European Journal of Pharmacology 661 (2011) 102–108

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Pulmonary, Gastrointestinal and Urogenital Pharmacology

Protective effects of hemin in an experimental model of ventilator-induced lung injury

Li An [1], Chang-Ting Liu [1], Xue-Bing Qin, Qing-Hui Liu, Yan Liu, Sen-Yang Yu *

Department of Pulmonary Medicine, Chinese PLA General Hospital, Beijing 100853, China

ARTICLE INFO

Article history:
Received 4 October 2010
Received in revised form 6 April 2011
Accepted 14 April 2011
Available online 28 April 2011

Keywords:
Ventilator-induced lung injury
Mechanical ventilation
Heme oxygenase-1
Oxidative stress
Inflammation

ABSTRACT

Mechanical ventilation is an indispensable life-support modality for critically ill patients with acute lung injury or acute respiratory distress syndrome. Unfortunately, mechanical ventilation even the protective ventilation strategies may evoke ventilator-induced lung injury. Heme oxygenase-1 (HO-1) has recently exhibited anti-inflammatory and anti-oxidative properties in vitro and in vivo. The effect of HO-1 in ventilator-induced lung injury has not been fully characterized. In this study, rabbits were subjected to high tidal volume ventilation to induce ventilator-induced lung injury, which was confirmed by histopathological alterations, increased bronchoalveolar lavage fluid protein content and lung wet-to-dry ratio. In contrast to the level of HO-1 expression in high tidal volume group, pretreatment with hemin, an inducer of HO-1, further up-regulated HO-1 expression. At the same time, these lung injury indexes were attenuated markedly. This pulmonary protection was accompanied by a decrease in bronchoalveolar lavage fluid neutrophil count and in lung myeloperoxidase activity. Besides, pretreatment with hemin prohibited the production of proinflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-8, and up-regulated the level of anti-inflammatory cytokine interleukin (IL)-10 in bronchoalveolar lavage fluid. Furthermore, a decreased malondialdehyde activity, a marker of oxidative stress and a robust increase in total antioxidant capacity were observed in hemin-treated animals. Our findings suggest that HO-1 up-regulation by hemin plays a protective role in ventilator-induced lung injury by suppression inflammatory process and oxidative stress.

1. Introduction

Acute lung injury or acute respiratory distress syndrome remains a major reason for intensive care unit admission. Mechanical ventilation is an indispensable therapy for acute lung injury or acute respiratory distress syndrome. Unfortunately, despite its lifesaving effects, mechanical ventilation may initiate or exacerbate lung damage in both healthy and diseased lungs, which is termed as ventilator-induced lung injury (VILI). Traditional high tidal volume ventilation causes pulmonary edema, neutrophil infiltration and alveolar-capillary barrier disruption, which are the hallmarks of ventilator-induced lung injury. Ventilation with low tidal volume has been designed to protect lungs from excessive stretch. But even low tidal volume cannot completely avoid regional alveolar distension for the highly spatial heterogeneity in the lungs of acute lung injury/acute respiratory distress syndrome (Mertens et al., 2009). In fact, there are still patients at risk of ventilator-induced lung injury despite this protective strategy. Therefore, adjuvant pharmacologic strategies based on pathophysiology are necessary to further eliminate or alleviate ventilator-induced lung injury.

The mechanisms responsible for ventilator-induced lung injury are complex. Redox imbalance attributable to overproduction of reactive oxygen species that overwhelm the antioxidant capacity has been suggested to involve in the development of ventilator-induced lung injury. In epithelial and endothelial cells, cyclic stretch has been observed to cause reactive oxygen species generation in a magnitude-dependant manner (Chapman et al., 2005; Birukov, 2009). A reduction in superoxide dismutase (SOD) and catalase along with an increase in malondialdehyde (MDA) was reported in rat lungs exposed to high tidal volume ventilation (Marín-Corral et al., 2010). In addition, injurious mechanical ventilation has been suggested to stimulate local inflammation reaction, characterized by neutrophil accumulation and inflammatory cytokines production, which also play a critical role in the development of ventilator-induced lung injury (Halbertsma et al., 2005).

Among the innate defenses of the lung, heme oxygenase-1 (HO-1) is a major inducible stress protein. HO-1 has been revealed to respond rapidly to diverse stimuli, including heat, oxidative stress, hypoxia, heavy metals, and several inflammatory cytokines (Ryter and Choi, 2009). HO-1 catalyzes the first and rate-limiting step in the oxidative degradation of heme to carbon monoxide (CO), biliverdin/bilirubin, and free iron. In the past few years, HO-1 and its end-products have

* Corresponding author at: Chinese PLA General Hospital, Department of Pulmonary Medicine, No. 28, Fuxing Road, Beijing 100853, China. Tel.: +86 10 66876232; fax: +86 10 66876250.
E-mail address: autumn2004@yahoo.cn (S.-Y. Yu).
[1] These authors contributed equally to the work.

0014-2999/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2011.04.032

demonstrated a multi-faced cellular protective role in vitro and in vivo. Enhanced HO-1 expression has showed to attenuate endotoxin-induced lung injury in mice by inhibiting tumor necrosis factor (TNF)-α and high-mobility group protein B1 (HMGB1) releasing (Gong et al., 2008). At low concentration, carbon monoxide inhalation has been observed to inhibit hyperoxia-induced reactive oxygen species production and endothelial cell death (Wang et al., 2007).

The role of HO-1 in the modulation of ventilator-induced lung injury has not been fully investigated. Therefore, in this study, we studied the effect of hemin as an HO-1 inducer on the lung injury caused by high tidal volume ventilation. We demonstrate that most signs of lung injury were alleviated upon hemin pretreatment, suggesting HO-1 as a promising pharmacologic target in ventilator-induced lung injury.

2. Materials and methods

2.1. Animals

Male New Zealand white rabbits (mean weight 2.50 ± 0.32 kg) were purchased from the experimental animal center of our hospital. They were fed with food and water ad libitum and were housed under controlled condition. The study was performed in accordance with the animal experimental guidelines of the national institute of health and with approval of the research committee of our hospital.

