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H_2S attenuates acute lung inflammation induced by administration of lipopolysaccharide in adult male rats

Fatma F. Ali[1], Heba A. Abdel-Hamid[1] and Nisreen D. M. Toni[2]

[1] *Medical Physiology Department, Faculty of Medicine, Minia University, Minia, Egypt*
[2] *Pathology Department, Faculty of Medicine, Minia University, Minia, Egypt*

Abstract. Hydrogen sulfide (H_2S) is gasotransmitter which plays an important role in human physiology. In this study, we aimed to check the effect of H_2S treatment on acute lung inflammation (ALI). Thirty-six adult male albino rats were used and divided into: control group, ALI group which was intraperitoneally (*i.p.*) injected with lipopolysaccharide (LPS) at a dose of 5 mg/kg body weight, ALI group treated by the H_2S donor; sodium hydrosulfide (NaHS) at a dose of 10 mg/kg body weight *i.p.* and ALI group treated by *i.p.* injection of 80 mg/kg body weight DL-propargylglycine (PAG) which is an inhibitor of endogenous H_2S synthesis. Serum was obtained to determine interleukin-6 (IL-6) levels. Lipid peroxides and total antioxidant capacity (TAC) levels were measured in lung. Lung histopathology and expression of inducible nitric oxide synthase (iNOS) were also done. Results showed that NaHS improved lung inflammation through its inhibitory effect on iNOS expression, decreasing the levels of IL-6 and lipid peroxides and increasing TAC levels. But, ALI was exacerbated with PAG administration. In conclusion, the results proved that H_2S has a protective effect against LPS induced ALI due to its anti-nitrative, anti-oxidant and anti-inflammatory properties.

Introduction

Inflammation is a rapid and strong action required to localize and remove the microorganisms (Allam-Ndoul et al. 2016). In lungs, the important organs for gas exchange, inflammation is dangerous for life. Microorganisms, exposure to toxins, allergens, air pollutants, or irritants may be the main reasons of acute lung inflammation. The underlying mechanism of inflammation is related to the activation of inflammatory cells which secrete inflammatory mediators to change the action of other types of cells (Yamada et al. 2016). There are two kinds of inflammation; acute like pneumonia (Shaver et al. 2016) and chronic type like asthma (Scherer and Chen 2016) which result in tissue damage (Robb et al. 2016).

H_2S is a colorless, flammable and water-soluble gas with the characteristic smell of rotten eggs. In the past several centuries, H_2S had been known only for its toxicity and environmental hazards (Kolluru et al. 2013). Recently, there has been growing evidence that H_2S has a broad range of physiological and pathophysiological functions, including induction of angiogenesis, regulation of neuronal activity, vascular relaxation, glucose homeostatic regulation, and protection against ischemia/reperfusion injury in heart, liver, kidney, lung and brain (Wu et al. 2015).

H_2S can be detected in a wide range of tissues and organs including the brain, thoracic aorta, lungs, liver, kidney, ileum, pancreatic islets, uterus, placenta and umbilical cord and several other organs, suggesting a pleiotropic role for the gaseous transmitter (Huang et al. 2015). Although H_2S can be generated nonenzymatically through reduction of elemental sulfur using reducing equivalents obtained from the oxidation of glucose, the majority of H_2S is believed to be produced through the action of cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS) and 3-mercaptopyruvate sulfurtransferase (3-MST) enzymes (Chatzianastasiou et al. 2016).

The role of H_2S in acute lung inflammation (ALI) is debatable. Several studies reported that H_2S gives protection against ALI (Campos et al. 2016; Zhang et al. 2016). But, Qu et al. (2014); Gaddam et al. (2016) have found the reverse. Due to these antagonistic results, the present

Correspondence to: Fatma Ali, Medical Physiology Department, Faculty of Medicine, Minia University, Minia, Egypt
E-mail: fatmafarrag2008@gmail.com

research was done to evaluate the effect of NaHS that serves as a donor of H_2S and PAG which is an irreversible inhibitor of the H_2S synthesizing enzyme CSE on ALI produced by LPS.

Materials and Methods

Ethical approval

The animal care and experiments protocol described in this study were complied with "Research Ethics Committee" Faculty of Medicine, Minia University, Egypt which are in accordance with the NIH Guide for Care and Use of Laboratory Animals (National Institutes of Health 1992).

