Life Sciences 232 (2019) 116650

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Inhalation of sodium hydrosulfide (NaHS) alleviates NO_2-induced pulmonary function and hematological impairment in rats

Zili Zhang[a,1], Jian Wang[a,c,1], Guihua Xu[b,1], Mingjing Ding[a,b], Fei Liu[a], Liang Yuan[a], Tao Wang[a], Jingyi Xu[a], Xiaohui Xie[a], Bingxian Deng[a], Dejun Sun[b,*], Wenju Lu[a,d,**]

[a] State Key Laboratory of Respiratory Disease, Guangzhou Institute of Respiratory Health, The First Affiliated Hospital, Guangzhou Medical University, Guangzhou, Guangdong 510180, PR China
[b] Department of Pulmonary and Critical Care Medicine, Inner Mongolia Autonomous Region People's Hospital, Hohhot 010017, Inner Mongolia Autonomous Region, PR China
[c] Division of Translational and Regenerative Medicine, Department of Medicine, The University of Arizona, Tucson, AZ 85721-0202, USA
[d] Department of Laboratory Medicine, The First Affiliated Hospital, Guangzhou Medical University, Guangzhou, Guangdong 510180, PR China

ARTICLE INFO

Keywords:
NO_2
Emphysema
Pulmonary function
NaHS

ABSTRACT

Background: Inhalation of NO_2 leads to a progressive airflow limitation and the development of emphysema-like lesions. We report on the efficacy of hydrogen sulfide (NaHS) for alleviating NO_2-induced pulmonary impairment.

Methods: Sprague Dawley rats were exposed to 20 ppm NO_2 for 6 h over six consecutive days for 75 days. At day 75, rats who had developed NO_2-induced emphysema were then divided into sodium hydrosulfide (NaHS) administrated group, placebo (NaCl) group and spontaneous recovery group for about one month (days 76–105); Pulmonary function (PF) and hematological and biochemical indices were measured at days 14, 45, 75, and 105.

Results: NO_2 exposure for 75 days was associated with a significant decrease in FEV_{100}/FVC%, an increased in functional residual capacity (FRC), and histologic evidence of emphysema, moreover; NO_2 exposure led to elevated triglyceride (TG), red blood cell (RBC), hemoglobin (HGB), and hematocrit (HCT) levels. Impaired rats treated with NaHS showed no further deterioration in PF compared to rats exposed to ambient air and elevated WBC, granulocyte and lymphocyte counts and HDL-C levels to rats given NaCl.

Conclusions: NO_2 exposure causes emphysema and a decline in PF in rats. NaHS could alleviate the PF decline as possible indicated by an elevation of HDL-C levels and leukocyte. NaHS has therapeutic potential for emphysema caused by air pollutant NO_2.

1. Introduction

Nitrogen dioxide (NO_2) is a common urban pollutant and a major public health concern worldwide. It is a pivotal marker in atmospheric quality monitoring, along with an underlying hazard in adverse effects exploration. Evidences reported that NO_2 was better to characterize the spatial variation of traffic-sourced air pollution than particulate matter with a diameter of 10 μm or less (PM_{10}) or fine particulate matter (particulate matter with an aerodynamic diameter less than or equal to 2.5 μm) ($PM_{2.5}$). Formed by the oxidation of nitric oxide (NO), NO2 exposure can impair Pulmonary Function (PF) and cause Premature Respiratory Death [1]. Moreover, studies have shown that NO_2 is associated with elevated respiratory symptoms and decreased PF in humans [2–4] and emphysema development in animal models [5–8]. Our earlier work also demonstrated the adverse effects of NO_2 on human health, especially among patients with chronic obstructive pulmonary disease (COPD) [9,10]. Notably, a reduction of NO_2 during the 2010 Asian games led to beneficial public health effects [10]. These indicate NO_2 is harmful to human health and may result in emphysema; however, further studies on medical treatments were badly limited.

Gaseous hydrogen sulfide (H_2S) has key roles in numerous physiological and pathological processes. Notably, H_2S can promote vasodilation and reduce blood pressure [11,12], while both endogenous and exogenous H_2S can activate endothelial angiogenesis [13]. As H_2S donors, sodium hydrosulfide (NaHS) protects cells from oxidative injuries caused by glutamate toxicity [14] and is anti-inflammatory [15]. While

* Correspondence to: D. Sun, 20 Zhaowuda Road, Hohhot 010017, Inner Mongolia Autonomous Region, PR China.
** Correspondence to: W. Lu, 151 Yanjiang Road, Guangzhou, Guangdong 510120, PR China.
E-mail addresses: nmg_sdj@163.com (D. Sun), wlu92@yahoo.com (W. Lu).
[1] Equal contribution authors.

https://doi.org/10.1016/j.lfs.2019.116650
Received 18 April 2019; Received in revised form 29 June 2019; Accepted 10 July 2019
Available online 11 July 2019
0024-3205/

studies have demonstrated that H_2S is involved in respiratory diseases, the underlying mechanisms still remain unclear. Nonetheless, studies have shown that NaHS and H_2S can protect against ventilator-induced acute lung injuries [16,17] and reduce airway inflammation and remodeling in a rat model of asthma [18]. Moreover, serum H_2S levels are reportedly lower in patients with acute exacerbation of COPD (AECOPD) than those with stable COPD [19].

In addition, it has ever reported that indoor levels of NO_2 could reach up to 4 ppm in power plants, refineries, and ice-skating rinks [20]; evidence indicates that mice exposed to NO_2 for 14 h each day for up to 25 days at a dose of 20 ppm NO_2 have increased mucus production and may develop airspace enlargement and progressive airway obstruction [8]; however, another sub-chronic, moderately high level of NO_2 exposure demonstrated that it could not produce an irreversible emphysematous lesion in the rat model (35 ppm for 25) [7]. Therefore, experiments currently designed in which rats were chronically exposed to 20 ppm NO_2 of practical importance for further study. We first established rat model of emphysema via from chronic NO_2 inhalation. Then, the results of biological effects on PF, hematological and biomedical indices were accordingly presented at different given times. Further, impaired rats were administrated with NaHS. We herein aimed to determine if NaHS can alleviate NO_2-induced pulmonary and hematological impairment in rats. Therefore, in this study, we explored the possibility that NaHS might be an economical and practical choice for emphysema treatment caused by air pollutant NO_2.

2. Methods

2.1. Study design

Rats were exposed to 20 ppm NO_2 or ambient air for 14, 45, or 75 days and thereafter treated daily with 80 mg/kg NaHS aerosol (161527-5, SIGMA, USA) or placebo (NaCl) during days 76–105. NaHS was dissolved in 0.85% NaCl. During treatment, rats receiving NaHS and NaCl were exposed to ambient air.

For each experiment time (days 14, 45, and 75 of NO_2 exposure, and one-month recovery period (days 76–105)), rats were anesthetized with pentobarbital (50 mg/kg body weight) and PF was measured. Immediately upon the onset of complete apnea, blood was drawn via cardiac puncture into vacuum containers containing EDTA as described previously [21]. Peripheral blood was used to measure hematological and biochemical indices. Lungs, heart, liver, spleen, and kidneys were excised and weighed. The remaining whole left lung was used for histology.

