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Journal of Thoracic Disease

J Thorac Dis. 2018 Jun; 10(6): 3232–3243.
doi: 10.21037/jtd.2018.05.93

PMCID: PMC6051853
PMID: 30069319

Hydrogen gas inhalation protects against cigarette smoke-induced COPD development in mice

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Abstract

Background

Chronic obstructive pulmonary disease (COPD) is a chronic lung disease with limited treatment options. Hydrogen (H_2) has been shown to be anti-oxidative and anti-inflammatory. This study aimed to evaluate the beneficial effects of H_2 inhalation on COPD development in mice.

Methods

A COPD mouse model was established in male C57BL mice by cigarette smoke (CS) exposure. The H_2 intervention was administered by atomisation inhalation. Lung functions were assessed by using Buxco lung function measurement system. The inflammatory cells were counted and the levels of IL-6 and KC in BALF were assayed with ELISA. The lung tissue was subjected to H&E or PAS or Masson's trichrome stain. Furthermore, 16HBE cells were used to evaluate the effects of H_2 on signaling change caused by hydrogen peroxide (H_2O_2). H_2O_2 was used to treat 16HBE cells with or without H_2 pretreatment. The IL-6 and IL-8 levels in cell culture medium were measured. The levels of phosphorylated ERK1/2 and nucleic NF-κB in lungs and 16HBE cells were determined.

Results

H_2 ameliorated CS-induced lung function decline, emphysema, inflammatory cell infiltration, small-airway remodelling, goblet-cell hyperplasia in tracheal epithelium and activated ERK1/2 and NF-κB in mouse lung. In 16HBE airway cells, H_2O_2 increased IL-6 and IL-8 secretion in conjunction with ERK1/2 and NF-κB activation. These changes were reduced by H_2 treatment.

Conclusions

These findings demonstrated that H_2 inhalation could inhibit CS-induced COPD development in mice, which is associated with reduced ERK1/2 and NF-κB-dependent inflammatory responses.

Keywords: Hydrogen gas, chronic obstructive pulmonary disease (COPD), cigarette smoke, oxidative stress, inflammation

Introduction

Chronic obstructive pulmonary disease (COPD) is the fourth-leading cause of death in the world (1), imposing a substantial economic and social burden (2). Cigarette smoking is the most common risk factor for COPD. A strong relationship has been identified between continuous tobacco use, oxidative stress, and exacerbation of COPD symptoms (3). Cigarette smokers have a higher prevalence of respiratory symptoms, lung function abnormalities, and COPD mortality rate than non-smokers (4). Currently, COPD drug therapy is used to relieve symptoms, improve athletic ability and health status as well as reduce the frequency and severity of exacerbation. However, there is no known pharmaceutical therapy for COPD that is able to reverse lung function decline (5-8). Therefore, it is necessary to develop new and effective medicine to prevent or treat COPD with fewer side-effects.

It has been shown that H_2, a physiologically regulatory gas molecule, exerts anti-inflammatory (9), antioxidant (10), anti-apoptotic (11) and signalling-regulating effects (12). Hydrogen gas is able to react with hydroxyl radicals (·OH) directly, which may contribute to its anti-inflammatory and anti-oxidative lesions. Clinical trials have confirmed that H_2 can be used to treat cerebral ischaemia, uraemia (13), diabetes (14), metabolic syndrome (15), erythema skin disease, pressure ulcers, malignancies, rheumatism, arthritis (16,17), side-effects associated with tumor radiotherapy and chemotherapy (18), mitochondrial muscle disease, and Parkinson's disease (19). Furthermore, inhalation of H_2 or ingestion of H_2-rich water can effectively prevent acute lung injury in mice induced by oxygen poisoning, ventilator injury, ischaemia-reperfusion injury, or sepsis (20,21). H_2-rich saline also attenuates cigarette smoke (CS)-induced airway mucus production in rats (22). Xiao *et al.* reported that H_2 saturated water reduced airway remodelling and airway mucus hypersecretion by reducing NF-κB activation in asthmatic mice (23). H_2 inhalation attenuated lung inflammatory responses in CS-induced COPD in rats. Furthermore, higher concentrations of H_2 showed better outcome compared to lower concentrations of H_2 (24). These findings strongly indicate that H_2 treatment may be beneficial in treating COPD. In the present study, we employed mouse model of COPD with CS exposures, and evaluated the effects of hydrogen gas on the development of COPD. We also examined the effects of hydrogen gas exposure on the activation of ERK1/2 and NF-κB-dependent inflammatory responses induced by hydrogen peroxide (H_2O_2) in human bronchial epithelial cells.