Animals

Thirty-six adult male albino rats from the local strain, of body weight ranging from 250–300 g and age ranging from 8–10 weeks were used throughout this study. They were settled under standard laboratory conditions with natural day/night cycle for two weeks before the beginning of the experiment. Rats were fed a standard diet of commercial rat chow (El-Gomhoria Company, Cairo, Egypt) and tap water *ad libitum* until the time of the experiment. Rats were classified haphazardly into the following groups (9 rats each): I. Control group, each rat was intraperitoneally (*i.p.*) injected with 0.5 ml of the vehicle; II. ALI group, rats received 5 mg/kg body weight LPS *i.p.* then after 24 h the occurrence of ALI was confirmed by a pathologist (Zhang et al. 2016); III. ALI+NaHS group, rats were treated with NaHS at a dose level of 10 mg/kg body weight *i.p.* (Tong et al. 2016), 1 h after induction of ALI (Ji et al. 2016); IV. ALI+PAG group, where each rat was *i.p.* injected with 80 mg/kg body weight of PAG (Wang et al. 2013) 1 h after induction of ALI (Ji et al. 2016).

Treatment protocol

LPS, NaHS and PAG were brought from Sigma Aldrich (USA), prepared on the day of the experiment and solved in sterile saline. Each drug dosage was chosen according to the principle of the preceding experiments.

Specimen collection

All rats were decapitated 24 h after experimental work. Blood samples were collected and centrifuged for ten minutes at 5000 rpm. The clear sera were stored at –20°C till the time of assay of serum IL-6 which was measured by enzyme-linked immunosorbent assay (ELISA) kit (Sigma Aldrich, USA).

Analysis of lung tissues

The chest of each rat was opened cautiously to take out the lung tissues. Lung tissues were stored at –80°C for subsequent measurement of lipid peroxides and total antioxidant capacity (TAC). Lipid peroxides determination is based on its reaction with thiobarbituric acid to form a pink complex with an absorption maximum at 535 nm (Heeba and Hamza 2015). TAC levels were measured by the obtainable colorimetric kit (Biodiagnostic, Egypt).

Histopathology of the lung

Multiple tissue biopsies of the lung were taken and fixed in 10% paraformaldehyde, embedded in paraffin and divided. Parts of 4 μm in thickness were stained with hematoxylin and eosin (H and E) stain using standard techniques and examined under light microscope by a pathologist with blind information of pathological data. Lung inflammation was quantitatively scaled according to (Christaki et al. 2011; Li et al. 2015) depending on alveolar collapse, alveolar edema, alveolar hemorrhage, alveolar neutrophils and macrophages infiltration, perivascular edema, perivascular hemorrhage and vascular congestion; ranging from 0–3: 0, equal normal; 1, equal mild; 2, equal moderate; 3, equal severe. The mean of the whole score in each group was measured to calculate the total score.

Immunohistochemistry assessment of iNOS

Xylene was used for deparaffinization then tissue rehydration was done by using descending grades of ethyl alcohol (100%, 95%, 80% and 70%) for 10 min each. Blocking of the endogenous peroxidase activity was done (using 0.5% H_2O_2 in methanol) for 25 min. Antigen retrieval (using 10 mM citrate buffer solution (pH 6)) was done. Samples were incubated with goat serum for 5 min followed by overnight incubation of samples at 4°C with anti-iNOS polyclonal antibody. Negative control slides were treated with PBS instead of using the iNOS. Incubation of the slides was done for 10 min with biotinylated secondary antibody. Incubation of the samples with streptavidin-peroxidase complex was done for 5 min followed by incubation with 3,3-diaminobenzidine tetrahydrochloride (DAB) for 3 min. Counterstaining of the slides was done by using the Meyer's haematoxylin for 1min then washing with water. In ascending grades of alcohol, tissues were dehydrated followed by clearance, mounting and finally covered with cover slips. With each run, positive and negative controls were done. Graded specific iNOS staining on a semiquantitative scale were done. Immunostaining was scored depending on the intensity of staining and the percentage of cells that stained positively. Staining scores

were calculated by multiplying the percentage of positive cells *per* section (0–100%) by the immunohistochemical staining intensity. The sections were classified according to staining intensity as 0 (negative or no abnormal staining), 1+ (weak staining), 2+ (moderate staining) or 3+ (strong staining), and scores ranged from 0 to 300. The staining scores obtained for 2 slides from the same specimen were calculated and the result was recorded as the score for that case (Korkomaz and Kolankaya 2013).

Statistical analysis of data

Data were expressed in the form of mean ± standard error of the mean (mean ± SEM). They were considered statistically significant values if the probability value (p value) less than or equal 0.05. Statistical analysis of data was performed by one-way analysis of variance (ANOVA). Graph pad prism software version 6 was used for all statistical analyses.