2.2. NO_2 exposure and animals

The NO_2 exposure system has been described and validated previously [22,23]. Dimensions of the custom-built Plexiglas chambers were 120 cm by 70 cm by 80 cm (Fig. 1). NO_2 was generated by mixing the gas phase of liquid NO_2 with nitrogen (N_2) in a 40 L Teflon gas cylinder (Special gas-Zhao Qing Gao Neng Da Chemical Industry Co., LTD, Guangdong, China). The mixing ratio of NO_2: N_2 depended on the desired exposure concentrations. For exposure, NO_2 supplied from gas cylinders was piped through Teflon tubing directly into the intake duct of the chamber. The NO_2 flow was controlled by a Panel (Piping) Type Flowmeter (Shanghai, China). However, once the mixture passed into the chamber by BIOLAK-Friox (8 in Fig. 1) after through Flow-ratio/Mass-flow controllers and Check valve, the mixture immediately mixed with air (through holes up in the chamber) to generate a quite homogeneous distribution of NO_2 + N_2 in the box because of the slight negative pressure equipment. Chamber concentrations of NO_2, O_2%, temperature, CO_x, pressure were measured continuously with a sensitive NO_x analyzer (Testo 340, Germany) and kept at a ± 5% tolerance limit. Once beyond the limit, the NO_x analyzer would raise the alarm.

N was 4 for 14 and 45 days of exposure except 75 and 76–105 days where n was or > 5. Experiments were done on Sprague Dawley male rats with an initial body weight of 180–200 g (Southern Medical University, Guangzhou, China). Subjects were held at the Central Animal Facility of Guangzhou Medical University. Rats were exposed to NO_2 continuously from 8 a.m. until 2 p.m., 6 consecutive days per week for 14, 45, or 75 days. Control rats whom were exposed to ambient air were housed in identical conditions and housing (equal or < 4 rats per cage). During the exposure, rats could freely move and had unlimited access to water and food. During rest periods, rats breathed for ambient air. The experiments protocol was approved by the institutional review board at the Guangzhou Medical University. Experiments were conducted in accordance with institutional guidelines on the Care and Use of Laboratory Animals (National Institutes of Health (China)).

2.3. Hematological indices

Peripheral venous blood samples were drawn after each ending point and measurements of hemoglobin (HGB), hematocrit (HCT), red blood cell (RBC) count, and total & differential leukocyte counts were made using a Mindray BC-2800 animal exclusive automated hematological analyzer. Leftover samples were centrifuged and aliquots of serum were kept at −80 °C until biochemical measurements of blood high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglyceride (TG) levels using an auto-analyzer (Chemray 240, RaytoInc, Shenzhen, China).

2.4. Pulmonary function

Spirometry was performed using a forced pulmonary maneuver system in accordance with the manufacturer's protocol (Buxco Research Systems, Wilmington, North Carolina, USA). Rats anesthetized with pentobarbital were given a tracheotomy, intubated, and then placed in the system body chamber. When the average breathing rate was 150 breaths per min, the functional residual capacity (FRC), total lung capacity (TLC), forced vital capacity (FVC), forced expiratory volume in 1 ms (FEV_{100}), forced expiratory volume in 0.5 ms (FEV_{50}), and chord compliance (Cchord) were measured. All relevant pulmonary function values were measured at least for three times.

2.5. Histopathology

The remaining left whole lung was infused, washed with phosphate-buffered saline, fixed with 4% paraformaldehyde, embedded in paraffin, sectioned to 4-μm thickness, and stained with hematoxylin and eosin (H&E). Slides were viewed with microscopy.

2.6. Statistical analyses

Quantitative data were expressed as the means and standard deviations (means ± SD) if normally distributed and median and interquartile range (IQR) if otherwise. At each given time (days 14, 45, or 75), pulmonary functions, hematological and biochemical indices, Δweight, and the ratio of different organs were compared between rats exposed to NO_2 and ambient air using the *t*-test. The One-way ANOVA with Bonferroni correction was used to compare pulmonary function, hematological and biochemical indices, Δweight, and the ratio of different organs among impaired rats administered NO_2 + NaHS or NO_2 + NaCl or spontaneous recovery group during the recovery period (76 to 105 days). All analyses were done with GraphPad Prism 5 software, using two-sided *P* values. Statistical significance was set at $P < 0.05$.

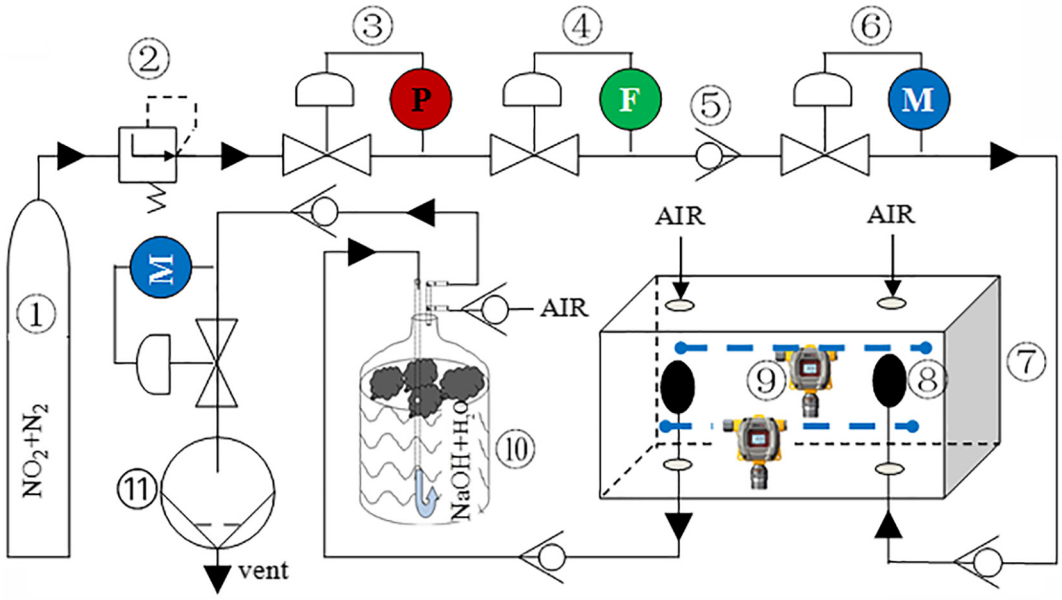

Fig. 1. Schematic diagram of the NO_2 generator and exposure equipment (schematic diagram provided by Professor Nan Sang, Shanxi University and drawn by the authors). 1: a 40-liter Teflon gas sampling container; 2: relief pressure valve; 3: gas pressure controller; 4: flow-ratio/mass-flow controller and check valve; 5: check valve; 6: mass-flow controller; 7: custom-built Plexiglas chambers (672L); 8: BIOLAK-Friox; 9: mobile monitor (showing values of NO_2, O_2, CO_2, temperature, pressure, and relative humidity etc.); 10: exhaust absorption bottle; 11: vacuum pump.

3. Results

3.1. Macroscopic examination

Lung tissues were first observed macroscopically. Rats exposed to NO_2 for 75 days showed the development of emphysema-like lesions, including a pale tan to grey appearance, pulmonary bullous, and elasticity attenuation (Fig. 2B) which control rats exposed to ambient air did not (Fig. 2A).

3.2. Histopathology

The effects of prolonged NO_2 exposure are observable in H&E-stained lung tissues (Fig. 3). B, D, and F were NO_2 exposure for 14, 45, and 75 days under sight fields of 100×, respectively, and A, C, and E were respective concurrent controls. H was 200× for H&E staining at 75 days of NO_2 exposure and G was control under a similar situation. Sections of lung tissue showed fragmented and free-floating alveolar septa (arrow) characteristic of an emphysema-like phenotype at H.