2.2. Drugs and solutions

Ferriprotoporphyrin IX chloride (hemin, Sigma, USA) was dissolved in 0.2 M NaOH, neutralized with 1 M HCL, adjusted to 0.5 mg/ml concentration with phosphate-buffered saline (PBS), and sterilized by filtration.

2.3. Experimental procedure

Rabbits were anesthetized with pentobarbital sodium (30 mg/kg) administered through the ear vein and additional anesthetic was given as needed. Tracheotomy was performed and a canula was inserted into the trachea. The animals were subjected to a rodent ventilator (Harvard Apparatus, Holliston, MA, USA) with initial parameters of a low tidal volume of 8 ml/kg, a positive end expiratory pressure (PEEP) of 2 cm H_2O, frequency of 40/min and inspiratory oxygen fraction of 40%, defined as normal ventilation in rabbits. After 30 min of initial ventilation, rabbits (n = 24) were randomly divided into 3 groups, each with 8 rabbits. (1) low tidal volume group: rabbits were ventilated with the initial ventilator settings for 4 h. (2) high tidal volume group: rabbits were ventilated with a high tidal volume (40 ml/kg, 8 breaths per min, inspiration:expiration = 1:2 and 0 PEEP) for 4 h. (3) Hemin pretreatment group: Hemin was administered intraperitoneally 48 h prior to the experiment (40 μmol/kg, 2 times/day, 2 days).Ventilator settings were the same as high tidal volume group.

2.4. Measurements

2.4.1. Bronchoalveolar lavage fluid neutrophil count and protein assay

The left lung was lavaged with 5 ml physiological saline for 3 times. The recovered volume was centrifuged and the supernatant was analyzed for protein concentration. Cell deposits were resuspended in 1 ml of saline, the neutrophils were counted by using giemsa staining.

2.4.2. Wet-to-dry ratio of the lung tissue

The wet weight of the total left lung was first determined. The dry weight was measured after desiccation for 24 h in an oven at 80 °C. The wet-to-dry ratio was calculated by dividing the wet by the dry weight to assess lung water accumulation.

2.4.3. Histological assessment of lung injury

The posterior portion of the right lower lobe was excised and immersed in 10% formaldehyde for at least 24 h, then embedded in paraffin, sliced at 4 μm and stained with hematoxylin and eosin. A semi-quantitative morphometric analysis of lung injury was performed using five grades from 0 to 4 (minimal, mild, moderate, severe, maximal) for 4 items: alveolar capillary congestion, hemorrhage, infiltration or aggregation of neutrophils infiltration into airspace or the vessel wall, thickness of the alveolar wall/hyaline membrane formation (Kim et al., 2008). A total histological lung injury score was obtained by adding the individual scores in five high-power fields (magnification ×400) randomly. Comparison was made by averaging the total values in each group.

2.4.4. Lung tissue malondialdehyde (MDA), total antioxidative capacity and myeloperoxidase (MPO) assay

Pulmonary malondialdehyde, total antioxidative capacity and myeloperoxidase activity were determined with commercial kits (Nanjing Jiancheng Bioengineering Institute, China). Lung tissue was homogenized and the supernatant was used for measurement. Oxidative stress was evaluated through the levels of malondialdehyde and total antioxidative capacity in lung tissue homogenates as an indicator of lipid peroxidation and antioxidant capacity, respectively. To determine malondialdehyde level, the samples were treated with thiobarbituric acid (TBA), which reacted with malondialdehyde to produce a red complex. The concentration of malondialdehyde was calculated by the absorbance at 532 nm. The total antioxidative capacity of the lung tissue was the sum of enzyme (superoxide dismutase, catalase, glutathione) and non-enzymatic antioxidant (bilirubin, uric acid). The total antioxidative capacity kit was based on the ability of antioxidants in samples to reduce Fe^{3+} to Fe^{2+}, which was then chelated with porphyrin to produce a purple complex. The total antioxidative capacity was quantified by measuring the absorbance at 520 nm. Lung tissue myeloperoxidase activity was measured to evaluate activated neutrophil accumulation. Myeloperoxidase activity was measured from the absorbance (at 460 nm) changes resulting from decomposition of H_2O_2 in the presence of *o*-dianisidine.

2.4.5. Enzyme-linked immunosorbent assay (ELISA) for cytokines measurement

The sandwich ELISA kits (Invitrogen Corporation, CA) were employed to measure TNF-α, IL-8 and IL-10 in bronchoalveolar lavage fluid. Each sample was processed according to the manufacturer's instructions. Results were expressed as picograms per milliliter bronchoalveolar lavage fluid.

2.4.6. mRNA and protein expression of HO-1

For isolation of the total RNA from tissue samples, lung tissue (100 mg) was homogenized in 1 ml TRIZOL (Invitrogen Life Technologies, Carlsbad, CA). Five micrograms of total RNA was used to synthesize cDNA with a Reverse Transcription Reagents kit (Tianwei Biotechnology, Beijing, China). Polymerase chain reaction (PCR) was done with GeneAmp PCR 2400 system (Perkin Elmer, US). The cycling reactions containing 1 μl cDNA, 10 μl 2× Taq PCR MasterMix (Tianwei Biotechnology, Beijing, China), 7 μl double distilled water, 1 μl of HO-1 forward primer (5′-CAGGTGACTGCCGAGGGTTTTA-3′) and 1 μl of HO-1 reverse primer (5′-GGAAGTAGAGCGGGGCGTAG-3′). β-actin was used as the standard gene with a forward primer (5′-CCCATCTACGAGGGCTACGC-3′) and a reverse primer (5′-CAGGAAGGAGGGCTGGAACA-3′). PCR samples were run on a 1.5% agarose gel and photographed. Densitometric analyses were performed using the Image software.