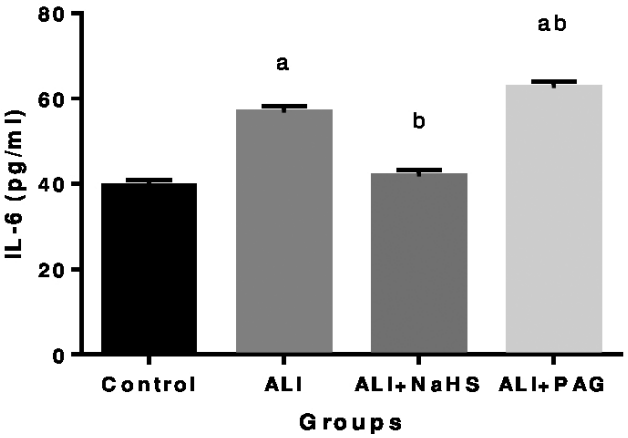

Figure1. Effect of acute lung inflammation (ALI) with or without H_2S treatment on serum interleukin (IL)-6 level. Values are expressed as mean ± SEM of 9 rats in each group. [a] $p \leq 0.05$, significant difference from control group; [b] $p \leq 0.05$, significant difference from ALI group.

Results

Lung oxidative state

The results obviously proved that administration of LPS in ALI group caused significant rise in lung lipid peroxides level and decrease in lung TAC level in comparison with control group. When the H_2S donor (NaHS) was given to ALI rats, significant decrease in lung lipid peroxides level and increase in TAC level were reported in comparison with ALI group. But, treatment with PAG caused a significant rise in lung lipid peroxides level and decrease in TAC level in comparison with both control and ALI groups (Table 1).

Serum IL-6 level

As regard serum IL-6, it was significantly increased in ALI group as compared to control animals. While NaHS treatment resulted in significant decrease in IL-6 level in ALI rats which was insignificantly different as compared to control group. But, PAG treatment led to significant rise in IL-6 level in comparison with both control and ALI groups (Figure 1).

Lung histopathology

Microscopic examination of lung sections of group I (Control) showed normal histologic pattern with inflated polygonal alveoli, thin septa in between, normal bronchioles and normal blood vessels. In group II (ALI) most of the studied sections were moderate to severe inflammation with alveolar wall thickening due to intense inflammatory cell permeation, narrowed alveolar spaces and dilated congested vessels. Bronchioles were partially obstructed by inflammation and shed epithelial cells. In group III (ALI+NaHS), the examined sections of lung appeared near to normal picture with little permeation of inflammatory cells and random thickening of the alveolar septa accompanied with slight narrowed alveolar spaces. In group IV (ALI+PAG), all specimens examined revealed severe inflammation of lung tissue with some fields revealed intense hemorrhage (Figure 2).

Table 1. Effect of acute lung inflammation (ALI) with or without H_2S treatment on lung oxidative state

Parameter	Group			
	Control	ALI	ALI+NaHS	ALI+PAG
Lipid peroxides (nmol/g tissue)	13.38 ± 1.47	24.57 ± 1.60[a]	18.73 ± 1.13[a,b]	30.58 ± 1.13[a,b]
TAC (μm/g tissue)	16.72 ± 1.31	7.61 ± 0.86[a]	12.14 ± 0.84[a,b]	3.63 ± 0.53[a,b]

Values are expressed as mean ± SEM (n = 9 rats in each group). [a] $p \leq 0.05$, total significant difference from control group; [b] $p \leq 0.05$, total significant difference from ALI group. ALI, acute lung inflammation; NaHS, sodium hydrosulfide; PAG, DL-propargylglycine; TAC, total antioxidant capacity.

Immunohistochemical assessment of iNOS

iNOS expression was only limited to the granulocytes and bronchiolar epithelial cells in control group which is a normal finding. In group II (ALI), iNOS expression was significantly increased in comparison with control group. In group III (ALI+NaHS), iNOS expression was significantly increased in comparison with control group but significantly decreased in comparison with ALI group. However, administration of PAG (ALI+PAG) led to significant increase in iNOS expression in comparison with both control and ALI groups (Table 2) and (Figure 3).

