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Exogenous ghrelin ameliorates acute lung injury by modulating the nuclear factor κB inhibitor kinase/nuclear factor κB inhibitor/nuclear factor κB pathway after hemorrhagic shock

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ABSTRACT

Previous studies have shown that ghrelin, a peptide produced in the stomach, attenuates acute lung injury (ALI) in various animal models, and that some of these effects are associated with inhibition of the nuclear factor κB signaling pathway. This study investigated whether ghrelin exerts beneficial effects on hemorrhagic shock (HS)-induced ALI by modulating nuclear factor κB inhibitor kinase/nuclear factor κB inhibitor/nuclear factor κB (IKK/IκBα/NF-κB) pathway activity. HS was induced in male SD rats by withdrawing blood to a mean arterial pressure (MAP) of 40 mm Hg for 1 h; rats then received ghrelin (10 nmol/kg) or vehicle intravenously and were resuscitated with the shed blood and an equal volume of Ringer lactate solution followed by observation for 2 h. After resuscitation, samples were collected and analyzed for lung histopathology, wet to dry weight ratio (W/D), bronchoalveolar lavage fluid (BALF) protein, neutrophil infiltration, plasma inflammatory cytokines (TNF-α and IL-6), and cytoplasmic phosphorylated IKKβ, IκBα, phosphorylated IκBα and nuclear NF-κB expression. Compared to those in the two sham groups, lung injury, W/D, BALF protein, neutrophil infiltration, plasma TNF-α and IL-6 levels, and IKK/IκBα/NF-κB pathway activation were significantly increased in HS rats. After ghrelin administration, all parameters analyzed were decreased compared to those without ghrelin in HS rats. Moreover, ghrelin alleviated the decreased MAP after resuscitation compared to that in HS rats. Exogenous ghrelin attenuates the inflammatory response and acute lung injury after HS. These beneficial effects appear to be mediated through inhibition of IKK/IκBα/NF-κB signaling.

1. Introduction

Hemorrhage is the most common cause of death after trauma, contributing to 30–40% of trauma-related mortality [1,2]; an estimated 1.5 million deaths per year worldwide result from hemorrhage after physical trauma [3]. Hemorrhagic shock (HS) and resuscitation trigger an uncontrolled systemic inflammatory response that can lead to the development of multiple organ failure [4]. In patients developing multiple organ failure, acute lung injury (ALI) and its severe form acute respiratory distress syndrome (ARDS) are thought to further promote the development of other organ dysfunction and death [5].

The excessive production of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and neutrophil sequestration and activation play a central role in the pathogenesis of ALI and ARDS [6,7]. The activation of nuclear factor κB (NF-κB) signaling was demonstrated to have important functions in the development of ALI after hemorrhage because inhibition of its activation is associated with decreased expression of proinflammatory cytokines and the sequestration of neutrophils [8,9].

Ghrelin, a 28-amino-acid acylated peptide produced and secreted predominantly by the X/A-like enteroendocrine cells of the stomach [10], is a novel endogenous ligand for the growth hormone secretagogue receptor (GHS-R) [11]. Previous studies reported that ghrelin inhibits proinflammatory cytokine production, mononuclear cell binding, and NF-κB activation in human endothelial cells in vitro, as well as endotoxin-induced cytokine production in vivo [12]. This suggests an anti-inflammatory function for ghrelin. Wu and coworkers [13] for the first time reported that exogenous ghrelin attenuates sepsis-induced ALI and decreases pulmonary levels of proinflammatory cytokines in rats, and that the protective effect of ghrelin was mediated by inhibition of NF-κB activation.

