Original Research

Hydrogen-rich saline inhibits tobacco smoke-induced chronic obstructive pulmonary disease by alleviating airway inflammation and mucus hypersecretion in rats

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Impact statement

This study was designed to evaluate protective effect of hydrogen-rich saline, a novel antioxidant, on tobacco smoke (TS)-induced chronic obstructive pulmonary disease (COPD) in rats and explore the underlying mechanism. Our results suggest that administration of hydrogen-rich saline improves lung function and alleviates morphological impairments of lung through alleviating inflammation, reducing oxidative stress and lessening mucus hypersecretion in TS-induced COPD rats.

Abstract

Chronic obstructive pulmonary disease induced by tobacco smoke has been regarded as a great health problem worldwide. The purpose of this study is to evaluate the protective effect of hydrogen-rich saline, a novel antioxidant, on chronic obstructive pulmonary disease and explore the underlying mechanism. Sprague-Dawley rats were made chronic obstructive pulmonary disease models via tobacco smoke exposure for 12 weeks and the rats were treated with 10 ml/kg hydrogen-rich saline intraperitoneally during the last 4 weeks. Lung function testing indicated hydrogen-rich saline decreased lung airway resistance and increased lung compliance and the ratio of forced expiratory volume in 0.1 s/forced vital capacity in chronic obstructive pulmonary disease rats. Histological analysis revealed that hydrogen-rich saline alleviated morphological impairments of lung in tobacco smoke-induced chronic obstructive pulmonary disease rats. ELISA assay showed hydrogen-rich saline lowered the levels of pro-inflammatory cytokines (IL-8 and IL-6) and anti-inflammatory cytokine IL-10 in bronchoalveolar lavage fluid and serum of chronic obstructive pulmonary disease rats. The content of malondialdehyde in lung tissue and serum was also determined and the data indicated hydrogen-rich saline suppressed oxidative stress reaction. The protein expressions of mucin MUC5C and aquaporin 5 involved in mucus hypersecretion were analyzed by Western blot and ELISA and the data revealed that hydrogen-rich saline down-regulated MUC5AC level in bronchoalveolar lavage fluid and lung tissue and up-regulated aquaporin 5 level in lung tissue of chronic obstructive pulmonary disease rats. In conclusion, these results suggest that administration of hydrogen-rich saline exhibits significant protective effect on chronic obstructive pulmonary disease through alleviating inflammation, reducing oxidative stress and lessening mucus hypersecretion in tobacco smoke-induced chronic obstructive pulmonary disease rats.

Keywords: Chronic obstructive pulmonary disease, hydrogen-rich saline, inflammation, mucus hypersecretion

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Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality in western countries and it has increased rapidly in developing countries. Cigarette smoking is the most predominant risk factor of COPD and accounts for more than 95% of cases in developed countries. The global initiative for chronic obstructive lung disease (GOLD) defines COPD as ''a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually associated with an abnormal inflammatory response of the lungs to noxious particles or gases.''[1] The airway inflammation of COPD patients involves the influx of macrophages, neutrophils and T lymphocytes, and the increase of inflammatory cytokines. Oxidative

stress is an important factor in driving this inflammation. The chronic inflammation can result in spasm, hyperplasia, and narrow of bronchial wall. Furthermore, inflammation in lung also leads to overproduction and hypersecretion of mucus, which is mainly composed of mucin 5AC (MUC5AC), and the reduction of mucociliary clearance. Therefore, bronchoconstriction and airflow limitation are aggravated. Recent studies suggest correlation between mucin secretion and aquaporin 5 (AQP5) expression. AQPs is a family of integral membrane proteins which functions as selective water transporters.[2] AQP5 is localized at apical membrane of Type I alveolar epithelial cells, acinar epithelial cells in submucosal glands and large airway epithelia.[3] Deletion of AQP5 leads to more concentrated protein and mucus secretion in upper respiratory tract of mice.[4] As a result, airway clearance, which is critical to COPD patients, will be impaired by viscous and dehydrated glands fluid.[5]

Although the combination treatment of various bronchodilators and corticosteroids has exhibited good effect on the management of COPD, corticosteroids have much side effect, such as osteoporosis and pneumonia.[6] Thus, current studies have been increasingly focusing on searching for safe, natural therapeutic alternatives for COPD.[7] Hydrogen (H_2), as the lightest and most abundant chemical element, reduces selectively cytotoxic oxygen radicals, and may potentially serve as a novel antioxidant in preventive and therapeutic applications.[8] For example, the pretreatment with hydrogen-rich water mitigated aspirin-induced gastric lesions via lessening oxidative stress and inflammatory reactions.[9] Hydrogen-rich saline suppressed the production of several proinflammatory mediators through inhibiting the activation of p38 and NF-κB.[10] Drinking hydrogen-rich water had anti-oxidative effect on aging periodontal tissues.[11] These observations lead us to speculate that hydrogen-rich saline may play a protective role in the pathogenesis of COPD.

