Four fields of vision (10 * 100) were obtained inside and outside of the epithelial cells in each small airway, with the diameter of the field of vision confirmed to be 140 μm. The number of neutrophils, lymphocytes, monocytes and eosinophils were tallied with the average value calculated. The number of the cells was represented by cell number/mm^2. Based on He Quanying's scoring of 8 pathological indicators, 4 small airways were reviewed in each section, followed by calculation of the total score.