Although the beneficial effects of ghrelin in the lung have been

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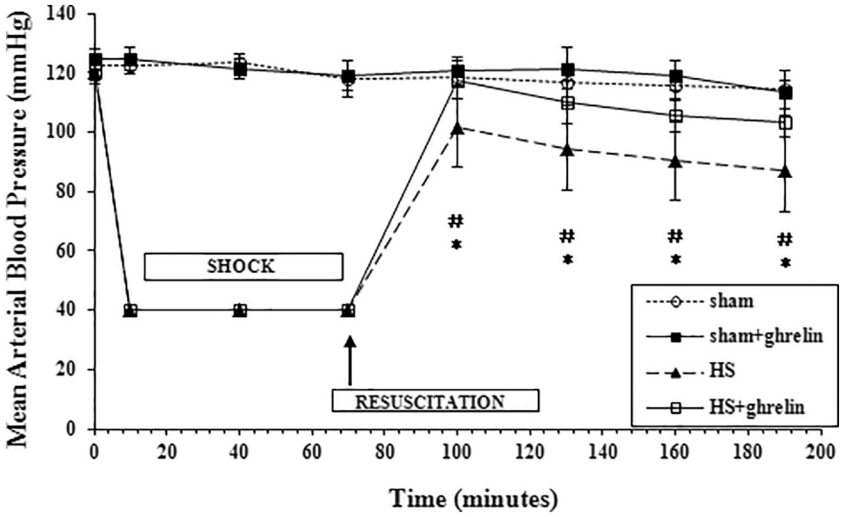

Fig. 1. Effect of exogenous ghrelin on mean arterial pressure (MAP) in a rat model of hemorrhagic shock (HS).
HS caused a marked decrease in MAP, which was elevated after ghrelin administration. N = 8 for each group; $*P < 0.01$ versus time-matched value in the sham and sham + ghrelin groups; $\#P < 0.01$ versus time-matched value in the HS group.

investigated, the effect of ghrelin on ALI in a model of HS has not been evaluated. Nuclear factor κB inhibitor kinase (IKK) is known as a gateway to NF-κB activation and transcription [14]. The purpose of this study was to determine whether ghrelin can attenuate lung injury following HS and whether this function was related to the regulation of IKK and downstream nuclear factor κB inhibitor (IκBα)/NF-κB pathway activation.

2. Materials and methods

2.1. Animals

Thirty-two male Sprague-Dawley (SD) rats (Vital River Laboratories, Beijing, China) weighing 300–350 g were housed in a controlled environment with a 12-hour light/dark cycle and free access to water and food for at least 1 week; then, rats were fasted overnight before the experiments but allowed free access to water. All procedures were approved by the Institutional Animal Care and Use Committee of Harbin Medical University.

2.2. Experimental protocols

Rats were anesthetized via intraperitoneal injection of 60 mg/kg sodium pentobarbital. The animals then underwent tracheostomy and cannulation. During the entire procedures, animals inspired 50% O_2 (LiMing Gas Corporation, Harbin, China) at a flow rate of 2 L/min. A venous catheter (PE-50 tubing) was inserted into the left femoral vein for fluid infusion and medication. The bilateral femoral arteries were cannulated and used for measuring mean arterial pressure (MAP) and drawing blood. Continuous arterial blood pressure was measured (AS/3, Datex, Helsinki, Finland) during the procedures. Arterial blood gas analyses were performed using a conventional analyzer (Rapid Lab 348, Bayer, Medfield, MA, USA). Heparin (1000 units/kg body weight) was added to the blood through the vein. Following these procedures, the rats were allowed to stabilize for 15 min, and vital signs were recorded.

Blood was then drawn over a 10-min period until a mean arterial pressure (MAP) of 40 mm Hg was achieved; this level was maintained for 60 min by withdrawing, or reinfusing shed blood through the femoral artery. After the shock period, rats were resuscitated by reinfusing all of the shed blood (2 mL/kg/min) supplemented with an equal volume of Ringer lactate solution (1 mL/kg/min), which was followed by a period of observation for 2 h. During the experiment, the rectal temperature of the rats was monitored and maintained at 37 °C using either a heating pad or a heat lamp.