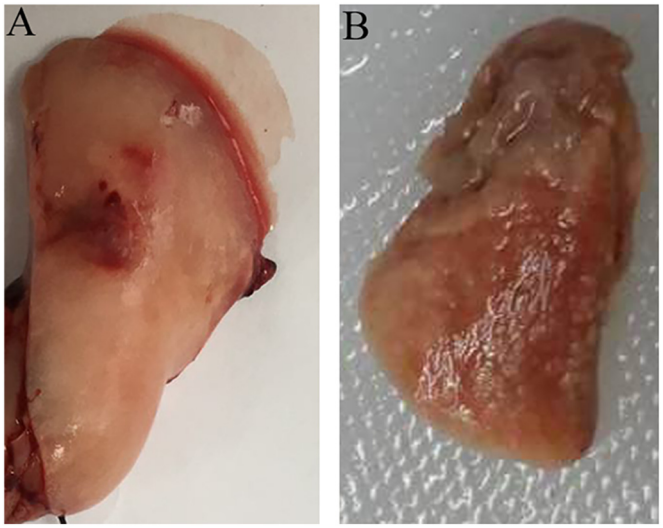

Fig. 2. B was NO_2-induced lung tissues of emphysema rats confirmed by macroscopic examination. Lung tissues of rats exposed to NO_2 for 75 days showed the development of emphysema-like lesions, including a pale tan to grey appearance, pulmonary bullous, and elasticity attenuation which control rats exposed to ambient did not (A).

3.3. Pulmonary function

Dynamic spirometry was evaluated at after 14, 45 or 75 days of NO_2 exposure. The FEV_{100}/FVC%, FEV_{50}/FVC%, and VC (Vital Capacity) were significantly lower at 45 days of NO_2 inhalation compared to ambient air rats ($P < 0.05$), and the same patterns were also exhibited at 75 days' ending point ($P < 0.05$); Furthermore, the FRC was significantly greater after 75-days NO_2 exposure relative to rats exposed to ambient air for an equivalent duration (Figs. 4 and S1 and Table S1). After a one month recovery period (76–105 days) wherein NO_2 exposure ceased, FRC impairment was alleviated by NaHS ($P > 0.05$) (Figs. 5 and S2 and Table S2). Comparisons between NO_2 + NaHS and NO_2 + NaCl showed no significant changes on these items ($P > 0.05$).

3.4. Hematological indices

Of the biochemical measurements, TG was elevated after 75 days NO_2 exposure compared with air exposure ($P < 0.05$) (Fig. 6 and Table S1). Meanwhile, of the hematological tests, levels of RBC, HGB, and HCT were elevated and PDW decreased after NO_2 exposure at 75 days ($P < 0.05$, for all) (Fig. 7 and Table S1). During days 76–105, emphysema like rats treated with NaHS, had greater HDL-C levels to that of rats treated with NaCl ($P < 0.05$) (Fig. 8 and Table S2); meanwhile, WBC, granulocyte, and lymphocyte counts also had somewhat increased ($P < 0.05$, for all) (Fig. 9 and Table S2).

3.5. Ratio of different organs and Δweight changes

ΔWeight also was evaluated at 14, 45 and 75 days of NO_2 exposure. Especially at 45 and 75 days of NO_2 inhalation, Δweight has already shown statistically significant decreased compared to ambient air rats

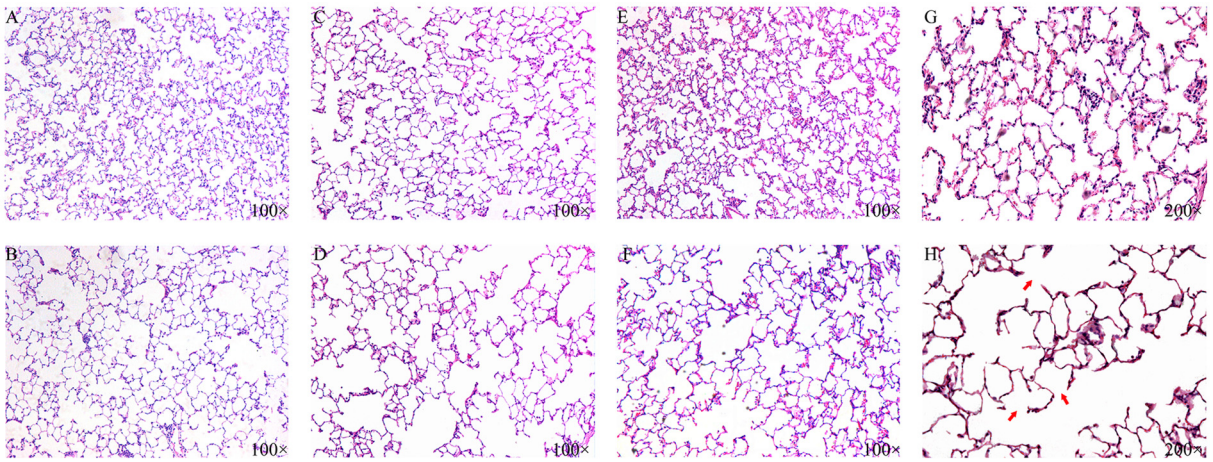

Fig. 3. Histologic features of H&E staining (A–F: 100-times magnification; G and H: 200-times magnification) in the lung of rats exposed to ambient air or 20 ppm NO_2. B, D, and F were NO_2 exposure for 14 days, 45 days and 75 days, respectively. A, C, and E were respective concurrent controls. H was 200-times magnification of H&E-stained tissues after 75 days of NO_2 exposure and G was control under similar situation. Sections of lung tissue showed fragmented and "free-floating" alveolar septa (arrow) characteristic of emphysema at H. N = 4 for 14 and 45 days of NO_2 exposure, and n = 5 for 75 days of inhalation.

($P < 0.05$) (Fig. 10 and Table S1). There were no significant changes to the ratio of organ (Heart, Liver, Spleen, and Kidney) to body weight and Δweight between NO_2-exposed rats treated with NaHS or NaCl during the recovery period ($P > 0.05$) (Fig. 11 and Table S2).

4. Discussion

The present study established a rat model of emphysema induced by chronic exposure to NO_2 and thereafter we firstly investigated the effects of NO_2 on PF, hematological and biochemical indices and attempted to explore the (protective) effects of NaHS on the NO_2-induced lung impairment. The findings suggest that prolonged exposure to 20 ppm NO_2 causes emphysema and a decline in PF in rats, which could be alleviated by NaHS treatment.

Measurements of PF and lung histology have offered a general insight into the overall response of the respiratory system to NO_2 exposure. At 45 days of exposure, rats exhibited a significant decrease in FEV_{100}/FVC%. However, the effect was not accompanied with a corresponding severe response on lung histology. We therefore prolonged exposure to the same levels of NO_2 until 75 days, at which histological and functional changes consistent with the diagnosis of emphysema were observed. After a one month of rest, reversibility of the impairment on PF was identified in the recovery rats, even if there was not a statistically significant improvement. We speculate that it is possible that the mitigation effects observed in the recovery rats would develop into a change after stopping exposure to NO_2. For instance, if the rest periods prolonged long enough, the situation might be improved largely on PF values for showing progressively better conditions while compared to the controls group.