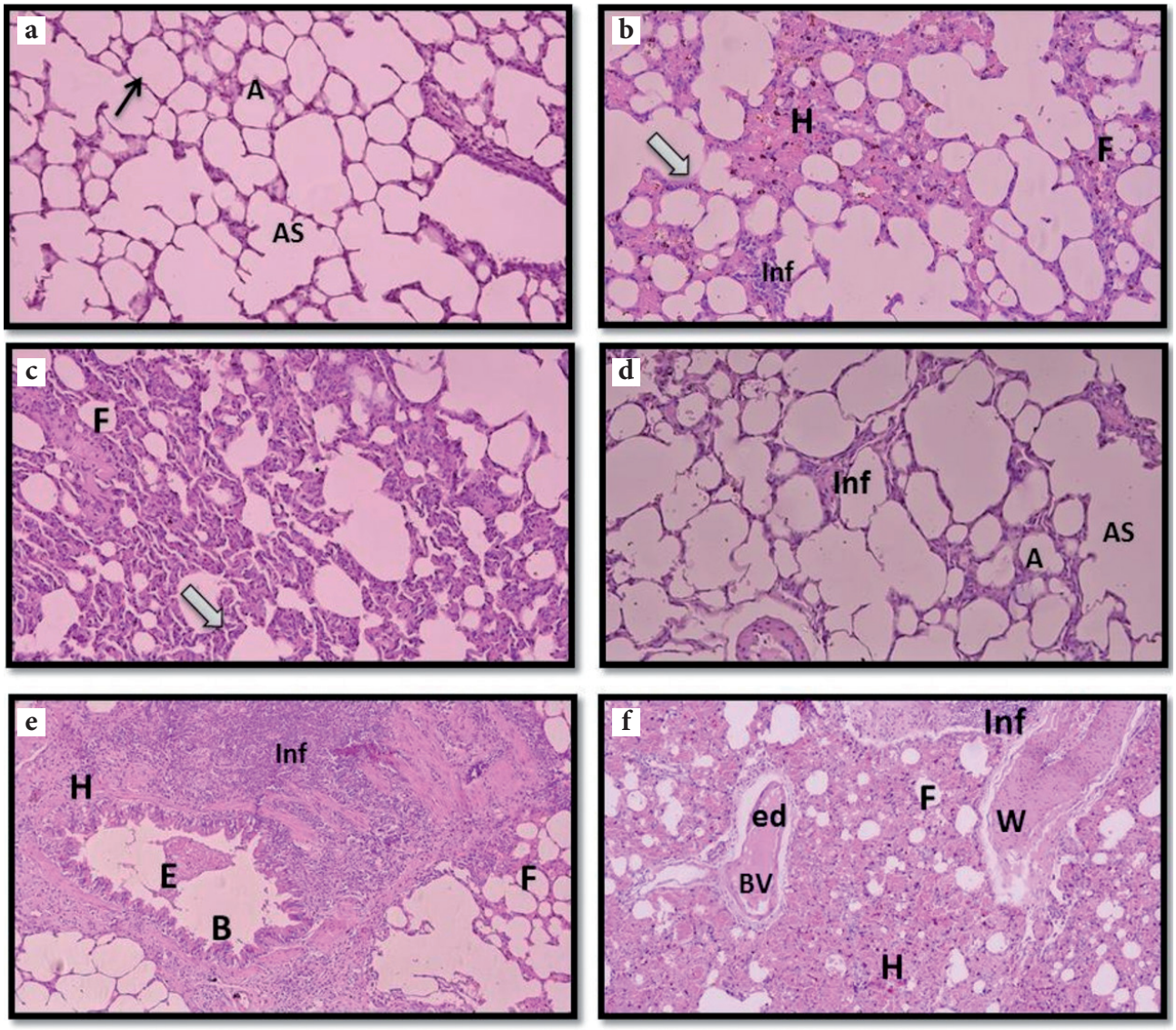

Figure 2. Histopathological examination of lung sections of different groups. Group I (control): **a.** Normal lung architecture with polygonal alveoli and alveolar sacs (AS). The alveoli (A) appeared well inflated with thin intact interalveolar septa (arrow). Group II (ALI): Moderate lung inflammation. The interalveolar septa are thickened **(b,** thick arrow**)** due to inflammatory infiltration **(**Inf) and hemorrhage (H) exhibited narrow alveolar spaces. Some alveoli are overinflated leading to destructed alveolar septa. **c.** Moderate infiltration of the alveoli with inflammatory cells (thick arrow), also fat droplets (F) between cellular infiltrations were found. Group III (ALI+NaHS): **d.** Lung inflammation in this group was minimal with few foci of inflammatory cells in the alveolar septa (Inf), otherwise the lung alveoli (A) and alveolar sacs (AS) look normal. Group IV (ALI+PAG): Severe lung inflammation. **e.** The alveoli are mostly replaced by inflammatory cells **(**Inf) with areas of hemorrhage (H). The bronchioles (B) are infiltrated with inflammation and the cavity show shed epithelial cells (E). **f.** Extensively infiltrated alveoli and interalveolar septa with inflammatory cells (Inf). Intra-alveolar exudate was also detected. Congested blood vessels (BV) appeared with markedly thickened wall (W) and edema (ed). Some fields revealed intense hemorrhage (H).