Methods

Reagents

All cell culture reagents were purchased from Gibco (Carlsbad, CA, USA). Masson's trichrome stain kit (HT15), Cell Counting Kit-8 [96992], and H_2O_2 solution (7722-84-1) were obtained from Sigma-Aldrich (St Louis, MO, USA). Periodic acid-Schiff (PAS) kit was purchased from Shanghai Sun Biotechnology Co., Ltd (Shanghai, China). β-actin antibody (sc-47778) was obtained from Santa Cruz Biotechnology (Dallas, TX, USA). NF-κB (ab32536) antibody was obtained from Abcam (Cambridge, UK). Antibodies against ERK1/2 (#4695), phosphorylated ERK1/2 (#8544), and histone H3 (#9728) were obtained from Cell Signaling Technology (Beverly, MA, USA).

Animals and CS exposure

Wild type C57BL/6J male mice (6–8 weeks old) were purchased from the Nanjing BioMedical Research Institute of Nanjing University (Nanjing, China). Animals were housed in the specific pathogen-free facilities and the Animal Care and Use Committee of Guangzhou Medical University approved all experimental protocols. And all methods were performed in accordance with the guidelines and regulations approved by the Animal Care and Use Committee of Guangzhou Medical University. Animals were randomized into the following three groups: control group (CTL, normal air inhalation without CS exposure), CS exposure (CS), CS exposure and H_2 inhalation (CS + H_2). To establish the COPD mouse model, mice were exposed to CS (9 cigarettes/h, 2 h per exposure, twice per day, 6 days per week) in a whole-body exposure chamber for 90 days, as we previously described (25,26). The cigarettes used in this study were the Plum brand, produced by Guangdong Tobacco Industry Co., Ltd. (Guangzhou, China). Each cigarette yields 11 mg tar, 1.0 mg nicotine, and 13 mg carbon monoxide (CO). For H_2 treatment, after exposure to CS for 60 days, mice were treated with H_2. H_2 and oxygen (O_2) were generated by electrolysing deionised water with a H_2 apparatus (Shanghai Asclepius Meditech, Shanghai, China). Fresh mixture of H_2 (66.7%) and O_2 (33.3%) gases was diluted with nitrogen (N_2) separated from air to a mixture containing the H_2 (42%), O_2 (21%) and N_2 (37%) and delivered through a rubber tube at a flow of 3.8 L/min to the CS-exposed mice put in a sealed chamber connecting to the outside air through a hole. The H_2 inhalation was performed for one hour per session, twice per day with interval of 6 to 8 hours. The mice in control group were put in the sealed chamber and given air. The concentrations of H_2, O_2 and CO_2 in the chamber were monitored at the beginning and by the end of each inhalation to ensure the stability of each air component. The animals were then subjected to lung function assessment before dissection for further analysis on day 91.

Lung function measurement

Lung function was measured using a Forced Pulmonary Maneuver System (Buxco Research Systems, Wilmington, NC, USA) following the manufacturer's protocol, as we previously described (25).

Hematocrit measurement

At the end of the chronic CS exposure, hematocrit was measured according to the method as we previously described (25).

Bronchoalveolar lavage fluid (BALF) collection and cell counting

Every mouse lung was lavaged with 0.6 mL of saline three times. Total cells in BALF were collected and counted using a haemocytometer. The cells were then subjected to giemsa staining for differential counting of neutrophils, macrophages, and lymphocytes under a microscope.