Materials and methods

Hydrogen-rich saline production

Hydrogen-rich saline was prepared as described previously.[12] Briefly, hydrogen was dissolved in normal saline for 2 h under high pressure (0.4 MPa) to a supersaturated level using hydrogen-rich water-producing apparatus. The saturated hydrogen-rich saline was freshly prepared in an aluminum bag every week, sterilized by gamma radiation and stored under atmospheric pressure at 4°C to maintain the concentration of hydrogen at higher than 0.6 mM.

Animal and experimental design

Twenty-four male Sprague-Dawley (SD) rats weighting 180–200 g were purchased from Shanghai Laboratory Animal Co. Ltd and housed in rooms maintained at constant temperature (21 ± 2°C) and humidity (55 ± 15%) with a 12-h light/dark cycle. They are allowed to have food and drink water ad libitum. All procedures were done in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and efforts were made to minimize the number of animals and their suffering. The rats were randomly divided into three groups: sham control group (Control), TS group and hydrogen-rich saline treatment group (TS+H) ($n = 8$ per group).

The experimental model of COPD was established as previously described with slight modification.[13,14] Rats in TS and TS+H groups were treated with TS generated from five cigarettes for 30 min, twice daily for 12 weeks. The cigarettes used in this experiment were unfiltered and contained 13 mg of tar oil and 1.2 mg of nicotine per cigarette (DA QIAN MEN from Shanghai Tobacco Group Co., Ltd, China). The rats were placed in 80 cm × 60 cm × 50 cm perspex chambers (four rats/chamber), in which a cigarette burn box lay at the bottom. The cigarette burn box has a slide glass door for controlling air supply and handling cigarette. The smoke concentration in the chamber was controlled by air control hole and cigarette burning speed and kept almost constant during exposure. Rats in control group were exposed to air in the same perspex chambers. From the ninth week, rats in TS+H group received 10 ml/kg hydrogen-rich saline intraperitoneally (i.p.) twice daily 30 min prior to TS exposure for four weeks.[15,16] However, rats in control and TS groups were administrated i.p. with 10 ml/kg normal saline each time.

Measurement of lung function

Lung function testing was performed with buxco pulmonary function analysis system by the modification method of Wright *et al*.[17] The major procedure involves the following: At the end of 12th week, rats were anesthetized with chloral hydrate (0.42 g/kg). Then their tracheas were cannulated and the rats were placed supine in a small animal plethysmograph (Beijing Yuehongda Co.Ltd, China). The lungs were inflated with air at a volume of 5-fold tidal volume and then rapidly deflated to a pressure of −35 cm H_2O. Lung airway resistance (RL), lung compliance (CL) and forced expiratory volume in 0.1 s/forced vital capacity ($FEV_{0.1}$/FVC) were determined.

Sample processing

After measurement of lung function, rats were sacrificed by anesthesia with an overdose of chloral hydrate and blood was collected from the abdominal aorta. The right bronchus was clamped to prevent lavage fluid entering right lung. The left lung was lavaged thrice with 2 mL of normal saline and bronchoalveolar lavage fluid (BALF) was collected and stored at −80°C. The right lung was taken out and flushed with normal saline. The right low lung was immediately fixed for histological analysis and the right upper lung was stored at −80°C.

Histological analysis

The right lower lung excised was fixed in 4% formalin for 48 h and embedded in paraffin. Serial 4 mm sections from blocks were stained with hematoxylin and eosin (H&E). Histopathological assessment was performed blind under a light microscope. Five randomly selected fields from the peripheral and central regions of the lung were

photographed, and the morphometric analysis was performed as described previously.[18] The total alveolar area, A (T), and alveolar tissue thickness, A (t), were determined, and the ratio of A (t)/A(T) was calculated. Neutrophils on sections were also counted.