NO_2 is relatively insoluble (0.037 mL per mL H_2O at 35 °C), and thus a large fraction of inhaled NO_2 is deposited in the pulmonary alveoli [24]. NO_2 inhalation can subsequently be delivered via lung tissue absorption and transferred across the blood-gas interface to the blood, potentially leading to systemic effects on biochemical and hematological indices, especially, the previous related studies never comprehensively investigated these systemic effects of NO_2 [5,25,26]. We currently found that levels of RBC, HGB and HCT were elevated after 75 days of NO_2 exposure. This could be caused by numerous factors. First, impaired PF could cause hypoxemia in rats, which could lead to elevated RBC, HGB, and HCT levels in response. Second, erythropoietin (EPO) is the main hematopoietic hormone acting on progenitor red blood cells via stimulation of cell growth, differentiation, and anti-apoptosis. In view of the plausible link shown between EPO and diesel exhaust particles (DEPs) by Kim HJ [27], it may, therefore, be possible

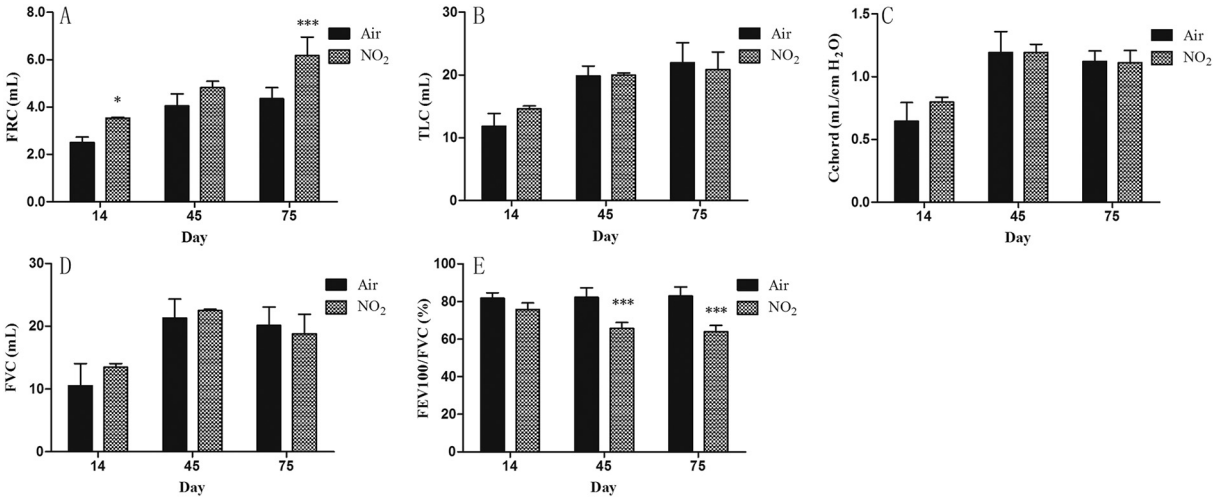

Fig. 4. Histogram (A–E) were pulmonary function index in rats at different given time (14, 45, and 75 days, respectively) exposed to NO_2 or ambient air. For NO_2 exposure (fine grid) vs ambient air (black color), $P < 0.05$. N = 4 for 14 and 45 days of NO_2 exposure, and n = 5 for 75 days of inhalation.

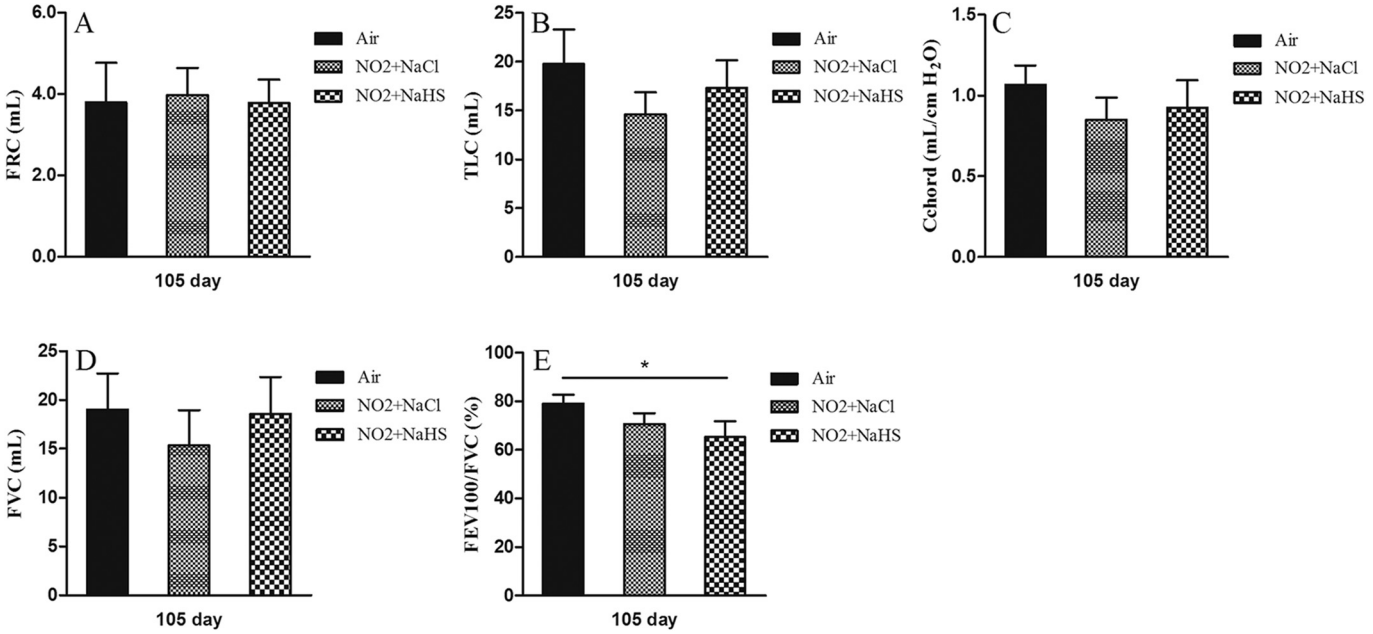

Fig. 5. Histogram (A–E) were pulmonary function index in rats with different treatments exposed to ambient air during the recovery periods (76–105 days). NaCl (fine grid), NaHS (coarse grid), and ambient air (black color) were included, $P < 0.05$, n ≥ 5 for each group.

that NO_2 exposure could lead to an increase in RBC, HGB, and HCT levels by upregulation of EPO. Third, Hypoxia Inducible Transcription factor-α (HIF-α), which adapts cellular hypoxia condition, can regulate the expression of many downstream genes including the EPO gene [28]. Thus, NO_2 might also play a vital role on the expression of HIF-α. Polycythemia would deteriorate pulmonary perfusion due to increased blood viscosity in lungs with decreased vascular beds, and the early damage of lungs was very common with enhanced EPO production, with time increased, emphysema might be possible. Secondary polycythemia is usual in serious COPD patients. It may be indicative of a compensatory physiologic response to hypoxemia. Polycythemia can contribute to pulmonary hypertension, reduced cerebral blood flow, and increase the risk of venous thromboembolic disease [29,30]. Furthermore, the detrimental effects on lipid metabolism by NO_2 inhalation also have been identified with elevated TG levels. This impaired effect could become no significant change without NO_2 exposure for a month. According to the literature, 70% of NO_2 absorbed in the lung is converted nitrite (NO_2^-), and it is thought that the toxicity of NO_2 was mainly due to the reaction of oxidizing acids with readily oxidizable pulmonary and extra-pulmonary tissues instead of water [31]. It could bind to lung tissue components and spread into circulation medium. Nonetheless, the underlying mechanisms remain unclear.