2.3. Experimental groups

Rats were randomly divided into four groups (n = 8 per group) as follows: sham operation group (sham group), sham operation plus ghrelin group (sham + ghrelin group), Hemorrhagic shock group (HS group), Hemorrhagic shock plus ghrelin group (HS + ghrelin group). Sham group animals underwent all surgical procedures without hemorrhage and resuscitation. Rats in the sham + ghrelin group underwent the same procedure as the sham group, except that ghrelin was administered at the same time as resuscitation. Rats received 10 nmol/kg ghrelin (Phoenix Pharmaceuticals, Belmont, CA) dissolved in 500 μL of saline or saline alone via the femoral vein before the initiation of resuscitation.

2.4. Tissue sample collection

At the end of the resuscitation period, animals were euthanized; and lungs and plasma samples were harvested. Bronchoalveolar lavage of the right lung was then performed. The left upper lobes were fixed with 10% formaldehyde solution for pathologic specimen preparation. The mid lobes were used for obtaining the wet lung weight, and the remaining lung tissue was cut into small pieces and stored at −70 °C.

2.5. Histological analysis

Lung tissue was fixed in 4% paraformaldehyde and embedded in paraffin. Sections were stained with hematoxylin-eosin (H&E). Morphological changes were analyzed by an experienced pathologist in a blinded fashion. A scoring system to grade the degree of lung injury was used based on the following histological features: edema, hyperemia and congestion, neutrophil margination and tissue infiltration, intraalveolar hemorrhage and debris, and cellular hyperplasia. Each feature was graded as absent, mild, moderate, or severe, with a score of 0–3. The total score was calculated for each animal [13].

2.6. Lung wet to dry weight ratio

After resuscitation, the left middle lobes were immediately weighed and then placed in an oven at 80 °C for 72 h to determine the dry weight. The wet-to-dry weight (W/D) ratios were then determined.

2.7. Bronchoalveolar lavage fluid (BALF) preparation

The BALF of the right lung was obtained by washing the lung three times with 3 mL of cold saline every time. Approximately 90% (8 mL) of