Measurement of inflammatory factors in BALF and serum

The concentrations of IL-8, IL-6, IL-10, and TNF-α which can be secreted during the process of inflammation were determined by ELISA kits (American R&D) according to the manufacturer's instructions. Triplicate repeated samples were analyzed in a single assay and concentration was expressed as pg/ml.

Measurement of MDA in lung tissue and serum

The level of malondialdehyde (MDA) in lung tissue and serum, a parameter reflecting oxidative stress, was determined by a spectrophotometric measurement of thiobarbituric acid-reactive substances (TBARS) according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China). Lung (100 mg) was homogenized in 2 ml of 10 mM phosphate buffer (pH 7.4). After centrifugation at 12,000*g* for 20 min, the MDA content in the supernatant was measured using the kit. Serum MDA level was also determined with similar procedures.

Measurement of MUC5AC in BALF and lung tissue

MUC5AC protein level in collected BALF and homogenized lung tissue was assayed using a rat MUC5AC ELISA kit (American R&D). Meanwhile, MUC5AC expression in lung tissue was further verified by Western blot analysis.

Western blot analysis for MUC5AC and AQP5 expression

Western blot analysis was performed in accordance with standard protocols. The lung tissue was homogenized with RIPA lysis buffer. The equal amounts of protein were subjected to 8% to 12% SDS-PAGE and then transferred onto polyvinylidene fluoride (PVDF) membrane (Millipore, Boston, MA). After blocked with 1% bovine serum albumin (BSA) in Trisbuffered saline and Tween 20 (TBS-T) at room temperature for 60 min, the membranes were incubated with MUC5AC (Abcam), AQP5 (Abcam) or β-actin (Abcam) antibodies (1:1000 dilution). Immunodetection was accomplished with horseradish peroxidase-conjugated secondary antibodies (1:2000 dilution) and an enhanced chemiluminescence kit (Thermo, Rockford, USA). The IOD value of immunoreactive bands was calculated using Image-Pro Plus software and normalized by housekeeping protein β-actin.

Statistical analysis

Statistical analysis was performed via one-way analysis of variance (ANOVA) followed by Student–Newmann–Keuls multiple comparison tests with the SPSS 13.0 software for Windows. Results are shown as means ± SD for at least three independent experiments.

Results

Hydrogen-rich saline improved lung function in TS-induced COPD rats

RL is the opposition to airflow caused by the forces of friction. In COPD, narrowed airway and increased mucus will increase RL. CL means per unit change of pulmonary pressure caused by lung volume change. In emphysema, due to structural damage and degradation of elastin of alveolar wall, CL will decrease. $FEV_{0.1}$/FVC refers to the ratio of $FEV_{0.1}$/FVC and it will be reduced in obstructive ventilatory disorder. Table 1 shows TS-induced changes of lung function. Compared with control group, RL was promoted by 170%, CL and $FEV_{0.1}$/FVC were lowered by 48% and 17%, respectively in TS group, which indicated a successful COPD model in rats. However, lung function of TS+H group was improved compared with the TS group, with evidence of significant decrease in RL and increase in LC and $FEV_{0.1}$/FVC.

Hydrogen-rich saline alleviated morphological impairment of lung in TS-induced COPD rats

Figure 1 shows representative histological images from lungs of rats. Control group exhibited normal lung structure, whereas TS group demonstrated alveolar wall thickening, extensive leukocyte infiltration around bronchial and bronchiolar airways and around blood vessels, pulmonary alveoli consolidation and localized emphysema and other pathological manifestations. After treatment with hydrogen-rich saline for four weeks, the impairment was lessened.

Hydrogen-rich saline abated lung inflammation and oxidative stress in TS-induced COPD rats

Hydrogen-rich saline decreased the levels of inflammatory cytokines in BALF and serum of TS-induced COPD rats. COPD is currently believed to be an exaggerated inflammatory response to inhaled irritants, in particular, cigarette smoke, which causes progressive airflow limitation. In this process, inflammatory factors play an important role. In this study, the levels of four major inflammatory

Table 1 Comparison of lung function in different groups

	Control group	TS group	TS+H group
RL (cm H_2O/ml/s)	0.44 ± 0.08	1.19 ± 0.22**	0.55 ± 0.08##
CL (ml/cm H_2O)	0.25 ± 0.07	0.13 ± 0.04**	0.21 ± 0.04#
$FEV_{0.1}$/FVC (%)	29.11 ± 3.20	24.23 ± 3.75*	30.70 ± 3.58##