To test the protein expression of HO-1, lung tissue segments were homogenized in ice-cold. Then the homogenates were centrifuged at 10,000 *g* for 10 min at 4 °C. An aliquot of the supernatant was used to

determine protein concentration. Protein aliquots were mixed with 4× buffer and were electrophoresed on 10% sodium dodecyl sulfate–polyacrylamide gels and transferred electrophoretically on nitrocellulose transfer membranes. The membranes were then incubated with 1:1000 anti-HO-1 (ABcam, UK) in 5% non-fat dry milk overnight at 4 °C and then washed with TBST. The membranes were later incubated with horseradish peroxidase-conjugated goat anti-rabbit antibody (1:2000 dilution) for 50 min at room temperature and washed with TBST. The blots were visualized with the ECL Western blot detection system (Applygen Technologies Inc, China). Autoradiogram signals were quantified by densitometry scanning, and the values were normalized to the housekeeping β-actin.

2.4.7. Immunohistochemistry for HO-1

Immunohistochemical stains were performed applying a two-step streptavidin-peroxidase (SP) technique to detect the distribution of HO-1. The slides were deparaffinized routinely. Then the endogenous peroxidase was sealed by hydroperoxide and unspecial antigen combining sites were blocked by goat serum. Slides were incubated with anti-HO-1 primary antibody (1:250) at 4 °C overnight. Then the specimens were further incubated with anti-rabbit second antibody conjugated with horseradish peroxidase (Zhongshan Golden Bridge Biotechnology, China) at 37 °C in wet chamber for 20 min. Finally, sections were detected with a diaminobenzidine (DAB) substrate mixture and counterstained by hematoxylin. A dark-brown DAB signal indicated positive staining of HO-1.

2.5. Statistical analysis

For statistical analysis we used the statistical software SPSS 13.0. Results were presented as mean ± standard error (S.D.). Comparisons of continuous variables between groups were performed using *t* test and one-way analysis of variance. Statistical difference was defined as a P value <0.05.

3. Results

3.1. Hemin up-regulates HO-1 mRNA and protein expression

In our study, the mRNA and protein expression of HO-1 were up-regulated after high tidal volume ventilation for 4 h, while very low HO-1 expression could be detected in rabbits received low tidal volume ventilation. Administration of hemin, a strong inducer of HO-1, caused greater HO-1 mRNA (Fig. 1A) and protein expression (Fig. 1B).

Immunohistochemistry staining was used to reveal the distribution of HO-1. In the low tidal volume group, the immunoreactivity of HO-1 was minimal (Fig. 2A). The high tidal volume group showed an elevated expression of HO-1, and the positive staining was found in neutrophils and macrophages. Besides, a moderate expression of HO-1 was observed in alveolar epithelial cells (Fig. 2B). The hemin group showed the highest level of HO-1 expression (Fig. 2C).

3.2. Hemin administration alleviates oxidative stress in ventilator-induced lung injury

Malondialdehyde at low level was measured in the lung from low tidal volume group. In contrast, malondialdehyde level increased 3-fold in high tidal volume group when compared to low tidal volume group, indicating injurious ventilation provoked an oxidative stress in the lung. Pretreatment with hemin decreased malondialdehyde level by 46% compared with high tidal volume group (Fig. 3A).

Total antioxidant capacity is the representative of enzyme and nonenzyme antioxidants of the lung tissue. Compared with rabbits received low tidal volume ventilation, high tidal volume ventilation provoked a slight increase in the total antioxidant capacity. In contrast, hemin treatment prior to high tidal volume ventilation produced a robust enhancement in total antioxidant capacity in hemin pretreatment group, which was nearly 2-fold of that in high tidal volume group (Fig. 3B). The results suggested that the up-regulation of HO-1 expression by hemin therapy is associated with an increase in antioxidants and a decrease in oxidative stress.

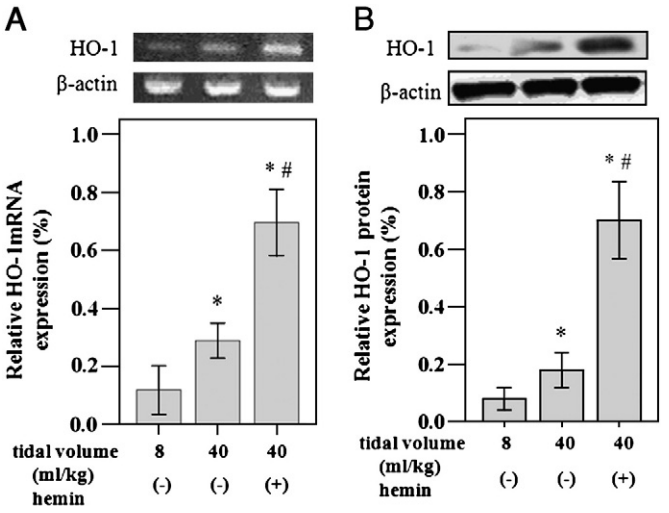

Fig. 1. Effects of ventilation and hemin pretreatment on the expression of HO-1 mRNA and protein in lung tissue. (A) Representative RT-PCR for HO-1 and densitometric analysis of HO-1/β-actin. HO-1 mRNA expression was significantly elevated by high tidal volume ventilation compared with that by low tidal volume ventilation. Hemin treatment enhanced HO-1 mRNA expression in rabbits receiving high tidal volume ventilation. (B) Representative Western immunoblot for HO-1 and quantitative analysis of relative HO-1 levels, normalized by β-actin. HO-1 protein expression was significantly elevated by high tidal volume ventilation compared with that by low volume ventilation. Hemin therapy greatly enhanced HO-1 protein expression in the rabbits receiving high tidal volume ventilation. Data represent mean ± S.D.; n = 8 rabbits/group. * $P<0.05$ vs. low tidal volume group; # $P<0.05$ vs. high tidal volume group.

3.3. Hemin therapy suppresses neutrophil infiltration and cytokine release

Activated neutrophils contribute to the development of ventilator-induced lung injury. The bronchoalveolar lavage fluid study demonstrated a significant elevation of neutrophil count in high tidal volume group compared with in low tidal volume group. Hemin pretreatment dramatically decreased neutrophil counts in rabbits received high tidal volume ventilation by more than 50% (Fig. 4A). We also examined neutrophil activity using myeloperoxidase assay. Likewise, exposure to high tidal volume ventilation induced a significant rise in myeloperoxidase activity. Administration of hemin prior to high tidal volume ventilation significantly reduced myeloperoxidase activity compared with rabbits subjected to high tidal volume ventilation alone, the reduction magnitude was about 36% (Fig. 4B).