The protective effects of NaHS on the NO_2-induced lung impairment were found. Treatment with NaHS following after exposure to 20 ppm NO_2 improved PF, though the effect was not notable. Evidence suggests that NaHS could protect against cigarettes smoke-induced pulmonary injuries, airway inflammation and remodeling, and thereby prevent the development of emphysema and pulmonary hypertension [32]. However, its roles in the pathogenesis of NO_2-induced emphysema remain unclear. Our findings suggest that NaHS have therapeutic potential for

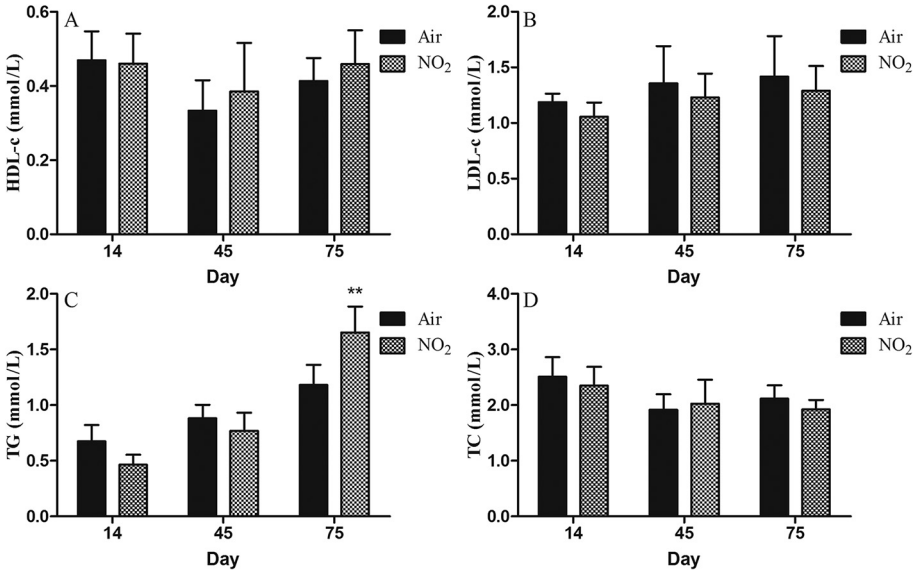

Fig. 6. Histogram (A–D) were biochemical index (HDL-C, LDL-C, TG, and TC, respectively) in rats exposed to NO_2 or ambient air at different time point (14, 45, and 75 days). For NO_2 exposure (fine grid) vs ambient air (black color), $P < 0.05$. N = 4 for 14 and 45 days of NO_2 exposure, and n = 5 for 75 days of inhalation.

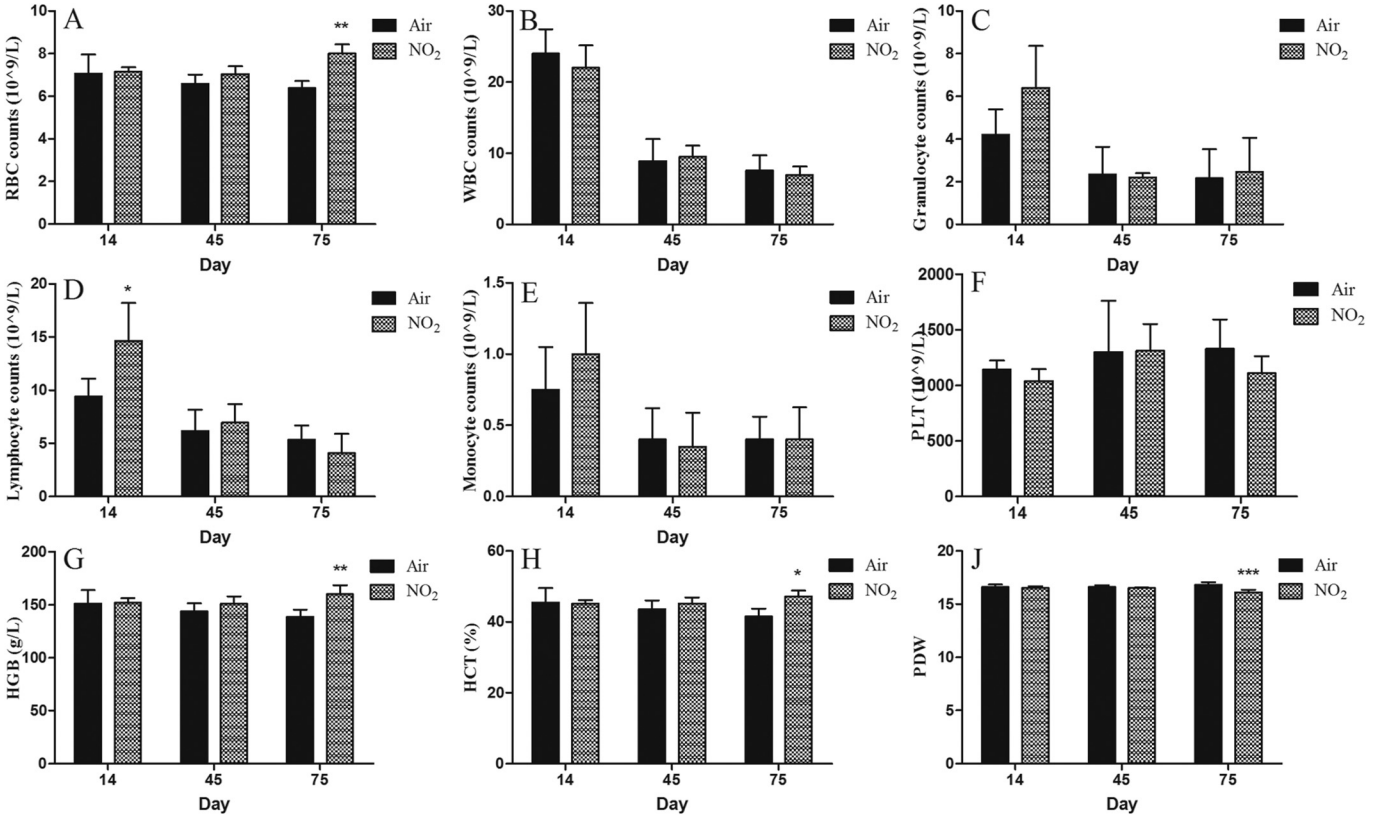

Fig. 7. Histogram (A–J) were hematological index (RBC, WBC, lymphocyte, monocyte, and granulocyte, PLT, HGB, HCT, and PDW, respectively) in rats at different given time (14, 45, and 75 days) exposed to NO_2 or ambient air $P < 0.05$. N = 4 for 14 and 45 days of NO_2 exposure, and n = 5 for 75 days of inhalation.

emphysema caused by NO_2. Examinations of hematological parameters found that NaHS treatment could result in relatively higher significant productions of WBC, lymphocyte, granulocyte, as well enhanced HDL-C level compared to NO_2 exposure with untreated. It might be that slightly elevated levels of these indices are associated with immune functions. Although NaHS mainly plays a pivotal role in vasodilation and endothelial angiogenesis and protects against oxidative injuries and inflammation [13], its protective effects on lung impairment caused by NO_2 firstly identified and should be considered in future studies. The exact mechanism would be deeply studied in the next work.