Histological staining and morphological analysis

After sacrifice, the left lungs of mice were fixed in 10% neutral buffered formalin for 24 h and then dehydrated and embedded in paraffin. The samples were cut into 4-μm sections and stained with H&E for histological examination. Lung sections were stained with PAS to determine goblet cell density or Masson's trichrome stain for collagen deposition with commercial kits following the manufacturers' instructions. Alveolar enlargement and destruction were determined by the average linear intercept as previously described (27). Image-Pro Plus version 6.0 software (Media Cybernetics, Rockville, MD, USA) was used to assess the mean linear intercept, reflecting the ratio of total length of alveoli to the number of alveoli per field under light microscopy. Similarly, to quantify collagen deposition in the small airway according to a previously described protocol (28), we used images of all airways with a diameter of 50–499 μm in lungs, including the blue layer outside the airway, which is the layer of deposited collagen, because larger airways are not associated with increased collagen deposition in CS-exposed mice. The area of the collagen layer deposited around small airways was calculated as follows: collagen thickness = collagen area/total bronchial area. Semi-quantitative analyses of the area stained positive area for goblet cells in the airway epithelium were defined using Image-Pro Plus. At least three fields per mouse were used.

Cell culture and treatment

16HBE were purchased from the Cell Bank of the Chinese Academy of Science (Shanghai, China) and cultured in DMEM medium containing 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified incubator at 37 °C with 95% (v/v) air and 5% (v/v) CO_2. For treatment with H_2O_2 plus H_2, 16HBE cells were seeded in 6-well plates at 60~70% confluence, then, the cells were serum starved for 12 h, and exposed to H_2 (42%) for 1 h prior to 24 h of treatment with H_2O_2 (0.1 mM) in 2 mL medium containing 1% FBS. After all these treatments, cells were washed twice with PBS, cell lysates were harvested for Western blot analysis. The supernatant was stored at −80 °C for detecting the pro-inflammatory cytokines.

Cell viability assay

The cell viability of 16HBE cells upon H_2O_2 treatment was determined by using a CCK8 assay kit (Sigma, USA). The cells (5,000 cells/well) were seeded in 96-well plates, which were treated with H_2O_2 (0–0.8 mM in medium containing 1% FBS) for 24 h. Subsequently, 10 μL of CCK8 solution was added to each well for an additional 2 h. Finally, we recorded the absorbance at 450 nm using a microplate absorbance reader (Thermo Scientific, MA, USA). Cell viability was presented as percentage of cells without H_2O_2 treatment.

Preparation of nuclear protein extracts

Nuclear proteins from lung tissues or 16HBE cells were extracted using the NE-PER Nuclear and Cytoplasmic Reagents (Thermo Fisher Scientific) containing a protease inhibitor cocktail according to the manufacturer's instructions. Histone H3 served as the loading control in Western blotting of nuclear proteins.

Western blotting

Mouse lung tissues and cells were homogenized in RIPA lysis buffer containing 1% protease inhibitor cocktail (Sigma-Aldrich), 5 μM EDTA, and 200 μM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride to extract the total protein. Equal amounts of protein in homogenate samples were separated in SDS-PAGE (Bio-Rad Laboratories, Hercules, CA, USA) and blotted with primary antibodies and corresponding peroxidase-conjugated secondary antibodies. The bound antibody signal was developed using an Immun-Star HRP chemiluminescent kit (Bio-Rad Laboratories). Western blot image was obtained by Tanon 5200 Chemiluminescence Imaging System (Shanghai Tanon Science & Technology, Shanghai, China). Semi-quantitative analyses of immunoblots were performed using the Image J.

Enzyme-linked immunosorbent assay (ELISA)

Levels of interleukin (IL)-6,keratinocyte chemoattractant (KC), tumour necrosis factor-α (TNF-α), IL-8, Muc5ac and Muc5b in BALF or in cell culture medium were measured by ELISA. Briefly, human or mouse IL-6 (88-7066/88-7064), mouse TNF-α (88-7324), and human IL-8 (88-8086) were measured using the ELISA kits from eBioscience (San Diego, CA, USA) following the manufacturer's protocols. KC in BALF was detected with ELISA using capture and detection antibodies obtained from R&D Systems (Minneapolis, MN, USA) according to a protocol described previously (29). Muc5ac and Muc5b in BALF was also detected as described previously (30). The capture antibodies for Muc5ac (sc-21701) and Muc5b (sc-135508) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA), and the corresponding detection antibody was obtained from KPL, Inc. (Gaithersburg, MD, USA). The signal was developed using a TMB Substrate Reagent Set (BD Biosciences, Franklin Lakes, NJ, USA).