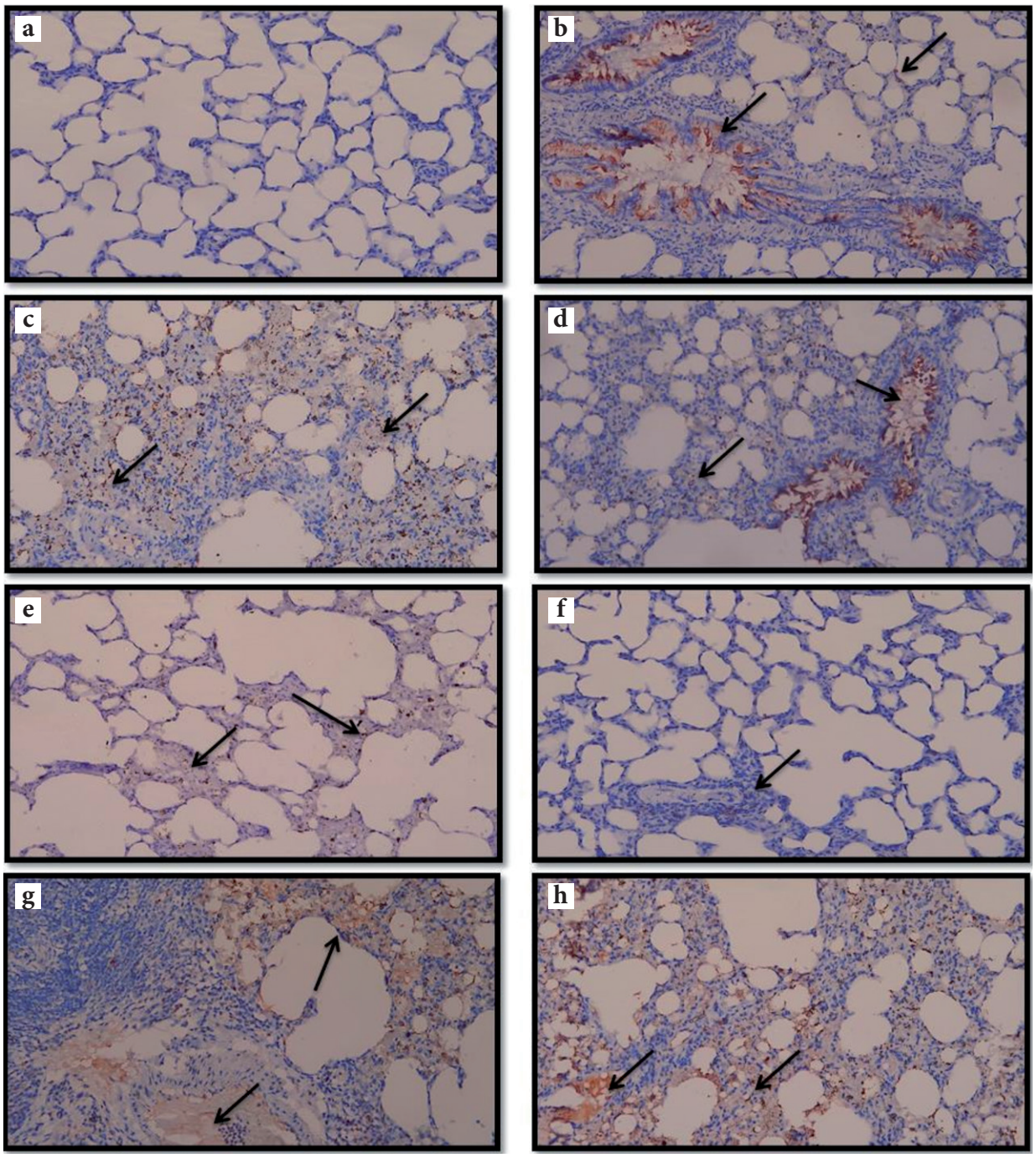

Figure 3. Immunohistochemical assessment of iNOS in lung sections of different groups. Immunostaining for iNOS (marked by arrows) was upregulated in parallel with the severity of inflammation. Group I (Control): The iNOS expression was confined to granulocytes and in bronchiolar epithelial cells (**b**) marked by arrows; the other components of the lung showed no expression (**a**). Group II (ALI): **c.** Moderate iNOS expression that is distributed all over the inflamed lung (marked by arrows); **d.** Moderate iNOS expression related to the inflammatory infiltration of the alveoli in addition to the normally expressed iNOS of bronchioles (marked by arrows). Group III (ALI+NaHS): **e**. Mild infiltration of the alveolar septa with inflammatory cells marked by arrows; **f.** Minimal inflammation and mild iNOS expression (marked by arrows). Group IV (ALI+PAG): **g.** Excessive thickening of the alveolar walls which are densely infiltrated with inflammatory cells with high positivity for iNOS (marked by arrows), with most of the alveolar cavities were occluded; **h.** The strongest iNOS expression intensity among the studied cases related to the dense inflammatory infiltrate (marked by arrows), resulting in narrowing of most of the alveolar cavities.

Table 2. Immunohistochemical staining intensity, percentage of positively stained cells and score for iNOS expression in lung specimens of different groups

Parameter	Group			
	Control	ALI	ALI+NaHS	ALI+PAG
Staining intensity	0	2+	1+	3+
Percentage of positive stained cells	0	63[a]	33[a,b]	90[a,b]
Staining score	0	126[a]	33[a,b]	270[a,b]

Values are expressed as mean ± SEM (n = 9 rats in each group). [a] $p \leq 0.05$, total significant difference from control group; [b] $p \leq 0.05$, total significant difference from ALI group. ALI, acute lung inflammation; NaHS, sodium hydrosulfide; PAG, DL-propargylglycine; 0, negative or no abnormal iNOS expression.

Discussion

LPS is an endotoxin found in the external membrane of gram negative bacteria and causes an intense inflammatory response in mammals (Kwon et al. 2016). During the present study, LPS was given for induction of ALI in rats which was confirmed by histopathological examination. Numerous studies have found that its administration resulted in significant inflammatory cells infiltration and tissue damage by producing different pro-inflammatory cytokines and reactive oxygen species (ROS) in the lung (Yoo et al. 2013; Lu et al. 2016; Yan et al. 2016; Zhang et al. 2016). The increased permeability of inflammatory cells may be due to impairment and disruption of the integrity of the endothelial barrier caused by nitrosative stress.