5. Conclusions

The present findings suggest that a 20 ppm NO_2 chronic exposure causes emphysema and a decline in PF in rats. NaHS could alleviate the PF decline as probably by an relative elevation of HDL-C levels and leukocyte. NaHS have therapeutic potential for emphysema caused by air pollutant NO_2.

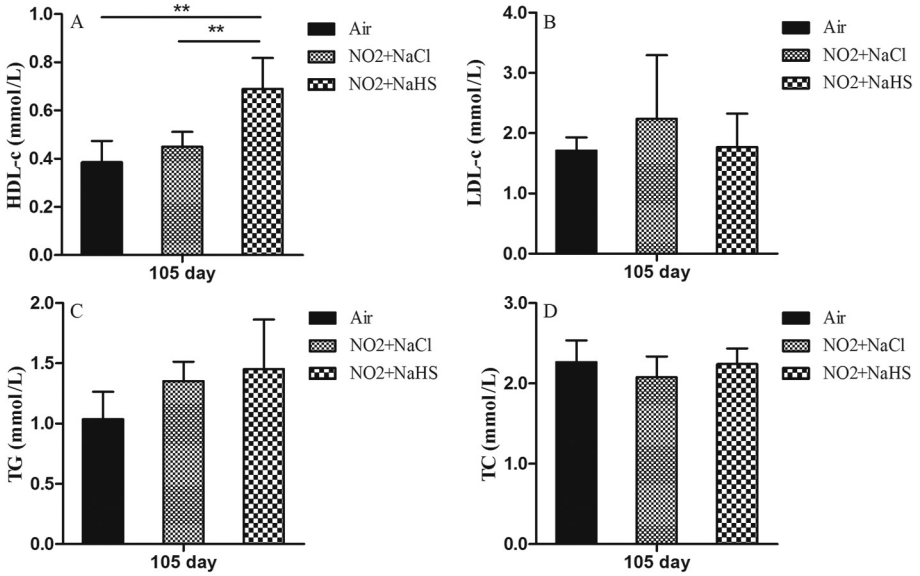

Fig. 8. Histogram (A–D) were biochemical index (HDL-C, LDL-C, TG, and TC, respectively) in rats with different treatments at one-month recovery period exposed to ambient air (76 to 105 days). NaCl (fine grid), NaHS (coarse grid), and ambient air (black color) were included, $P < 0.05$, n ≥ 5 for each group.

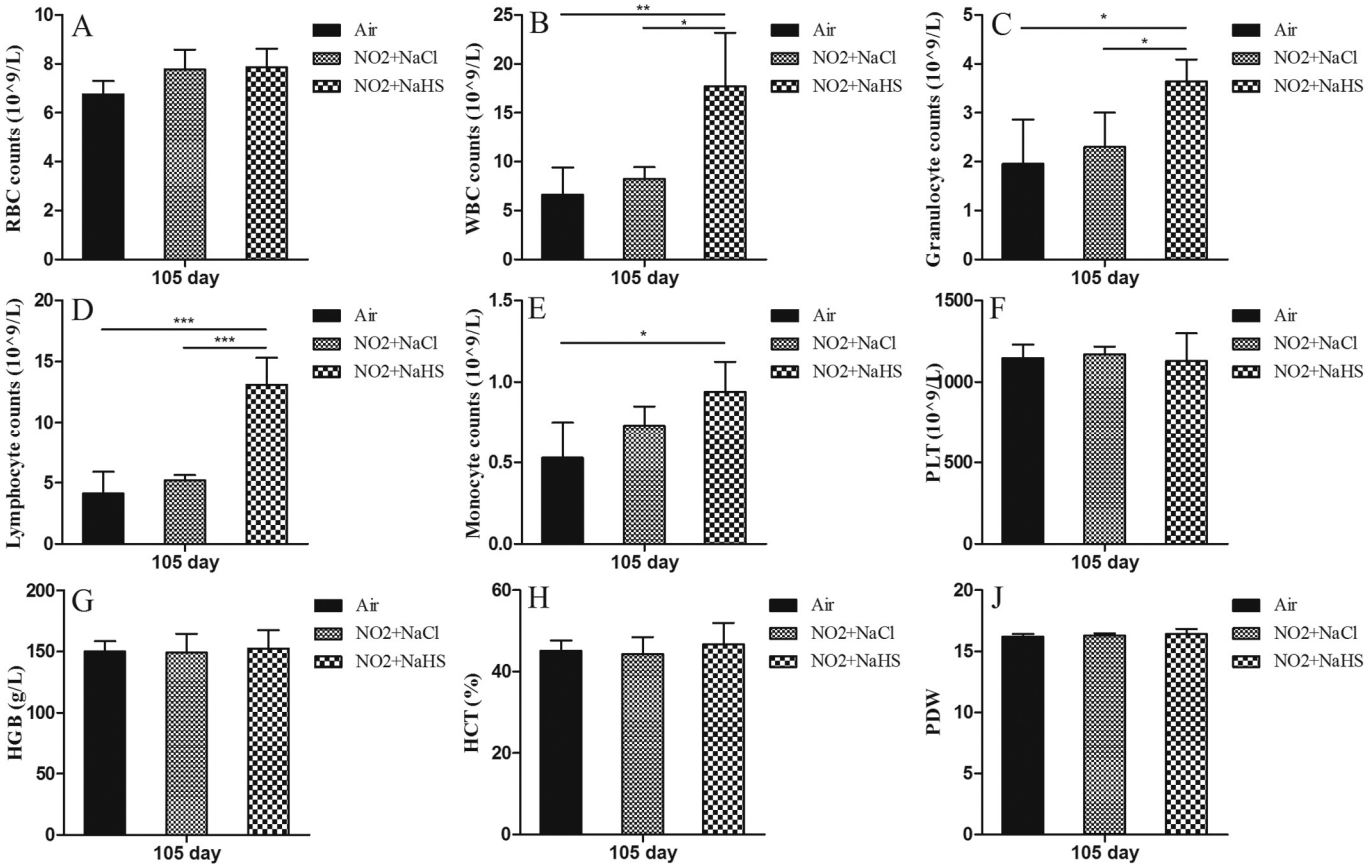

Fig. 9. Histogram (A–J) were hematological index in rats with different treatments during the recovery periods (76 to 105 days). NaCl (fine grid), NaHS (coarse grid), and ambient air (black color) were included, $P < 0.05$, n ≥ 5 for each group.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.116650.