Note: Lung airway resistance (RL), lung compliance (CL), and the ration of forced expiratory volume in 0.1 s/forced vital capacity ($FEV_{0.1}$/FVC) were measured via buxco pulmonary function analysis system at the end of 12 weeks. Data are presented as means ± SD ($n = 8$).
*$P < 0.05$, **$P < 0.01$ vs. control group; #$P < 0.05$, ##$P < 0.01$ vs. TS group.
TS: tobacco smoke; FVC: forced vital capacity; FEV: forced expiratory volume.

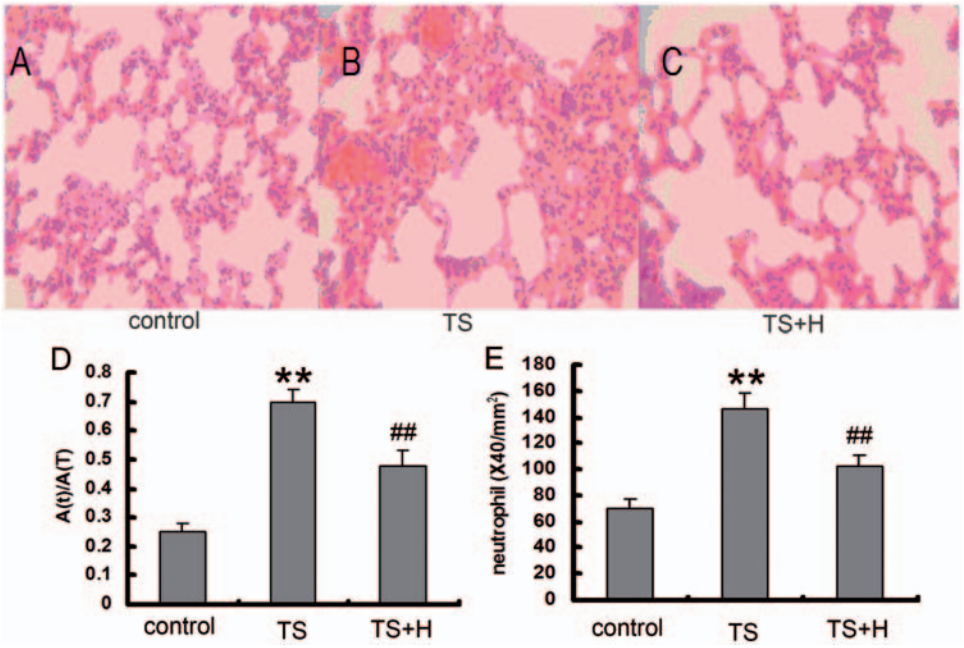

Figure 1 Hydrogen-rich saline alleviated morphological impairment of lung in TS-induced COPD rats. Sprague-Dawley rats were made COPD models via TS exposure for 12 weeks and rats were treated with 10 ml/kg hydrogen-rich saline intraperitoneally twice daily during the last 4 weeks. At the end of 12th week, lung tissues were collected. Histological changes were detected using H&E staining. (a–c) shows the representative H&E staining photos from each group (original magnification × 200); (d) shows the ratio of alveolar tissue thickness/total alveolar area, A (t)/A(T), from each group; (e) shows the number of neutrophils on lung sections from each group. Data are presented as means ± SD ($n = 8$). $^{**}P < 0.01$ versus control group; $^{\#\#}P < 0.01$ versus TS group. (A color version of this figure is available in the online journal.)

factors (IL-8, IL-6, IL-10 and TNF-a) were measured in BALF and serum. Compared with control group, tobacco smoking increased the levels of IL-8 in BALF and serum by 58% and 63%, respectively, increased the levels of IL-6 in BALF and serum by 13% and 52%, respectively, and increased the level of IL-10 in BALF by 12%. Compared to TS group, hydrogen-rich saline decreased the concentrations of IL-8 in BALF and serum by 23% and 18%, respectively, decreased IL-6 in BALF and serum by 13% and 57%, respectively, and lowered IL-10 in BALF and serum by 16% and 19%, respectively (Figure 2). However, there was no significant difference in the content of TNF-α in BALF and serum among the three groups.