Previous studies discovered the presence of both constitutive nitric oxide synthase (c-NOS) and iNOS in rat and human lungs (Syed et al. 2016). In spite of the fact that nitric oxide (NO) is involved in anti-inflammatory, antimicrobial and antioxidant actions, excess NO production may be deleterious (Rao et al. 2016). NO is similar to H_2S as it may act as a pro-inflammatory or anti-inflammatory agent according to its concentration. Under physiological conditions, small amounts of NO derived from c-NOS are believed to be beneficial. But large amounts of NO derived by iNOS may contribute to the injury observed in different experimental models of inflammation (Antosova et al. 2017).

Liu et al. (2016) reported that the toxic effects of excess NO formation may be related to its products rather than NO itself. It can react with ROS to form the strong oxidant peroxynitrite ($ONOO^-$) (Oostwoud et al. 2016). $ONOO^-$ and/or ROS can lead to lung injury by several mechanisms including: first, deoxyribonucleic acid strand damage associated with point mutations (Zhang et al. 2016); second, proteins oxidation that changes protein action (Davies 2016) and third, oxidative damage of lipid accompanied by the formation of pro-inflammatory molecules such as nuclear factor kappa B (NF-κB) (Santo et al. 2016).

Previous researchers documented that LPS activates the excess production of NO *via* upregulation of iNOS as detected in the present study and by others (Huang et al. 2016; Zhang et al. 2016; Wei et al. 2017). Another study reported that the iNOS gene is a highly inducible gene and its transcription is readily upregulated by inflammatory cytokines (Nassi et al. 2016). Excess cytokines production might be caused by the LPS ability to stimulate mitogen activated protein kinase (MAPK) (Peng et al. 2016) and NF-κB (Jang et al. 2017) pathways. This is compatible with the LPS induced increase in serum IL-6 level in the present study which is also in agreement with Liu and Cheng (2016) and Weifeng et al. (2016). Yılmaz et al. (2016) demonstrated that cytokines caused acute lung damage by affecting phospholipids, surfactant and increased vessel permeability. Therefore, LPS induced ALI is most probably related to an increase of both cytokines and nitrosative stress levels (Zhang et al. 2016).