Statistical analysis

Data were processed with Graphpad Prism 5 software and presented as mean ± SEM. In the animal experiment, “n” represents animal number, while in the cell experiment, “n” represents the number of experiments repeated. The significance of difference among groups was evaluated by One-way ANOVA and Bonferroni-Holms test. $P<0.05$ was considered statistically significant.

Results

H_2 inhalation attenuates CS-induced lung function decline in mice

COPD mice were established by CS exposure for 90 days (*Figure 1A*). Compared with control mice with normal air inhalation, the CS exposed mice presented typical COPD-like lung function decline (*Figure 1B,C,D,E,F,G*) indicated by increases in FRC, total lung capacity (TLC), Chord compliance (Cchord), FVC, and resistance (RI), as well as a decrease in the FEV50/FVC ratio. Although difference of FVC and RI between CS group and CS+H_2 group is not significant, CS-caused increases in FRC, TLC, Cchord and decrease in the FEV50/FVC were attenuated by H_2 inhalation. As shown in *Figure 1H*, CS exposures significantly increased hematocrit value in blood, which was ameliorated by H_2 administration. Altogether, these results demonstrate that H_2 inhalation ameliorates CS-induced mouse lung function decline and hypoxia-induced hematocrit elevation.

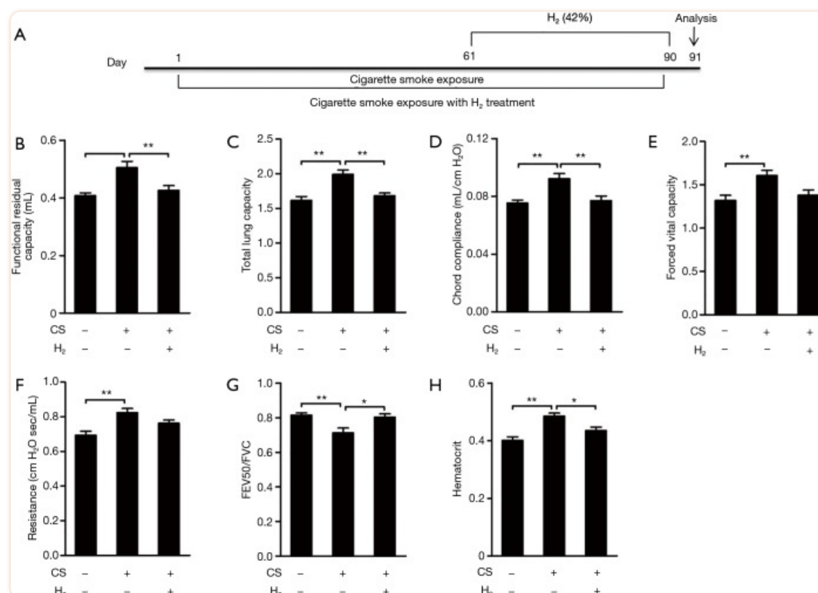

Figure 1

H_2 inhalation inhibited CS-induced lung function decline in mice. (A) C57BL/6J mice were exposed to CS for 90 days. Mice were then treated with vehicle or 42% H_2, and subjected to lung function assessment; (B,C,D,E,F,G) Lung function parameters, functional residual capacity (FRC), total lung capacity (TLC), chord compliance (Cchord), forced vital capacity (FVC), resistance index (RI), and the FEV50/FVC ratio were measured; (H) Hematocrit an indicator of chronic hypoxia, was measured. Data presented as mean ± SEM, n=10 in CTL group, n=8 in CS group, n=8 in CS + H_2 group. *, $P<0.05$; **, $P<0.01$.