Hydrogen-rich saline reduced MDA levels in serum and lung tissue of TS-induced COPD rats. Oxidative stress plays an important part in inflammatory process of COPD. MDA is one of the most reliable and widely used indices of oxidative stress. In our study, we determined the levels of MDA in serum and lung tissue of rats. As shown in Figure 3, compared with that of control group, the content of MDA in serum and lung tissue was remarkably increased in TS group. The treatment of hydrogen-rich saline for four weeks significantly lowered MDA levels by 49% and 19% respectively.

Hydrogen-rich saline lessened mucus hypersecretion in airway of TS-induced COPD rats

Hydrogen-rich saline down-regulated mucin MUC5AC expression in BALF and lung tissue of TS-induced COPD rats. Mucins are complex glycoproteins which provide viscoelastic properties of mucus and MUC5AC is the most prominent mucin in respiratory tract. In this study, we determined the effect of hydrogen-rich saline on TS-induced expression of MUC5AC in BALF and lung tissue of rats by ELISA and Western blot. The results indicated that compared with control group, TS promoted the expression of MUC5AC in both BALF and lung tissue while hydrogen-rich saline attenuated this up-regulation (Figure 4).

Hydrogen-rich saline up-regulated AQP5 expression in lung tissue of TS-induced COPD rats. AQP5 is an apical membrane water channel located in airway and alveolar epithelium to respond to luminal environment and participate in diverse epithelial responses. In the progress of COPD, AQP5 plays a key role. Compared with control group, protein expression of AQP5 was markedly declined in TS-induced rat COPD models, which was in accordance with previous study.[19] The down-regulation was suppressed by the treatment of hydrogen-rich saline (Figure 5).

Discussion

This study was to investigate beneficial effect of hydrogen-rich saline on COPD in rat models and explore the underlying mechanism. In this study, the stable COPD rat models were replicated by TS inhalation and hydrogen-rich saline was administered intraperitoneally. The data indicated hydrogen-rich saline can improve pulmonary function