In the current study, a significant increase in lipid peroxides accompanied by a significant decrease of TAC levels in the lung were observed after LPS injection as found by Weifeng et al. (2016), Huang et al. (2016) and Zhang et al. (2016). Wang et al. (2016) mentioned that elevated lipid peroxides level could be attributed to oxidative stress which initiates lipid peroxidation of the membrane bound polyunsaturated fatty acids, leading to impairment of the membrane functional and structural integrity. Increased ROS released from the inflammatory cells may invade the active site of antioxidant enzymes and make them lose their function to scavenge ROS. So, ROS levels get increased and antioxidant enzyme levels get decreased (Huang et al. 2016).

Several studies reported that the role of H_2S in lung injury is conflicting. Qu et al. (2014) suggested that inhibition of H_2S improves ALI with acute pancreatitis. But, another research found that H_2S donor decreased the inflammatory response in sepsis induced lung injury (Ang et al. 2011). The concentration and the release rate of exogenous H_2S donors are the key factors in determining whether H_2S acts anti-inflammatory or pro-inflammatory. At high concentration and rapid releasing rate, H_2S is noxious. But, at small concentration and slow releasing rate, H_2S is protective (Wu 2013; Zhang et al. 2013).

In our results, NaHS treatment has resulted in improvement of ALI as compared to ALI group which was evident by lung histopathology. NaHS treatment could have decreased $ONOO^-$production and subsequently decreased oxidative stress, lipid peroxidation, and IL-6 and hence it improved lung inflammation. Benetti et al. (2013) found that H_2S reacts with ROS produced in the inflammatory states to oppose the oxidative stress. H_2S dissociated to yield HS (hydrosulfide) anions which are powerful one electron chemical reductants capable of scavenging free radicals by single hydrogen atom transfer or single electron transfer. It may also react with peroxynitrites and generates thionitrate isomer, which can decompose and serve as an NO donor (Xie et al. 2016). In addition, H_2S could inhibit ROS production indirectly by decreasing expression and activity of the ROS generators, NADPH oxidases (Nox), where Nox1, Nox2, and Nox4 are predominantly expressed in the lung. Moreover, previous studies showed their involvement in lung injury (Spassov et al. 2017).

ROS are widespread and continuously produced in living organisms. Many of them are necessary to carry out particular cellular and biological reactions. When they are overproduced, cellular damage may occur. ROS are counterbalanced in the body by a net of antioxidants (Xie et al. 2016). Recently, Sestito et al. (2017) reported that administration of NaHS has resulted in increased expression of anti-oxidants such as glutathione peroxidase, superoxide dismutase, glutathione reductase and heme oxygenase-1 (HO-1) due to the role of H_2S in regulating the nuclear factor erthyroid 2-related factor 2 (Nrf2) which is a key transcription regulator of antioxidant genes.

In the presence of ROS, H_2S could induce Nrf2 expression and enhance its translocation to the nucleus and bind with antioxidant response elements (AREs) to increase the expression of anti-oxidant proteins. The mechanism of the upregulation of Nrf2 by H_2S may be due to its ability to inactivate the H_2S negative regulator, Kelch-like ECH-associated protein 1 (Keap1) (Streeter et al. 2013). In addition, Lohninger et al. (2015) also demonstrated that H_2S led to activation of Nrf2 in THP-1 macrophages through inhibition of the p38 MAPK, suggesting that the p38 MAPK pathway may be involved in H_2S induced Nrf2 signalling pathway.

Polysulfides, sodium tri- and tetra sulfide (Na_2S_3 and Na_2S_4), the products of H_2S oxidation, are formed endogenously in mammalian cells and tissues. They could also act as potent scavengers of superoxide anion radical in comparison to H_2S (Misak et al. 2018). Polysulfides enhance transcription of antioxidant genes *via* sulfuration of cysteine residue in Keap1 and stimulate Nrf2 nuclear translocation (Kimura 2015). Other forms of polysulfides include glutathione polysulfides (GSSH) and cysteine polysulfides. GSSH can be produced by addition of sulfur atom to glutathione from H_2S by sulfide quinone oxidoreductase (SQR) action in mitochondria. GSSH are highly nucleophilic and reducing agents. They can reduce cytochrome c and scavenge hydrogen peroxide directly more efficiently than glutathione. Cysteine polysulfides are generated from the reaction of H_2S with cysteine and have an action similar to GSSH (Koike et al. 2017). All previous mechanisms concerning the effects of both H_2S and its products; polysulfides on ROS production and antioxidant gene expression are compatible with the decreased lung lipid peroxides associated with increased lung TAC levels in our results which is also in agreement with Campos et al. (2016), Zhang et al. (2016), Li et al. (2016) and Song and Wang (2016).