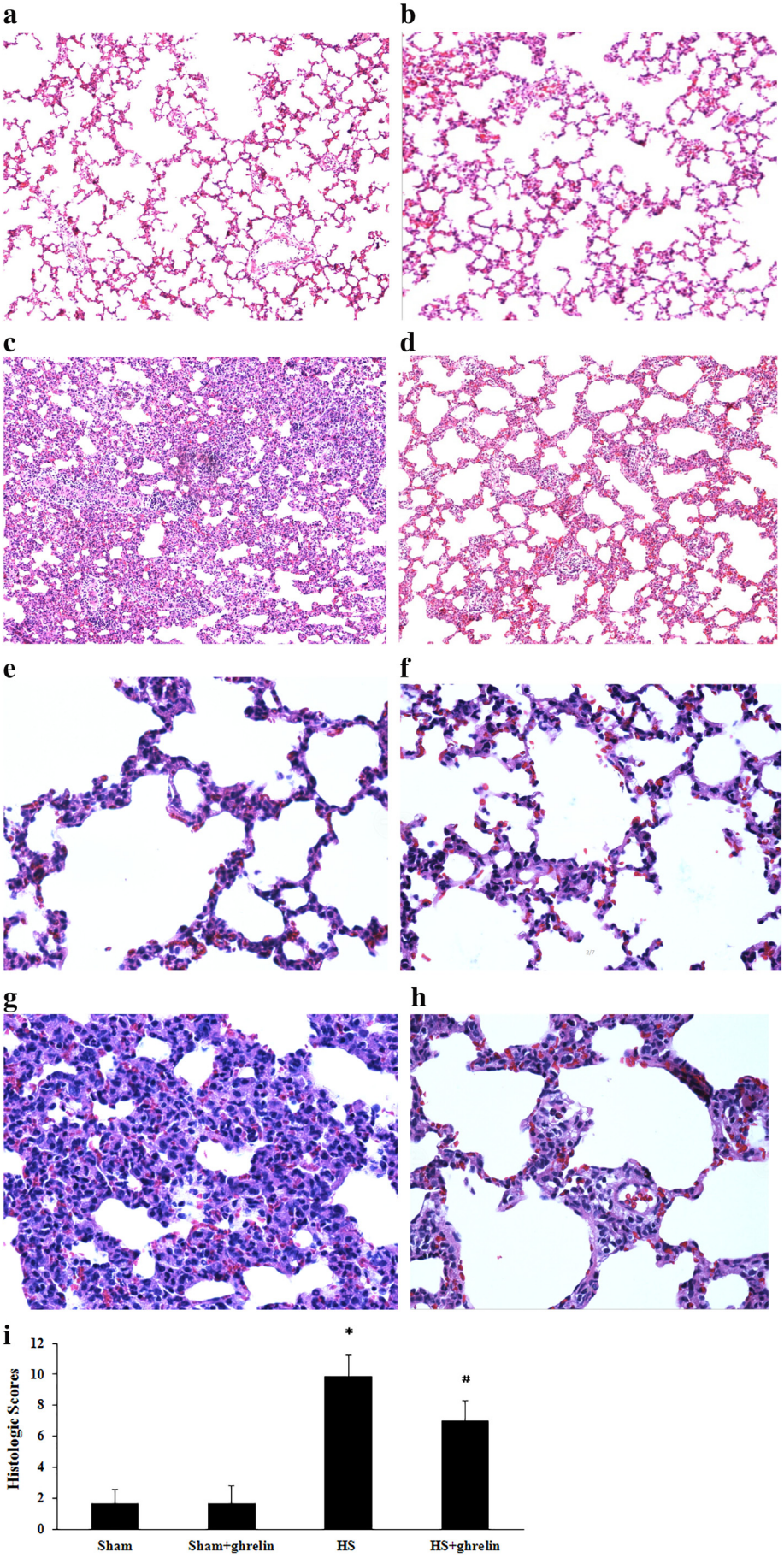

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Fig. 2. Effect of ghrelin on lung histology during hemorrhagic shock (HS)-induced acute lung injury in rats.
Tissues were prepared for hematoxylin and eosin staining. (a–d, 100× magnification; e–h, 400× magnification). (a, e) Sham group. (b,f) Sham + ghrelin group. (c, g) HS group. (d, h) HS + ghrelin group. i, histopathological scoring of lung injury.
*$P < 0.01$ versus the sham and sham + ghrelin groups; #$P < 0.01$ versus the HS group. Data are presented as mean ± SD (n = 8) and were compared using one-way ANOVA and the Student-Newman-Keuls test.

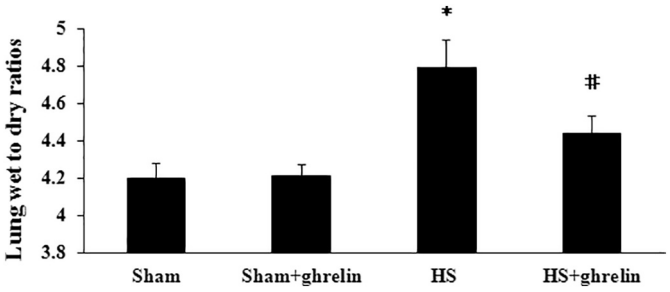

Fig. 3. Effect of ghrelin on hemorrhagic shock (HS)-induced lung wet to dry weight ratios in rats.
After resuscitating the animals, left middle lung lobes were weighed and dried at 80 °C for 72 h to determine wet-to-dry weight ratios; *$P < 0.01$ versus the sham and sham + ghrelin groups; #$P < 0.01$ versus the HS group. Data are presented as the mean ± SD (n = 8) and were compared by one-way ANOVA and the Student-Newman-Keuls test.