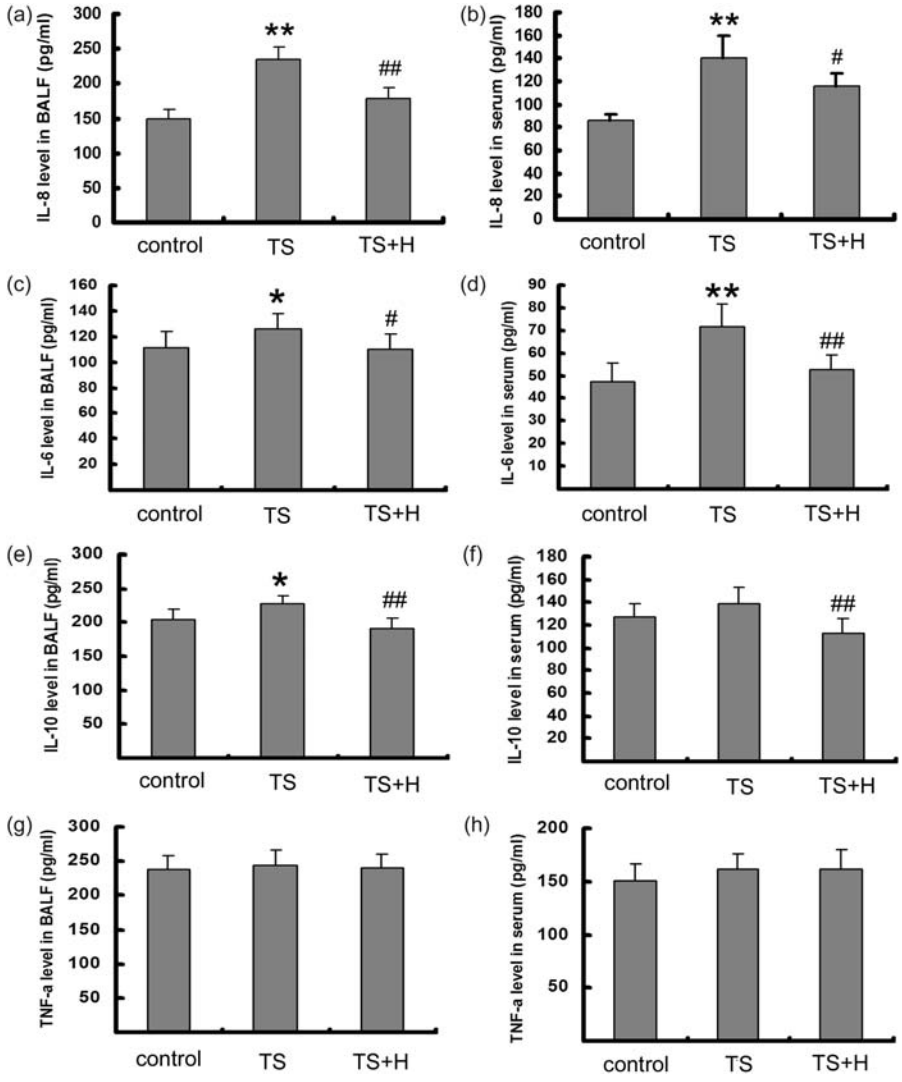

Figure 2 Effect of hydrogen-rich saline on the levels of inflammatory cytokines in BALF and serum of COPD rats after treatment for four weeks. The contents of IL-8 (a and b), IL-6 (c and d), IL-10 (e and f), and TNF-a (g and h) in BALF and serum from each group were measured by ELISA and expressed as pg/ml. Data are presented as means ± SD ($n = 8$). $^{*}P < 0.05$, $^{**}P < 0.01$ versus control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus TS group

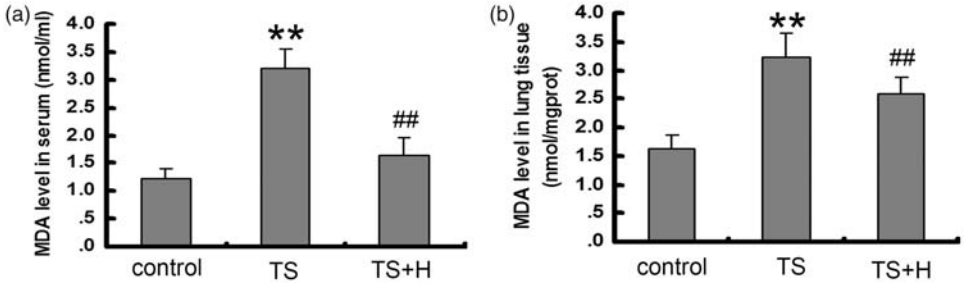

Figure 3 Hydrogen-rich saline reduced MDA level in serum and lung tissue of TS-induced COPD rats. (a) Shows the content of MDA in serum; (b) shows the content of MDA in lung tissue. Data are presented as means ± SD ($n = 8$). $^{**}P < 0.01$ versus control group; $^{\#\#}P < 0.01$ versus TS group

and mitigate lung pathological impairment in COPD rats, the curative mechanism is probably related to alleviating inflammation, reducing oxidative stress and lessening mucus hypersecretion.

COPD is characterized by airflow limitation which is not fully reversible. The increase of RL and reduction of FEV/FVC ratio are important clinical indicators of obstructive lung disease. With continuous cigarette smoking, emphysema develops in COPD patients due to protease/antiproteinase imbalance,[20] secretion of neutrophil elastase,[21] and small airway obstruction,[22] which will result in structural damage of alveolar wall and decrease