Pulmonary cytokine production is potential effector molecules that modulate inflammatory response aggravating lung injury. We therefore examined the expression of TNF-α and IL-8, two important pro-inflammatory cytokines during high tidal volume ventilation. Both TNF-α and IL-8 rose markedly in high tidal volume group when compared with low tidal volume group. Hemin pretreatment markedly suppressed TNF-α and IL-8 production induced by high tidal volume ventilation. IL-10 was measured as a representative anti-inflammatory cytokine. In contrast to a markedly increase in pro-inflammatory cytokines, there was only a slight increase in IL-10 level in high tidal volume group, indicating a pro-inflammtory shift caused by high tidal volume ventilation. However, ELISA analysis revealed a significant up-regulation in IL-10 level in hemin-treated rabbits followed high tidal volume ventilation (Fig. 5).

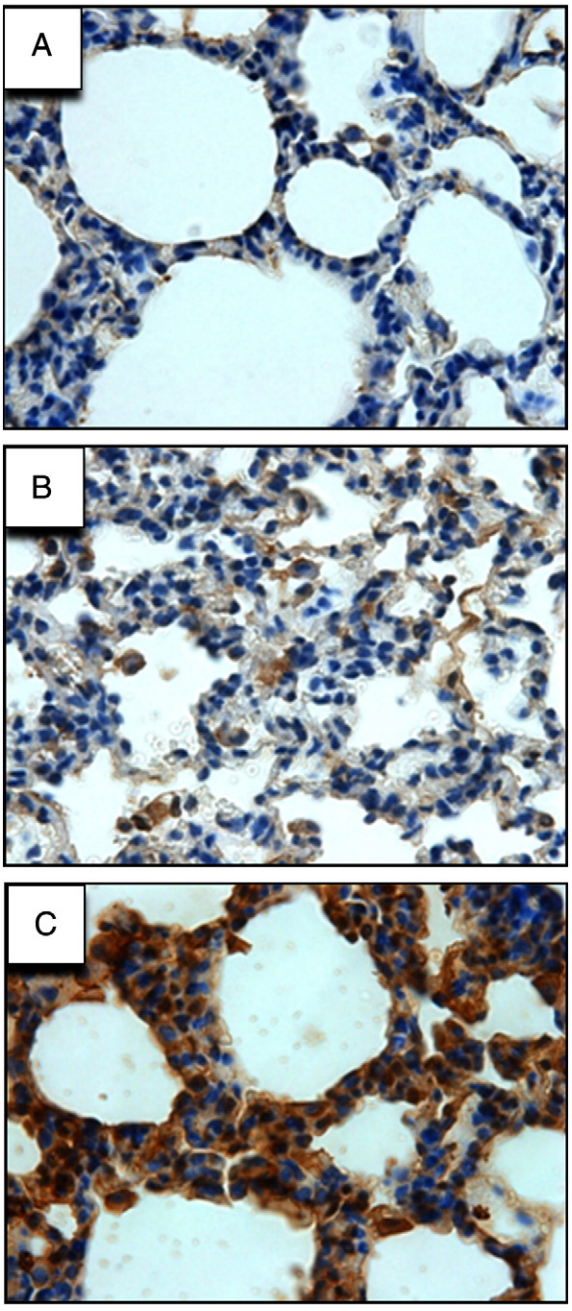

Fig. 2. Immunodetection of HO-1 localization in paraffin sections of lungs. (A) Rabbits received low tidal volume ventilation showed a mild expression of HO-1, mainly in epithelial cells. (B) Moderate staining of HO-1 was found in high tidal volume ventilated rabbits, HO-1 positive stained cells were found in neutrophils, macrophages and alveolar epithelial cells in ventilator-induced lung injury group. (C) A strong staining of HO-1 was found in hemin group. (A–C) Magnification (×400).

Taken together, these results demonstrate that hemin therapy may greatly suppress the inflammatory response provoked by high tidal volume ventilation through up-regulation of HO-1 expression.

3.4. Hemin therapy abrogates vascular permeability and lung injury

We assessed lung vascular permeability using bronchoalveolar lavage fluid protein assay and lung wet-to-dry ratio. We found a marked increase in bronchoalveolar lavage fluid protein level and lung wet-to-dry ratio in high tidal volume group as compared with

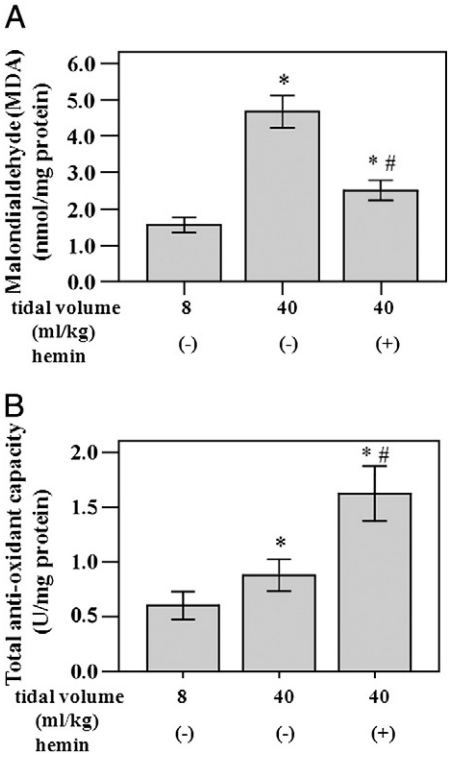

Fig. 3. Effect of hemin pretreatment on oxidative stress. (A) Basal levels of malondialdehyde in low tidal volume group were comparably low, whereas malondialdehyde in high tidal volume group was significantly elevated. Hemin therapy greatly attenuated malondialdehyde level. (B) The total antioxidant capacity in the lungs subjected to high tidal volume ventilation was elevated compared with that in rabbits receiving low tidal volume ventilation. Hemin treatment greatly enhanced the total antioxidant capacity in rabbits subjected to high tidal volume ventilation. Data represent mean ± S.D.; n = 8 rabbits/group. * $P<0.05$ vs. low tidal volume group; # $P<0.05$ vs. high tidal volume group.

low tidal volume group. Rabbits received hemin prior to high tidal volume ventilation exhibited a significantly decreased level of bronchoalveolar lavage fluid protein concentration as well as lung wet-to-dry ratio, the magnitude of reduction was 50% and 25%, respectively (Fig. 6A, B). These data reflect a decreased lung vascular permeability by hemin pretreatment.