Abbreviations

PF	pulmonary function
NO_2	nitrogen dioxide
NO_x	nitrogen oxides
NaHS	sodium hydrosulfide
SD	Sprague-Dawley
WBC	white blood cell
RBC	red blood cell
HGB	hemoglobin
HCT	hematocrit

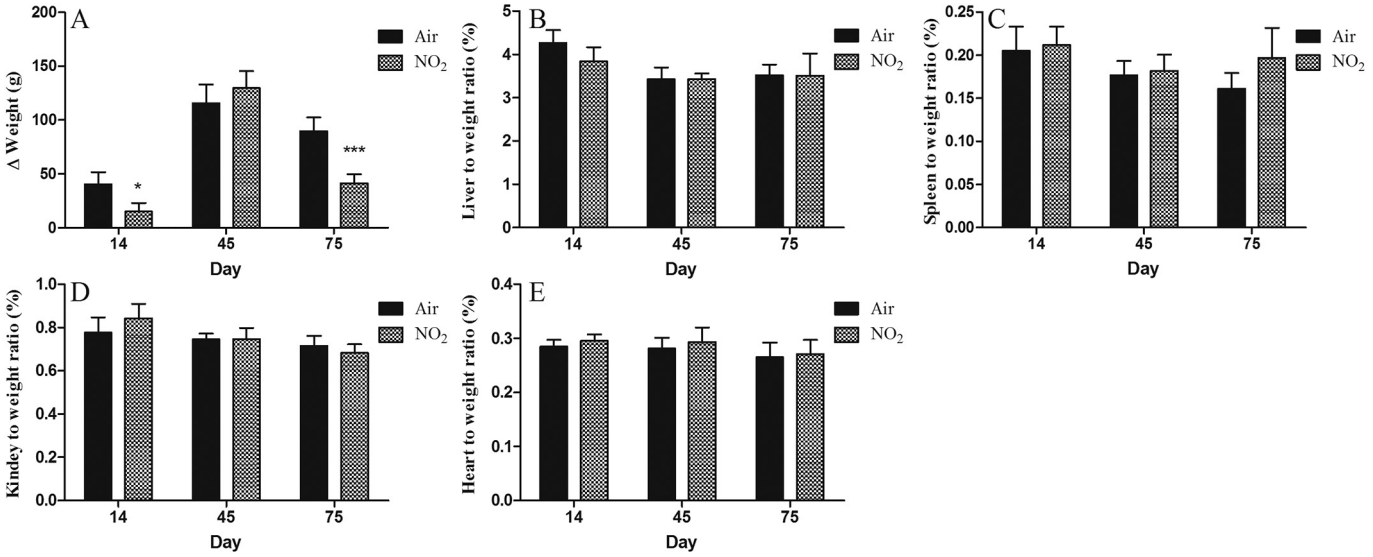

Fig. 10. Histogram (A–E) were Δweight and ratio of different organs (Liver, Spleen, Kidney, and Heart, respectively) to their own weight at different given time (14, 45, and 75 days) exposed to NO_2 or ambient air. For NO_2 exposure (fine grid) vs ambient air (black color), $P < 0.05$. N = 4 for 14 and 45 days of NO_2 exposure, and n = 5 for 75 days of inhalation.

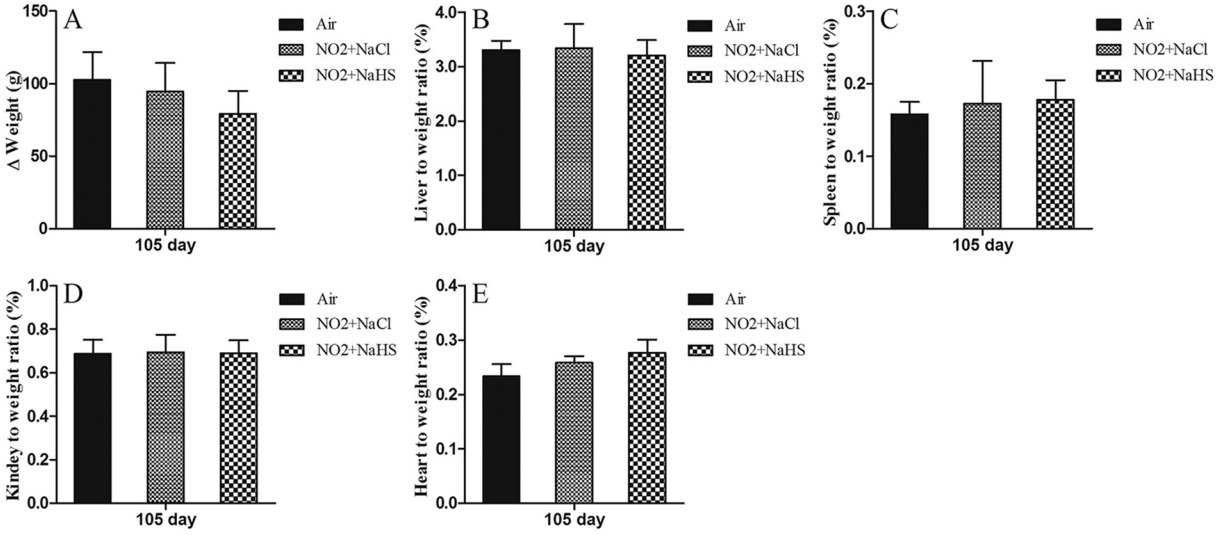

Fig. 11. Histogram (A–E) were Δweight and ratio of different organs (Liver, Spleen, Kidney, and Heart, respectively) to weight in rats with different treatments during the recovery periods (76 to 105 days). NaCl (fine grid), NaHS (coarse grid), and ambient air (black color) were included, $P < 0.05$, n ≥ 5 for each group.

HDL-C	high-density lipoprotein cholesterol
LDL-C	low-density lipoprotein cholesterol
GLU	glucose
TC	total cholesterol
PLT	platelets
TG	triglyceride
GSP	glycated serum protein
FRC	functional residual capacity
TLC	total lung capacity
FVC	forced vital capacity
FEV_{100}	forced expiratory volume in 1 ms
FEV_{50}	forced expiratory volume in 0.5 ms
Cchord	chord compliance
Ti	inspiratory time
Te	expiratory time
VC	vital capacity
PEF	peak expiratory flow

Funding source

This work was supported by grants from the National Natural Science Foundation of China (81520108001, 81700043, 81770043, and 81220108001), Guangdong Natural Science Foundation (2016A030313593), Guangzhou Science and Technology Programs for Science Study (201607020030), the National Key R&D Project (2016YFC 0903700), the 973 Key Scheme of China (2015CB553406), Guangdong Province Universities and Colleges Pearl River Scholar Funded Scheme (2014, for WL), Guangdong Province Universities and Colleges Key Grant for Innovative Research (cxzd1142), Guangzhou Department of Education (1201620007, 13C08, and 12A001S), Open Project of State Key Laboratory of Respiratory Disease (SKLRD-OP-201808, SKLRD-QN-201719), Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program (2017BT01S155), and the National Key R&D Program of China (2018YFC1311900). The funding sponsors had no role in the study.

Author contributions

Conceived and designed the experiments: WJL, JW and ZLZ; performed the experiments: ZLZ, DJS, GHX, MJD, FL, LY, TW and JYX; analyzed the data: ZLZ; contributed reagents/materials/analysis tools: ZLZ, JYX and DJS; the paper was written by ZLZ; reviewed and revised by WJL TW, DJS, XHX and BXD.

Ethics approval and consent to participate

The experiments protocol was approved by the institutional review boards of Guangzhou Medical University and conducted in accordance with our institutional guidelines on the Care and Use of Laboratory Animals (National Institutes of Health).

Declaration of Competing Interest

None.

Acknowledgments

We thank Professor Nan Sang and Wei Yan (Shanxi University, Taiyuan, Shanxi, China) for their invaluable assistance in developing the NO_2 exposure system.