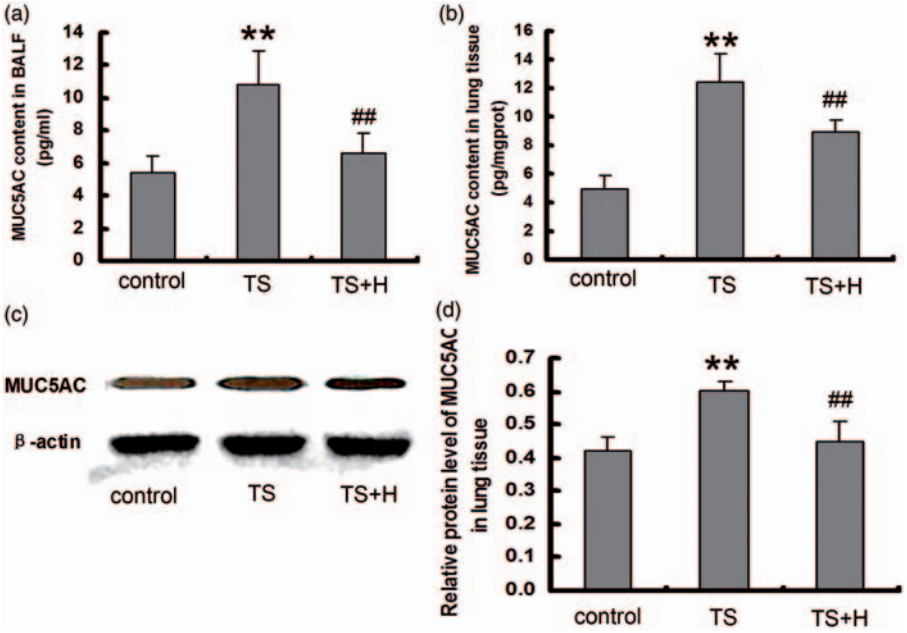

Figure 4 Hydrogen-rich saline down-regulated mucin MUC5AC expression in BALF and lung tissue of TS-induced COPD rats. The protein expression of MUC5AC from each group was analyzed by ELISA and Western blot. (a) Shows MUC5AC content in BALF by ELISA; (b) shows MUC5AC content in lung tissue by ELISA; (c) shows the representative image of MUC5AC expression in lung tissue by Western blot; (d) shows the IOD ratio of MUC5AC to β-actin in lung tissue by Western blot. Data are presented as means ± SD. $^{**}P < 0.01$ versus control group; $^{\#\#}P < 0.01$ versus TS group

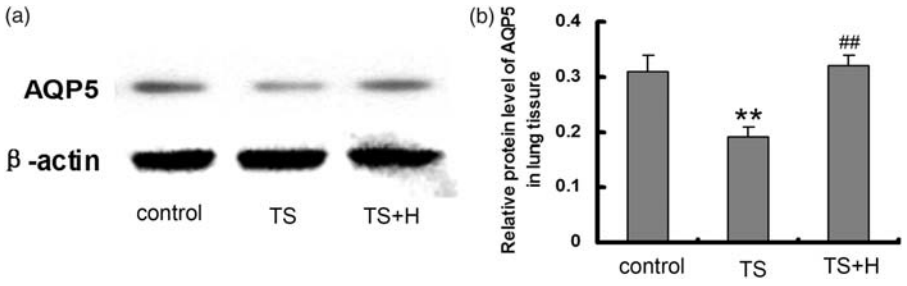

Figure 5 Hydrogen-rich saline up-regulated AQP5 expression in the lung tissue of TS-induced COPD rats. The protein expression of AQP5 was analyzed by Western blot and normalized to β-actin level. (a) Shows the representative image of AQP5 expression in lung tissue by Western blot. (b) Shows the IOD ratio of AQP5 to β-actin in lung tissue. Data are presented as means ± SD of at least three independent experiments. $^{**}P < 0.01$ versus control group; $^{\#\#}P < 0.01$ versus TS group

of CL. In our experiment, RL, CL, and $FEV_{0.1}$/FVC were tested, respectively. Data showed a significant decrease of CL and $FEV_{0.1}$/FVC concomitantly with an obvious increase of RL in TS group compared to control group, which demonstrated lung function declined to certain degree of severity in this model of COPD. Treatment with hydrogen-rich saline partly and significantly reversed lung functional decline, which suggested that the intervention was effective in ameliorating airflow obstruction of small airway.

The lung structural impairment was also observed in this study. Peribronchiolar inflammation with a large amount of inflammatory cells infiltration around small airways, thickening of alveolar wall, and localized emphysema was observed in COPD rats. These pathologic features of lungs indicated that rat model of TS-induced COPD was well developed. Treatment with hydrogen-rich saline alleviated morphological impairment in the challenged lungs, suggesting that this agent may help slow down the progression of the disease.