As reduced vascular permeability suggests protection from ventilator-induced lung injury, we next examined histological alterations in different groups. Lungs received low tidal volume ventilation were relatively normal at light microscopy. In contrast, there were obvious lung lesions including interstitial and alveolar edema, neutrophil infiltration and pulmonary hemorrhage in high tidal volume group. These changes were ameliorated markedly by hemin treatment (Fig. 7A, B and C). The above described microstructural alterations were quantified using a histological grading system in our study. The lung injury score in low tidal volume ventilation, high tidal volume ventilation, and hemin pretreatment group were 1.75 ± 0.89, 8.13 ± 0.99 and 4.38 ± 1.19 respectively, with significant differences between the groups (Fig. 8).

4. Discussion

Our data indicate that HO-1 overexpression by hemin pretreatment is protective against ventilator-induced lung injury. Injurious ventilation with high tidal volume without positive end expiratory pressure resulted in remarkable lung injury in rabbit, manifested by increased lung edema, elevated bronchoalveolar lavage fluid protein content and severe histological damage. In contrast, HO-1 up-regulation by hemin therapy reduced all above lung injury indexes markedly, with a significantly decreased oxidative stress and inflammatory process.

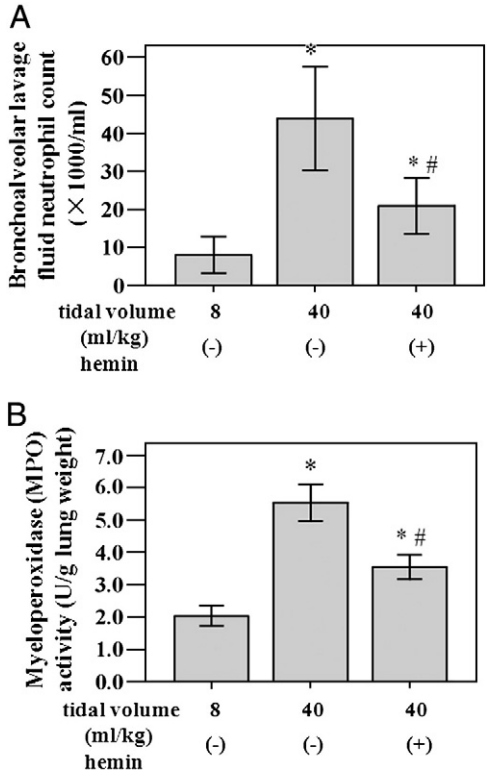

Fig. 4. The levels of neutrophil infiltration and myeloperoxidase activity in the lungs. (A) Bronchoalveolar lavage fluid neutrophil count was increased in high tidal volume group compared with that in low tidal volume group. Hemin therapy greatly attenuated bronchoalveolar lavage fluid neutrophil count. (B) Increased neutrophil infiltration in high tidal volume group was also detected with myeloperoxidase assay. Hemin treatment greatly decreased myeloperoxidase activity. Data represent mean ± S.D.; n = 8 rabbits/group. * $P<0.05$ vs. low tidal volume group; # $P<0.05$ vs. high tidal volume group.

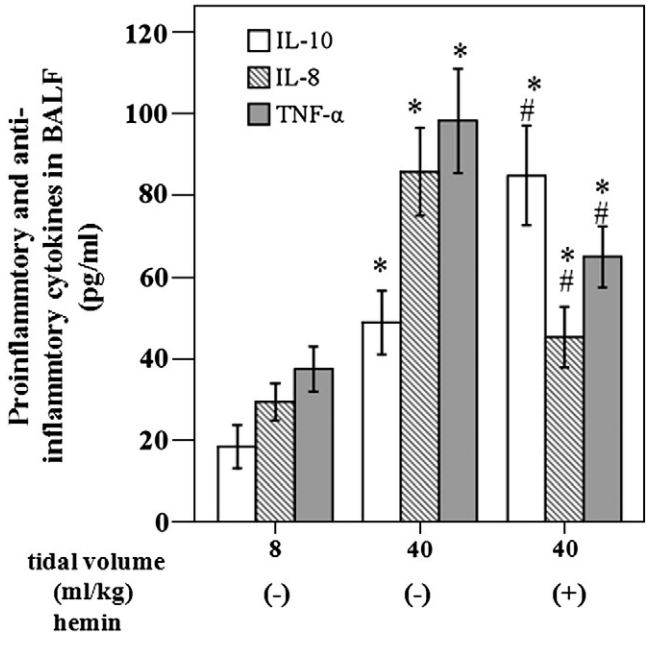

Fig. 5. Proinflammatory and anti-inflammatory cytokines in bronchoalveolar lavage fluid. High tidal volume ventilation induced a robust increase in proinflammatory cytokine levels (TNF-α and IL-8) and a slight increase in anti-inflammatory cytokine (IL-10) when compared with low tidal volume ventilation. Pretreatment of hemin caused a significant down-regulation of TNF-α and IL-8 along with an obvious up-regulation of IL-10. Data represent mean ± S.D.; n = 8 rabbits/group. * $P<0.05$ vs. low tidal volume group; # $P<0.05$ vs. high tidal volume group.