H_2 inhalation attenuates CS-induced emphysema, collagen deposition in the small airway and goblet cell hypertrophy and hyperplasia of airway epithelium

Figure 2A showed a typical pathological presentation of COPD, such as damaged alveolar walls and pulmonary bullae, in mouse lungs exposed to CS. Inhalation of H_2 significantly reduced structural damage of the lung and accumulation of leukocytes in both the alveolar walls and spaces. Goblet cells from the airway epithelium of CS-exposed mice, identified by PAS staining, contained large granular stores of PAS-positive substances, which was attenuated in the H_2 treatment group (Figure 2B). Blue staining displayed severe collagen deposition in the small airway (50–499 μm diameter) in CS-exposed mice, and these effects were reduced by H_2inhalation (Figure 2C). These results suggest that H_2 inhalation ameliorates CS-induced COPD pathological changes in mouse lung.

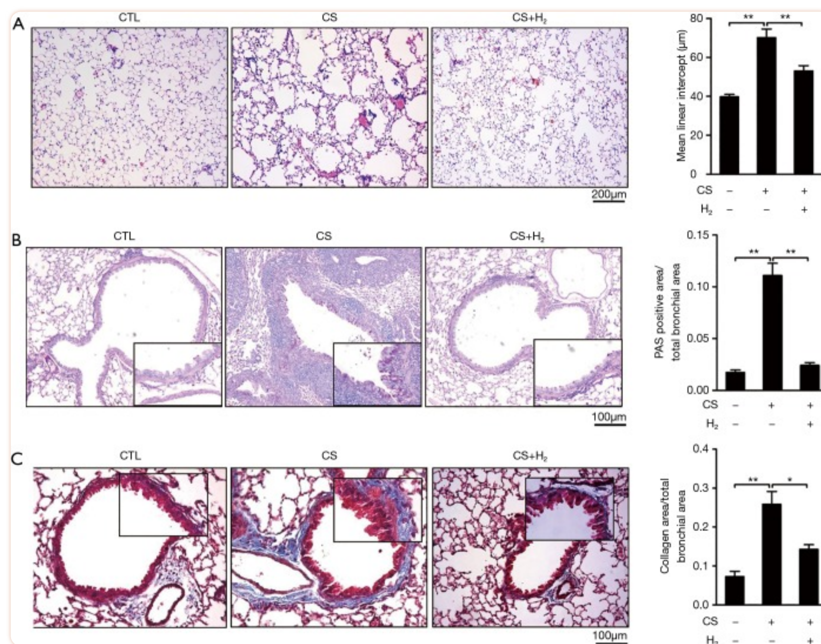

Figure 2

H_2 inhalation attenuated CS-induced emphysema, collagen deposition in the small airway and goblet cell hypertrophy and hyperplasia of airway epithelium. Comparison of H&E or Masson or PAS staining of mouse lung sections from control (CTL), CS and CS plus H_2 (CS + H_2) treated mice. Software IPP6.0 was used to assess the average linear intercept (Lm) of alveoli (A), goblet cell hyperplasia (B) and small airway remodelling (C) in at least three fields of lung section per mouse. Data presented as mean ± SEM, n=5 in each group. *, $P<0.05$; **, $P<0.01$.

H_2 inhalation reduces CS-induced airway inflammation and mucus hypersecretion in COPD mice

Figure 3A,B,C,D showed that CS exposure resulted in airway and lung inflammation, indicated by increases in total leukocyte number (i.e., neutrophils, macrophages, and lymphocytes) and higher levels of IL-6, TNF-α, and KC in BALF. H_2 treatment attenuated these effects. Levels of Muc5ac and Muc5b were increased significantly in BALF from the CS-exposed group compared to control

mice, which was reduced significantly by H_2 treatment (*Figure 3E,F*). These results demonstrate that H_2 inhalation attenuates CS-induced airway inflammation and mucus hypersecretion in COPD mice.