It has been confirmed that chronic inflammation in airway and lung tissue occurs in all the stages of COPD, in which the release of multiple cytokines plays an important role.[23] IL-8 is a potent attractant for neutrophils and has been demonstrated to be responsible for acute exacerbation and disease progression of COPD. IL-6 is found to accelerate the release of acute-phase proteins and worsen the underlying inflammatory condition.[24] The increased serum IL-6 level has been improved in patients with COPD[25] and IL-6 level in the induced sputum of COPD patients is significantly promoted as well compared with that of asthma patients.[26] TNF-α, one of early regulators in immune response, is responsible for stimulating the release of secondary pro-inflammatory cytokines.[27] IL-10 is an anti-inflammatory cytokine. However, there is controversy on the role of IL-10 in the pathogenesis of COPD. In the study by Gessner *et al.*,[28] smokers and COPD patients had detectable IL-10 in the exhaled breath condensate compared with healthy non-smokers, and steroid treatment

could reduce IL-10 level. In addition, Lim *et al*.[29] also found that airway macrophages from smokers produced more TNF-α and IL-10. Nevertheless, Takanashi *et al*.[30] found the reduction of IL-10 and IL-10 positive cells in the sputum and postulated that the reduced level of IL-10 within airway played a role in the pathogenesis of chronic airway inflammation of COPD.[30] In present study, our findings showed pro-inflammatory cytokines IL-8 and IL-6 and anti-inflammatory cytokine IL-10 in BALF and serum were markedly increased in TS-induced COPD rats compared with control group at the end of 12th week, whereas treatment with hydrogen-rich saline for four weeks significantly decreased the three cytokines. However, TNF-α remained unchanged among three groups. Thus, the role of IL-10 and the change of TNF-α in COPD are required to be further elucidated in future studies.

Oxidative stress is thought to play an important role in pathogenesis of COPD through direct injurious effects or versatile molecular mechanism associated with inflammation.[31] TS can not only produce a large amount of exogenous ROS but also induce endogenous ROS through inflammatory cells and impaired airway epithelial cells. MDA is a natural product of lipid oxidation. MDA testing revealed MDA level in lung tissue and serum of COPD rats was significantly elevated compared with normal rats, which was attenuated by administration of hydrogen-rich saline, a strong antioxidant.

Airway inflammation can result in mucus hypersecretion, which have been proposed to promote bacterial adhesion and inhibit bacterial clearance by impeding cilia function.[32] In addition, mucus hypersecretion might directly increase morbidity and mortality by obstructing airways and impairing gas exchange in COPD.[33] One of the main components of mucus is mucins which are major determinants of mucus viscoelasticity.[34] In all mucins, MUC5AC is produced mainly in airway epithelium by goblet cells.[35,36] It is the only mucin isolated from normal human airway secretions and is therefore proposed to be the key airway secretory mucin.[37] Previous studies indicated that inflammation activated epidermal growth factor receptor and stimulated IL-13 to induce both Clara and ciliated cells to transform to goblet cells, which led to up-regulation of MUC5AC expression and secretion and mucus hypersecretion.[38] In the present study, we also found the promotion of MUC5AC expression in BALF and lung tissue of COPD model rats, which was suppressed by the treatment of hydrogen-rich saline. Therefore, it can be referred that hydrogen-rich saline can weaken mucus hypersecretion via down-regulation of MUC5AC in COPD.

Besides, mucus viscosity is also regulated by the movement of water, bicarbonate, and glutathione. The decrease in any of these factors increases mucus viscosity, which is not favorable to the excretion of mucus. Aquaporins are water channel proteins that allow water to move rapidly through plasma membrane in response to osmotic/hydrostatic pressure gradients.[39] AQP5, a subtype of aquaporin family, is involved in fluid secretion of airway submucosal gland[40] and modulation of mucus viscosity.[41] In previous study, an attenuated expression of AQP5 was detected throughout bronchial tissue from COPD patients compared with healthy controls.[19] Furthermore, Chen *et al*.[42,43] found that AQP5 gene could regulate the expression of MUC5AC gene and proteins. In this study, our data showed AQP5 expression was also decreased accompanied with the increase of MUC5AC in lung tissue of TS-induced COPD rats compared with control group. The treatment of hydrogen-rich saline reversed the decline of AQP5 level in COPD rats, suggesting that hydrogen-rich saline might regulate mucus hypersecretion via AQP5 expression.

In conclusion, hydrogen-rich saline exhibits significant protective effect on lung function and pathological impairment in TS-induced COPD rats. Alleviating inflammation, reducing oxidative stress, and lessening mucus hypersecretion may be the mechanism contributing to inhibiting and delaying the procession of COPD. This study suggests that hydrogen-rich saline may be a valuable adjuvant therapy for COPD.

Authors' contributions: All authors participated in the design and laboratory experiments of the study. Zibing Liu and Wenye Geng analyzed data and wrote the manuscript. All authors approved the final version of the manuscript.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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