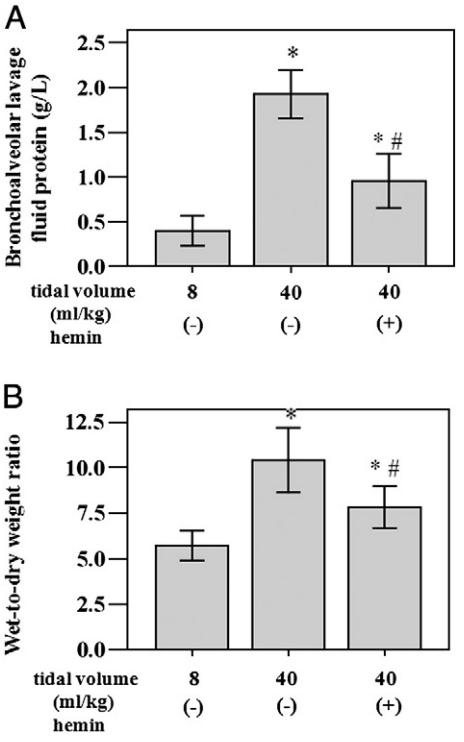

Fig. 6. Reduced pulmonary edema in hemin treated rabbits upon high tidal volume ventilation. (A) The total protein concentration in bronchoalveolar lavage fluid was elevated in high tidal volume group compared with that in low tidal volume group. Hemin treatment significantly decreased protein concentration in bronchoalveolar lavage fluid. (B) Lung wet-to-dry ratio was elevated in high tidal volume group compared with that in low tidal volume group. Hemin treatment markedly decreased lung wet-to-dry ratio upon high tidal volume ventilation. Data represent mean ± S.D.; n = 8 rabbits/group. * $P<0.05$ vs. low tidal volume group; # $P<0.05$ vs. high tidal volume group.

As an inducible stress protein, HO-1 induction is considered to be an adaptative cellular response to hazardous stimuli. Researchers have observed that HO-1 is increased in response to oxidative stress, hypoxia, heavy metals, and several inflammatory cytokines (Ryter and Choi, 2009). Exogenous up-regulation of HO-1 has showed beneficial role in various models of cellular and tissue injury recently (Abraham, 2003; Soares and Bach, 2009). As shown here, high tidal volume ventilation by itself was able to induce HO-1 expression both at mRNA and protein level. But this expression up-regulation was not robust enough to protect the rabbits from ventilator-induced lung injury development. However, our experiment clearly showed that pretreatment with hemin abundantly increased the expression of HO-1, resulting in significantly alleviated high tidal volume ventilation-associated pulmonary edema and histological damage, indicating a protective role of hemin against ventilator-induced lung injury. Since hemin is usually utilized as an inducer of HO-1, it seems reasonable to conclude that the demonstrated benefit effect of hemin is partly through HO-1 up-regulation. This is in accordance with another study by Zhao and Hu (2010). In their rat model of ventilator-induced lung injury, preinduction of HO-1 by hemin was associated with alleviated ventilator-induced lung injury, whereas administration of ZnPP (an HO-1 inhibitor) plus hemin abolished the protective effects of HO-1. Moreover, Hoetzel A and colleagues also illustrated that inhalation of carbon monoxide, a byproduct of HO-1 activity, prevented lung inflammation and lung injury following mechanical ventilation (Hoetzel et al., 2008). Taken together, these results suggest that induction of HO-1 may play a critical role in the protection against ventilator-induced lung injury.

Inflammatory response induced by deforming stress contributes to ventilator-induced lung injury. Neutrophil recruitment and activation are thought to play an important role in the development of ventilator-

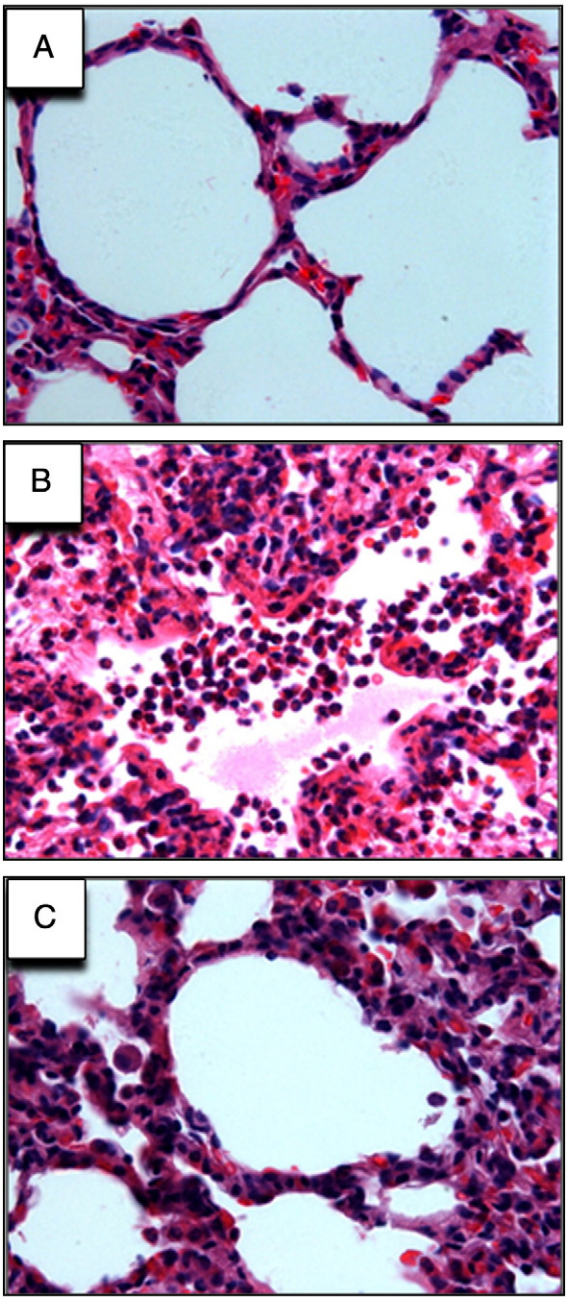

Fig. 7. The effect of therapeutic treatment with hemin on morphological changes in the lungs upon high tidal volume ventilation. (A) Lungs received low tidal volume ventilation were relatively normal at light microscopy. (B) Lungs subjected to high tidal volume ventilation exhibit alveolar wall thickening, inflammatory infiltrates, and interstitial and alveolar hemorrhage. (C) Treatment with hemin significantly attenuated lung injury. (A–C) Magnification (×400).