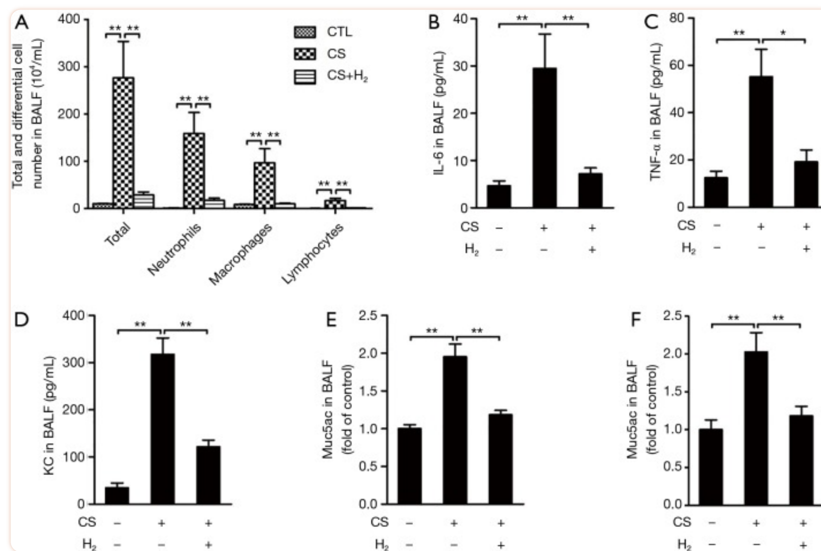

Figure 3

H_2 inhalation reduced CS-induced airway inflammation and mucus hypersecretion in COPD mice. Comparison of total cell count (associated with neutrophils, macrophages, and lymphocytes) (A) and levels of IL-6 (B), TNF-α (C), and KC (D) in BALF from control (CTL), CS and CS plus H_2 (CS + H_2) treated mice. Comparison of level of Muc5ac (E) and Muc5b (F) in BALF from control (CTL), CS and CS plus H_2 (CS + H_2) treated mice. Data presented as mean ± SEM, n=10 in CTL group, n=8 in CS group, n=8 in CS + H_2 group, *, P<0.05; **, P<0.01.

H_2 decreases H_2O_2-induced pro-inflammatory cytokine release in human bronchial epithelial cells

Under the exposure of 42% H_2, concentration of H_2 in cell culture medium gradually increased with the exposure time, and H_2 concentration in cell culture medium was saturated at 60 min (445±36 ppb) (*Figure 4A*). H_2O_2, an important factor inducing oxidative stress, is elevated in expired breath condensate of COPD patients compared with control subjects (31), we therefore we investigated the anti-inflammatory and antioxidant abilities of H_2 against H_2O_2-induced oxidative injury in human bronchial epithelial cells (16HBE). After incubating the cells with H_2O_2 for 24 h at concentrations of 0.1, 0.2, 0.4, and 0.8 mM, we observed a concentration-dependent decrease in cell viability (*Figure 4B*). While high concentrations of H_2O_2 (0.2–0.8 mM) decreased cell viability, lower concentration of H_2O_2 (0.1 mM) did not reduce cell viability, but enhanced the release of IL-6 and IL-8 to culture supernatant (*Figure 4C,D*). However, lower levels of IL-6 and IL-8 were observed in the supernatant from 16HBE cells pre-treated with H_2, compared with levels in the H_2O_2 group. These findings suggest that H_2 treatment prevented H_2O_2-induced pro-inflammatory cytokine release from 16HBE cells.

Figure 4

H_2 decreased H_2O_2-induced pro-inflammatory cytokine release in 16HBE cells. 16HBE cells were treated with H_2O_2 (0.1 mM) with or without H_2 (42%) exposure for 2 h, (A) the H_2 concentration in the cell culture medium was measured. 16HBE cells were treated with H_2O_2 (0.1 mM) with or without H_2 (42%) exposure for 24 h; (B) cell viability was measured using the CCK8 assay; (C,D) levels of IL-6 and IL-8 in cell culture medium were measured. Data presented as mean ± SEM, nC=5 in each group, **, P<0.01.

H_2 inhalation reduces activation of ERK1/2 and NF-kB in CS-exposed mouse lungs and H_2O_2-treated 16HBE cells

Oxidative stress and inflammation play a key role in the pathogenesis of COPD. MAPKs and NF-κB signal pathways play important roles in regulating CS-induced the secretion of pro-inflammatory mediators (32-34), To further elucidate how H_2 modulates CS-induced inflammation, we measured the action of ERK1/2 and NF-κB in lung tissue. As shown in *Figure 5A*, the phosphorylation of ERK1/2 and intranuclear accumulation of NF-κB were remarkably increased in lung tissue from the CS group. H_2 treatment significantly reduced these levels. In 16HBE cells, we observed that ERK1/2 phosphorylation and nuclear accumulation of NF-κB were significantly increased when treated with H_2O_2, while pretreatment with H_2 attenuated these molecular changes (*Figure 5B*). Altogether, these results suggest that H_2 protects against CS and oxidative stress-induced activation of NF-κB and MAPK signal pathways.