References

[1] P. Nafstad, L.L. Haheim, T. Wisloff, F. Gram, B. Oftedal, I. Holme, et al., Urban air pollution and mortality in a cohort of Norwegian men, Environ. Health Perspect. 112 (2004) 610–615.
[2] B. Brunekreef, S.T. Holgate, Air pollution and health, Lancet 360 (2002) 1233–1242.
[3] W.J. Gauderman, E. Avol, F. Gilliland, H. Vora, D. Thomas, K. Berhane, et al., The effect of air pollution on lung development from 10 to 18 years of age, N. Engl. J. Med. 351 (2004) 1057–1067.
[4] M.R. Masjedi, H.R. Jamaati, P. Dokouhaki, Z. Ahmadzadeh, S.A. Taheri, M. Bigdeli, et al., The effects of air pollution on acute respiratory conditions, Respirology 8 (2003) 213–230.
[5] J. Blank, J.E. Glasgow, G.G. Pietra, L. Burdette, G. Weinbaum, Nitrogen-dioxide-induced emphysema in rats. Lack of worsening by beta-aminopropionitrile treatment, Am. Rev. Respir. Dis. 137 (1988) 376–379.
[6] L.T. Juhos, D.P. Green, N.J. Furiosi, G. Freeman, A quantitative study of stenosis in the respiratory bronchiole of the rat in NO_2-induced emphysema, Am. Rev. Respir. Dis. 121 (1980) 541–549.
[7] D.M. Stavert, D.C. Archuleta, L.M. Holland, B.E. Lehnert, Nitrogen dioxide exposure and development of pulmonary emphysema, J. Toxicol. Environ. Health 17 (1986) 249–267.
[8] M. Wegmann, A. Fehrenbach, S. Heimann, H. Fehrenbach, H. Renz, H. Garn, et al., NO_2-induced airway inflammation is associated with progressive airflow limitation and development of emphysema-like lesions in C57bl/6 mice, Exp. Toxicol. Pathol. 56 (2005) 341–350.
[9] Z. Zhang, J. Wang, L. Chen, X. Chen, G. Sun, N. Zhong, et al., Impact of haze and air pollution-related hazards on hospital admissions in Guangzhou, China, Environ. Sci. Pollut. Res. Int. 21 (2013) 4236–4244.
[10] Z. Zhang, J. Wang, M. Guo, M. Xiong, Q. Zhou, D. Li, et al., Air quality improvement during 2010 Asian games on blood coagulability in COPD patients, Environ. Sci. Pollut. Res. Int. 23 (2015) 6631–6638.
[11] J.R. Koenitzer, T.S. Isbell, H.D. Patel, G.A. Benavides, D.A. Dickinson, R.P. Patel,

et al., Hydrogen sulfide mediates vasoactivity in an O_2-dependent manner, Am. J. Physiol. Heart Circ. Physiol. 292 (2007) H1953–H1960.
[12] W. Zhao, J. Zhang, Y. Lu, R. Wang, The vasorelaxant effect of H(2)S as a novel endogenous gaseous K(ATP) channel opener, EMBO J. 20 (2001) 6008–6016.
[13] A. Papapetropoulos, A. Pyriochou, Z. Altaany, G. Yang, A. Marazioti, Z. Zhou, et al., Hydrogen sulfide is an endogenous stimulator of angiogenesis, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 21972–21977.
[14] Y. Kimura, Y. Goto, H. Kimura, Hydrogen sulfide increases glutathione production and suppresses oxidative stress in mitochondria, Antioxid. Redox Signal. 12 (2010) 1–13.
[15] A. Esechie, L. Kiss, G. Olah, E.M. Horvath, H. Hawkins, C. Szabo, et al., Protective effect of hydrogen sulfide in a murine model of acute lung injury induced by combined burn and smoke inhalation, Clin. Sci. (Lond.) 115 (2008) 91–97.
[16] H. Aslami, A. Heinen, J.J. Roelofs, C.J. Zuurbier, M.J. Schultz, N.P. Juffermans, Suspended animation inducer hydrogen sulfide is protective in an in vivo model of ventilator-induced lung injury, Intensive Care Med. 36 (2010) 1946–1952.
[17] S. Faller, S.W. Ryter, A.M. Choi, T. Loop, R. Schmidt, A. Hoetzel, Inhaled hydrogen sulfide protects against ventilator-induced lung injury, Anesthesiology 113 (2010) 104–115.
[18] Y.H. Chen, R. Wu, B. Geng, Y.F. Qi, P.P. Wang, W.Z. Yao, et al., Endogenous hydrogen sulfide reduces airway inflammation and remodeling in a rat model of asthma, Cytokine 45 (2009) 117–123.
[19] Y.H. Chen, W.Z. Yao, B. Geng, Y.L. Ding, M. Lu, M.W. Zhao, et al., Endogenous hydrogen sulfide in patients with COPD, Chest 128 (2005) 3205–3211.
[20] D.B. Peden, B. Boehlecke, D. Horstman, R. Devlin, Prolonged acute exposure to 0.16 ppm ozone induces eosinophilic airway inflammation in asthmatic subjects with allergies, J. Allergy Clin. Immunol. 100 (1997) 802–808.
[21] Z. Zhang, J. Wang, J. He, Z. Zheng, X. Zeng, C. Zhang, et al., Genetic variants in MUC4 gene are associated with lung cancer risk in a Chinese population, PLoS One 8 (2013) e77723.
[22] W. Yan, X. Ji, J. Shi, G. Li, N. Sang, Acute nitrogen dioxide inhalation induces mitochondrial dysfunction in rat brain, Environ. Res. 138 (2015) 416–424.
[23] Z. Zhang, J. Wang, F. Liu, L. Yuan, M. Ding, L. Chen, et al., Non-inflammatory emphysema induced by NO_2 chronic exposure and intervention with demethylation 5-Azacytidine, Life Sci. 221 (2019) 121–129.
[24] V. Mohsenin, Human exposure to oxides of nitrogen at ambient and supra-ambient concentrations, Toxicology 89 (1994) 301–312.
[25] P.J. Barth, B. Muller, U. Wagner, A. Bittinger, Quantitative analysis of parenchymal and vascular alterations in NO_2-induced lung injury in rats, Eur. Respir. J. 8 (1995) 1115–1121.
[26] J.E. Glasgow, G.G. Pietra, W.R. Abrams, J. Blank, D.M. Oppenheim, G. Weinbaum, Neutrophil recruitment and degranulation during induction of emphysema in the rat by nitrogen dioxide, Am. Rev. Respir. Dis. 135 (1987) 1129–1136.
[27] H.J. Kim, S.Y. Kim, J.Y. Kwon, Y.J. Kim, S. Hun Kang, W.H. Jang, et al., Identification of potential novel biomarkers and signaling pathways related to otitis media induced by diesel exhaust particles using transcriptomic analysis in an in vivo system, PLoS One 11 (2016) e0166044.
[28] Y. Wu, Z. Jiang, Q. You, X. Zhang, Application of in-vitro screening methods on hypoxia inducible factor prolyl hydroxylase inhibitors, Bioorg. Med. Chem. 25 (2017) 3891–3899.
[29] B.D. Kent, P.D. Mitchell, W.T. McNicholas, Hypoxemia in patients with COPD: cause, effects, and disease progression, Int. J. Chron. Obstruct. Pulmon. Dis. 6 (2011) 199–208.
[30] W. Macnee, Right heart function in COPD, Semin. Respir. Crit. Care Med. 31 (2010) 295–312.
[31] E.M. Postlethwait, A. Bidani, Mechanisms of pulmonary NO_2 absorption, Toxicology 89 (1994) 217–237.
[32] W. Han, Z. Dong, C. Dimitropoulou, Y. Su, Hydrogen sulfide ameliorates tobacco smoke-induced oxidative stress and emphysema in mice, Antioxid. Redox Signal. 15 (2011) 2121–2134.