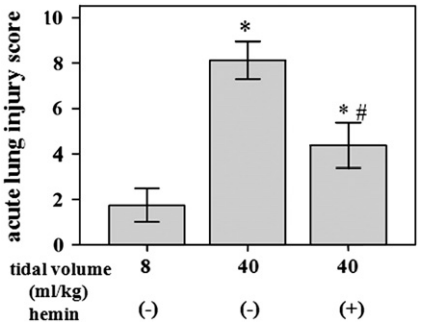

Fig. 8. Acute lung injury score in each group. Lung injury score in high tidal volume group was higher than that in low tidal volume group. Pretreatment of hemin decreased lung injury score significantly. Data represent mean ± S.D.; n = 8 rabbits/group. * $P<0.05$ vs. low tidal volume group; # $P<0.05$ vs. high tidal volume group.

induced lung injury (Hu et al., 2010). Kawano observed a remarkably attenuated degree of ventilator-induced lung injury in neutrophil-depleted animals (Kawano et al., 1987). Another study by Karzai demonstrated a worsened ventilator-induced lung injury after neutrophil stimulation with granulocyte colony stimulating factors (Karzai et al., 2005). As showed in our experiment, neutrophils migrated into lungs subjected to high tidal volume ventilation as manifested by an elevation in bronchoalveolar lavage fluid neutrophil count and lung myeloperoxidase activity. In addition, we also revealed that hemin-induced HO-1 overexpression attenuated this neutrophil infiltration caused by high tidal volume ventilation. Since neutrophils are believed to be the key inflammatory cell in ventilator-induced lung injury, inhibition of neutrophil recruitment to lungs may account in part for the alleviation of ventilator-induced lung injury by hemin.

Release of inflammatory mediators, apart from neutrophil accumulation, is another feature of ventilator-induced lung injury. The balance between pro- and anti-inflammatory cytokines is essential for directing the immune response. Recently, HO-1 has implicated as a modulator of innate immunity and inflammation (Jin and Choi, 2005; Takamiya et al., 2009). There have been previous reports that up-regulation of HO-1 contributes to protection against endotoxic shock by inhibition of TNF-α and augmentation of IL-10. (Tamion et al., 2006; Siner et al., 2007). In our experiment, there was an abundant increase in TNF-α and IL-8 in contrast to a slight increase in IL-10 in high tidal volume group, indicating a proinflammatory shift caused by high tidal volume ventilation. Furthermore, pharmacological up-regulation of HO-1 by hemin caused a markedly rise in IL-10 along with a decrease in TNF-α and IL-8, indicating its ability to inhibit inflammatory reactions. Together with previous studies, these results reveal that hemin induced HO-1 expression exerts its protective role in ventilator-induced lung injury through modulation of the inflammatory cytokines.

Beyond the inflammatory response, oxidative stress is a critical pathogenic mechanism of ventilator-induced lung injury. It is well accepted that deforming stress promotes the production of reactive oxygen species (Papaiahgari et al., 2007; Syrkina et al., 2008). In the present study, we investigate the redox status within the lung by measurement of malondialdehyde and total antioxidative capacity, which represent reactive oxygen species generation and the sum of the antioxidant in the lung respectively. Our data showed that high tidal volume ventilation caused a markedly rise in malondialdehyde while a slight increase in total antioxidative capacity, indicating an oxidative stress in the lung. In addition, hemin-induced HO-1 up-regulation decreased malondialdehyde level markedly, indicating a restriction of reactive oxygen species production. Interestingly, this was accompanied by an increase in total antioxidant capacity in hemin-treated high volume ventilated rabbits. These data indicate that up-regulation of the HO-1 may potentiate the oxidative stress in the lungs subjected to high tidal volume ventilation.

HO-1 is known to catalyze the rate-limiting step in the degradation of free heme, producing carbon monoxide, ferrous iron, and biliverdin/bilirubin. Induction of HO-1 is considered to be an adaptative survival response to oxidative stress and other toxic insults. But until now, the precise mechanism by which HO-1 exerts its protective roles is not fully elucidated. It is speculated that oxidative degradation of heme, production of anti-oxidant biliverdin/bilirubin, and sequestration of redox-active iron by ferritin could explain HO-1 mediated protection against oxidative stress (Ryter et al., 2007; Morse et al., 2009; Gozzelino et al., 2010). Besides, increased HO-1 levels lead to an enhanced

formation of carbon monoxide, which seems to be responsible for the most anti-inflammatory actions of HO-1 (Chhikara et al., 2009; Pae and Chung, 2009).

In summary, our study demonstrated a beneficial effect of hemin pretreatment on ventilator-induced lung injury via HO-1 up-regulation. These findings support the notion that HO-1 inducers may be useful in prevention of ventilator-induced lung injury.

Acknowledgements

We sincerely thank Dr. Jie Ma for her excellent technical support. This work was supported by Beijing Natural Science Foundation (No.7082088).