Figure 5

H_2 inhalation attenuated activation of ERK1/2 and NF-κB in CS-exposed mouse lungs and H_2O_2 treated 16HBE cells. (A) Levels of phosphorylated ERK1/2 in cytosolic protein fractions and NF-κB/p65 in nuclear protein fractions from lung tissue of mice. Data presented as mean ± SEM, n=4 in each group, **, P<0.01; (B) 16HBE cells were treated with hydrogen peroxide (0.1 mM) for 1 h with or without a 1 h H_2 pretreatment. Levels of phosphorylated ERK1/2 in cytosolic protein fractions and NF-κB/p65 in nuclear protein fractions from 16HBE cells were determined. Data presented as mean ± SEM, n=3 in each group, **, P<0.01.

Discussion

COPD is a leading cause of morbidity and mortality worldwide (1,35). The major risk factor for COPD is tobacco smoke, including exposure to second-hand smoke (36). In the present study, we showed that inhalation of high-concentration hydrogen gas ameliorated lung function decline and

COPD histopathological changes caused by CS exposure, including pulmonary emphysema, chronic bronchitis, and small airway remodelling. These histopathological changes lead to gas trapping and progressive airflow limitation. The protective role of H_2 in CS-exposed mouse lungs is attributable at least in part to its anti-inflammatory and anti-oxidant activities. CS-induced phosphorylation of ERK1/2 and nuclear accumulation of NF-κB in lungs were abolished by H_2 treatment. Furthermore, H_2 protection against H_2O_2-induced ERK1/2 and NF-κB-dependent inflammatory responses in 16HBE cells. Based on our findings, H_2 is a potential drug for COPD prevention, which could be conveniently delivered by inhalation.

In this study, the mouse model was established by CS exposure, a method that mimics the development of human COPD. Inflammation induced by CS is a critical factor in disease development. CS inhalation elicits acute and chronic inflammatory responses, which can potentially cause alveolar destruction. Once lung tissue damage exists and patients has difficulty in breathing. Smoking cessation may not significantly prevent further lung damage (37). The mechanisms for this amplified inflammation are not yet fully understood. Oxidative stress in the lung is likely to further trigger inflammation in COPD (37-39). Even with the present anti-inflammatory medications, we find it difficult to control airway inflammation Unlike the long-acting bronchodilators, it is not easy to find safe and effective anti-inflammatory treatment of COPD (40). So far, there is no treatments to effectively inhibit chronic inflammation in COPD.

Recent several studies have indicated that H_2 is a new type of medical treatment gas with anti-oxidant and anti-inflammatory properties by alleviating the secretion of inflammatory cytokines, such as IL-6 and TNF-a and some phosphorylating signal factors (41,42). Adding H_2 to haemodialysis solutions ameliorated inflammatory reactions by reducing the levels of MCP-1 and myeloperoxidase in plasma of haemodialysis patients (13), which brings the potential therapeutic effect to prevent lung diseases.

Our findings demonstrated that inhalation of 42% H_2 ameliorated COPD histopathological changes and improved lung function in CS-induced mouse COPD model. COPD is an inflammatory disease of respiratory system, and oxidative stress plays an important role in the process (39). The beneficial effects of inhaled H_2 are undoubtedly due, in part, to its antioxidant property (9). However, information on H_2 regulation *in vivo* is limited, and the signalling molecules involved require further investigation. Several studies have shown that NF-κB plays a pivotal role in COPD inflammation (37). Moreover, ERK1/2 has been reported to be involved in CS-induced inflammation by modulating NF-κB DNA binding activity in A549 cells (43). In the present study, we found that 16HBE cells pretreated with H_2 before exposure to H_2O_2 released lower levels of IL-6 and IL-8 and displayed reduced activation ofERK1/2 and NF-κB, suggesting that H_2 attenuated H_2O_2-induced inflammation. In vivo, CS-induced ERK1/2 phosphorylation and NF-κB nuclear translocation were abolished by H_2 treatment in mouse lung. Therefore, H_2 inhalation attenuates CS-induced oxidative stress, leading to reduced inflammatory responses and subsequent emphysema.