Kang et al. (2017) reported that H_2S decreased proinflammatory cytokines by suppressing the activation of NF-κB and MAPK dependent signaling and this is compatible with decreased serum IL-6 level with NaHS administration in the current study. A previous study documented that H_2S donor could inhibit the activity of NF-κB by inhibiting the degradation of its inhibitor nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα). It also inhibited the activity of all MAPK members including p38 MAPK, extracellular regulated kinase (ERK) and c-Jun NH2-terminal kinase (JNK) by inhibiting their phosphorylation (Wu et al. 2015). Recently, Shin et al. (2017) documented that suppression of NF-κB and MAPK pathways may contribute to attenuation of LPS induced inflammation in ALI. Thus, it has been suggested that inhibitors of both pathways may be useful as anti-inflammatory agents.

Administration of NaHS resulted in significant inhibition of lung iNOS expression in comparison with ALI group in this study which is compatible with Song and Wang (2016) who found that H_2S attenuated lung inflammation by inhibiting iNOS expression and reducing the production of pro-inflammatory NO. The possible mechanism is *via* overexpression of HO-1. The upregulated HO-1could suppressed NF-κB signaling pathway and pro-inflammatory cytokines induced iNOS expression (Hristova et al. 2016; Yang et al. 2017). Previous studies reported that H_2S has enhanced the activity of endothelial nitric oxide synthase (eNOS) by facilitating phosphorylation of its active site and dephosphorylation of the inhibitory site to increase the production of anti-inflammatory NO. This was confirmed when these effects were not observed in CSE knockout mice. On the other hand, it was found that NO increased H_2S production through enhancing expression of CSE enzyme. All previous effects indicate that there is a cross talk between H_2S and NO in inflammation (Miyamoto et al. 2017).

The present results show that lung inflammatory response and oxidative stress were exacerbated by the administration of PAG as compared to ALI group which is in agreement with Ning et al. (2013), Qi et al. (2014) and Jin et al. (2015). Recently, Tang et al. (2017) reported that inhibition of H_2S

synthesizing enzyme; CSE by PAG has resulted in reduction of plasma H_2S levels which was associated with aggravation of severity of lung inflammation, increase in lung myeloperoxidase activity and increase in plasma levels of tumor necrosis factor α, IL-6, and interleukin 1β . Wu et al. (2013) reported that NaHS administration significantly decreased lipid peroxidation, myeloperoxidase activity and interleukin 1β but increased interleukin 10 levels in lung tissues. In contrast, PAG administration further exacerbated lung injuries. Moreover, Meng et al. (2017) also documented that PAG has exacerbated lung damage through reduction of endogenous H_2S level, CSE expression and Nrf2 nuclear translocation which was associated with increased oxidative stress markers.

Zhao et al. (2014) reported that H_2S donors have been widely used in the field. These compounds are not only useful research tools, but also potential therapeutic agents. However, there are limitations associated with the choice of donors used to generate H_2S either *in vitro* or *in vivo*. The current available donors include H_2S gas, sulfide salts as NaHS and sodium sulfide (Na_2S), garlic-derived sulfur compounds, Lawesson's reagent/analogs, 1,2-dithiole-3-thiones and H_2S-hybrid nonsteroidal anti-inflammatory drugs, thiol-activated H_2S donors, photo-caged donors and thiamine acids.

Wallace and Wang (2015) elucidated that several attempts were done to develop novel therapeutics that aimed to release H_2S. Several trails that focus on exploiting the potent anti-inflammatory and cytoprotective actions of H_2S have been founded. H_2S-releasing derivatives of a number of drugs have been developed with a major focus on non-steroidal anti-inflammatory drugs (NSAIDs). The main actions of these drugs are analgesic and anti-inflammatory with less exposure to gastrointestinal ulceration that is normally caused by NSAIDs due to the benefit of H_2S release (Chattopadhyay et al. 2012). GYY4137, the slow releasing H_2S donor, is now widely used as a research tool to study the effects of H_2S. It has been shown to exert anti-inflammatory actions through its ability to reduce circulating levels of various pro-inflammatory cytokines and mediators (Li et al. 2009). *N*-acetylcysteine is a mucolytic drug used in the treatment of cystic and pulmonary fibrosis and also as an antidote for acetaminophen-induced liver damage. This drug can also generate H_2S and has been shown to elicit marked anti-inflammatory effects in rodents (Zanardo et al. 2006).

In conclusion, the present study proved that H_2S has a protective effect against LPS induced ALI due to its anti-nitrative, anti-oxidant and anti-inflammatory properties. These are based upon our results which include its suppressive effect on iNOS expression, decreasing the levels of both serum IL6 and lung lipid peroxides that were accompanied by increased TAC level in the lung.

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