References

Abraham, N.G., 2003. Therapeutic applications of human heme oxygenase gene transfer and gene therapy. Curr. Pharm. Des. 9, 2513–2524.
Birukov, K.G., 2009. Cyclic stretch, reactive oxygen species, and vascular remodeling. Antioxid. Redox Signal. 11, 1651–1667.
Chapman, K.E., Sinclair, S.E., Zhuang, D., Hassid, A., Desai, L.P., Waters, C.M., 2005. Cyclic mechanical strain increases reactive oxygen species production in pulmonary epithelial cells. Am. J. Physiol. Lung Cell. Mol. Physiol. 289, L834–L841.
Chhikara, M., Wang, S., Kern, S.J., Ferreyra, G.A., Barb, J.J., Munson, P.J., Danner, R.L., 2009. Carbon monoxide blocks lipopolysaccharide-induced gene expression by interfering with proximal TLR4 to NF-kappaB signal transduction in human monocytes. PLoS One 4, e8139.
Gong, Q., Yin, H., Fang, M., Xiang, Y., Yuan, C.L., Zheng, G.Y., Yang, H., Xiong, P., Chen, G., Gong, F.L., Zheng, F., 2008. Heme oxygenase-1 upregulation significantly inhibits TNF-alpha and Hmgb1 releasing and attenuates lipopolysaccharide-induced acute lung injury in mice. Int. Immunopharmacol. 8, 792–798.
Gozzelino, R., Jeney, V., Soares, M.P., 2010. Mechanisms of cell protection by heme oxygenase-1. Annu. Rev. Pharmacol. Toxicol. 50, 23–54.
Halbertsma, F.J., Vaneker, M., Scheffer, G.J., van der Hoeven, J.G., 2005. Cytokines and biotrauma in ventilator-induced lung injury: a critical review of the literature. Neth. J. Med. 63, 382–392.
Hoetzel, A., Dolinay, T., Vallbracht, S., Zhang, Y., Kim, H.P., Ifedigbo, E., Alber, S., Kaynar, A.M., Schmidt, R., Ryter, S.W., Choi, A.M., 2008. Carbon monoxide protects against ventilator-induced lung injury via PPAR-gamma and inhibition of Egr-1. Am. J. Respir. Crit. Care Med. 177, 1223–1232.
Hu, G., Malik, A.B., Minshall, R.D., 2010. Toll-like receptor 4 mediates neutrophil sequestration and lung injury induced by endotoxin and hyperinflation. Crit. Care Med. 38, 194–201.
Jin, Y., Choi, A.M., 2005. Cytoprotection of heme oxygenase-1/carbon monoxide in lung injury. Proc. Am. Thorac. Soc. 2, 232–235.
Karzai, W., Cui, X., Heinicke, N., Niemann, C., Gerstenberger, E.P., Correa, R., Banks, S., Mehlhorn, B., Bloos, F., Reinhart, K., Eichacker, P.Q., 2005. Neutrophil stimulation with granulocyte colony-stimulating factor worsens ventilator-induced lung injury and mortality in rats. Anesthesiology 103, 996–1005.
Kawano, T., Mori, S., Cybulsky, M., Burger, R., Ballin, A., Cutz, E., Bryan, A.C., 1987. Effect of granulocyte depletion in a ventilated surfactant-depleted lung. J. Appl. Physiol. 62, 27–33.
Kim, J.H., Suk, M.H., Yoon, D.W., Kim, H.Y., Jung, K.H., Kang, E.H., Lee, S.Y., Lee, S.Y., Suh, I.B., Shin, C., Shim, J.J., In, K.H., Yoo, S.H., Kang, K.H., 2008. Inflammatory and transcriptional roles of poly (ADP-ribose) polymerase in ventilator-induced lung injury. Crit Care. 12, R108.
Marín-Corral, J., Martínez-Caro, L., Lorente, J.A., de Paula, M., Pijuan, L., Nin, N., Gea, J., Andrés, E., Barreiro, E., 2010. Redox balance and cellular inflammation in the diaphragm, limb muscles, and lungs of mechanically ventilated rats. Anesthesiology 112, 384–394.
Mertens, M., Tabuchi, A., Meissner, S., Krueger, A., Schirrmann, K., Kertzscher, U., Pries, A.R., Slutsky, A.S., Koch, E., Kuebler, W.M., 2009. Alveolar dynamics in acute lung injury: heterogeneous distension rather than cyclic opening and collapse. Crit. Care Med. 37, 2604–2611.
Morse, D., Lin, L., Choi, A.M., Ryter, S.W., 2009. Heme oxygenase-1, a critical arbitrator of cell death pathways in lung injury and disease. Free Radic. Biol. Med. 47, 1–12.
Pae, H.O., Chung, H.T., 2009. Heme oxygenase-1: its therapeutic roles in inflammatory diseases. Immune Netw. 9, 12–19.
Papaiahgari, S., Yerrapureddy, A., Reddy, S.R., Reddy, N.M., Dodd-O, J.M., Crow, M.T., Grigoryev, D.N., Barnes, K., Tuder, R.M., Yamamoto, M., Kensler, T.W., Biswal, S., Mitzner, W., Hassoun, P.M., Reddy, S.P., 2007. Genetic and pharmacologic evidence links oxidative stress to ventilator-induced lung injury in mice. Am. J. Respir. Crit. Care Med. 176, 1222–1235.
Ryter, S.W., Choi, A.M., 2009. Heme oxygenase-1/carbon monoxide: from metabolism to molecular therapy. Am. J. Respir. Cell Mol. Biol. 41, 251–260.
Ryter, S.W., Morse, D., Choi, A.M., 2007. Carbon monoxide and bilirubin: potential therapies for pulmonary/vascular injury and disease. Am. J. Respir. Cell Mol. Biol. 36, 175–182.
Siner, J.M., Jiang, G., Cohen, Z.I., Shan, P., Zhang, X., Lee, C.G., Elias, J.A., Lee, P.J., 2007. VEGF-induced heme oxygenase-1 confers cytoprotection from lethal hyperoxia in vivo. FASEB J. 21, 1422–1432.
Soares, M.P., Bach, F.H., 2009. Heme oxygenase-1: from biology to therapeutic potential. Trends Mol. Med. 15, 50–58.
Syrkina, O., Jafari, B., Hales, C.A., Quinn, D.A., 2008. Oxidant stress mediates inflammation and apoptosis in ventilator-induced lung injury. Respirology 13, 333–340.
Takamiya, R., Hung, C.C., Hall, S.R., Fukunaga, K., Nagaishi, T., Maeno, T., Owen, C., Macias, A.A., Fredenburgh, L.E., Ishizaka, A., Blumberg, R.S., Baron, R.M., Perrella, M.A., 2009. High-mobility group box 1 contributes to lethality of endotoxemia in heme oxygenase-1-deficient mice. Am. J. Respir. Cell Mol. Biol. 41, 129–135.
Tamion, F., Richard, V., Renet, S., Thuillez, C., 2006. Protective effects of heme-oxygenase expression against endotoxic shock: inhibition of tumor necrosis factor-alpha and augmentation of interleukin-10. J. Trauma 61, 1078–1084.
Wang, X., Wang, Y., Kim, H.P., Nakahira, K., Ryter, S.W., Choi, A.M., 2007. Carbon monoxide protects against hyperoxia-induced endothelial cell apoptosis by inhibiting reactive oxygen species formation. J. Biol. Chem. 282, 1718–1726.
Zhao, W.J., Hu, W.W., 2010. A study of the role of heme oxygenase-1 expression in ventilator induced lung injury and its mechanism in rats. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue 22, 410–413.