Pulmonary function analysis is an important tool in the evaluation of mouse respiratory disease models. However, most commonly used pulmonary function variables of human are not routinely applied in mice. An invasive pulmonary function device (forced maneuvers system from Buxco Research Systems) was used to evaluate the mouse model of CS-induced COPD. CS exposure for 3

months caused airspace enlargement in mice. Similarly, statistically significant increases in FRC, Cchord, FVC, TLC and RI, as well as a decrease in the FEV50/FVC ratio were obtained in CS-exposed mice, which consisted with our previous studies (25,26). Furthermore, the CS-induced lung function decline in mice was attenuated by H_2 inhalation. From a clinical perspective, FVC is usually reduced in actual cases of CS-induced COPD. So it was remarkable that FVC was increased in CS-exposed mice. The mechanism for this is as follows. First, human lung function is measured during active respiration, however, mouse lung function was measured during forced passive respiration. Second, a statistically significant increase in TLC was obtained in CS-exposed mice when compared with the control group, which is similar in humans. Third, due to the larger lung volume upon maximal inflation in CS-induced mice, forced expiration maneuver resulted in significant increase of FVC, whereas FEV50/FVC (parameter for airflow obstruction) during expiration was significantly reduced.

Mucus hypersecretion is one of the most important factors in predicting the morbidity and mortality in COPD (44). In the present study, we demonstrated that H_2 inhalation significantly ameliorated CS-induced airway mucus production, which were identified by decreased hyperplasia of airway goblet cells and reduced levels of Muc5ac and Muc5b in BALF. This protective effect of H_2 on CS-induced mucus hypersecretion was, at least partially, due to its antioxidant ability (22).

Compared with other anti-inflammatory drugs for treating COPD, H_2 offers several advantages. First, it is nontoxic and reacts with the hydroxyl radical (·OH) to produce water in the body. Second, H_2 can easily penetrate membranes and diffuse into the cytosol and nucleus, making it highly effective in reducing cytotoxic substances (45). Third, there is no influence of H_2 on the physiologic properties (24,46). Fourth, H_2 treatment is simple and inexpensive. Altogether, inhaled H_2 is a promising, easily delivered and straightforward therapeutic option for COPD.

In conclusion, high-concentration H_2 inhalation abrogated CS-induced pulmonary emphysema, chronic bronchitis, and small airway remodelling in mice, as well as reduced lung function decline. This protective action is attributable to the anti-inflammatory and anti-oxidant actions of H_2. The inhibition of the ERK1/2 and NF-κB signalling pathways may be implicated in H_2's anti-inflammatory effects. This study provides a foundation for further investigation of the protective effects of H_2 on COPD. Although we have demonstrated the beneficial effects of H_2 on COPD in a mouse model, its efficacy in humans should be assessed in clinical trials.

Acknowledgements

Funding: This work was supported by grants from the National Natural Science Foundation of China (81670041, 81520108001, 81220108001, 81170052), the 973 Key Scheme of China (2015CB553406), the National Key R&D Project (2016YFC0903700), Guangdong Province Universities and Colleges Pearl River Scholar Funded Scheme (2014, W Lu), Guangdong Province Universities and Colleges Key Grant for Innovative Research (cxzd1142), Guangzhou Municipal Research Project (201607020030), Guangzhou Department of Education Innovative Team (13C08), and Guangzhou Department of Education Yangcheng Scholarship (12A001S).

Notes

Ethical Statement: Animals were housed in the specific pathogen-free facilities and the Animal Care and Use Committee of Guangzhou Medical University approved all experimental protocols. And all methods were performed in accordance with the guidelines and regulations approved by the Animal Care and Use Committee of Guangzhou Medical University.

Footnotes

Conflicts of Interest: The authors have no conflicts of interest to declare.

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