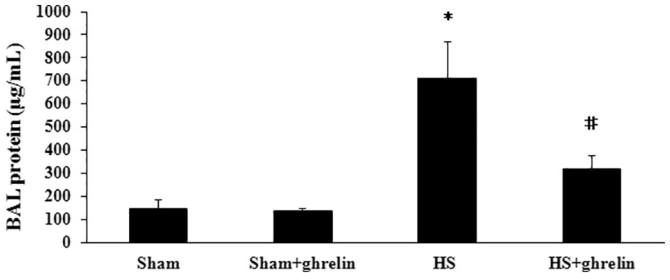

Fig. 4. Effect of ghrelin on protein content in the bronchoalveolar lavage fluid (BALF) after hemorrhagic shock (HS)-induced acute lung injury in rats.
BALF of the right lung was obtained, and protein content was measured by the bicinchoninic acid assay method; *$P < 0.01$ versus the sham and sham + ghrelin groups; #$P < 0.01$ versus the HS group. Data are presented as the mean ± SD (n = 8) and were compared by one-way ANOVA and the Student-Newman-Keuls test.

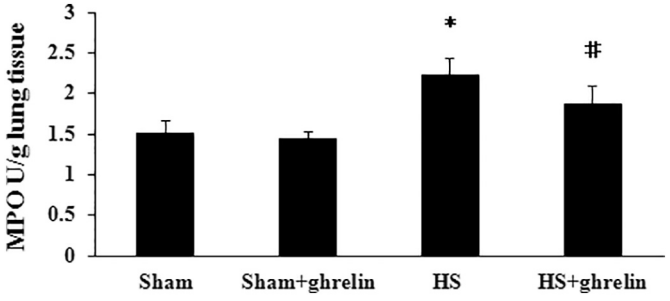

Fig. 5. Effect of ghrelin on lung neutrophil infiltration after hemorrhagic shock (HS)-induced acute lung injury in rats.
Frozen lung tissue was homogenized and myeloperoxidase activity, considered an index of pulmonary neutrophil sequestration, was then determined; *$P < 0.01$ versus the sham and sham + ghrelin groups; #$P < 0.01$ versus the HS group. Data are presented as mean ± SD (n = 8) and were compared by one-way ANOVA and the Student-Newman-Keuls test.

the total volume was retrieved. Then, the BALF was centrifuged at 450 × *g* at 4 °C for 10 min, and the supernatant was stored at −70 °C; BALF protein content was measured by the bicinchoninic acid assay method.

2.8. Pulmonary neutrophil sequestration

Myeloperoxidase (MPO) activity was considered an index of pulmonary neutrophil sequestration. Frozen lung tissue was homogenized in the available buffer to a concentration of 50 mg/mL. In brief, lung MPO activity was then determined according to the instructions of a commercial assay kit (Jiancheng Bioengineering Institute, Nanjing, China) using a UV-2100 spectrophotometer at 460 nm (Unico Company, Shanghai, China).

2.9. Plasma cytokine levels

Plasma concentrations of TNF-α and IL-6 were determined according to the instructions of enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN, USA).

2.10. NF-κB activity assay

NF-κB DNA-binding activity was measured based on the nuclear translocation and DNA binding of the p65 subunit from nuclear extracts of lung tissues according to the instructions of a commercially available ELISA kit (rat NF-κB p65 ELISA Kit, RayBiotech, USA). Briefly, nuclear proteins were extracted from frozen lung tissue using the nuclear extraction kit (Beyotime Institute of Biotechnology, Beijing, China) according to the manufacturer's instructions. Next, these proteins were incubated in 96-well plates coated with purified rat NF-κB p65 antibody, followed by addition of the HRP-labeled NF-κB p65 antibody to form the antibody-antigen-enzyme-antibody complex. The TMB substrate solution was added after extensive washing, the color of which turned blue because of HRP-mediated catalysis. The reaction was terminated by the addition of sulfuric acid and the color change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of NF-κB p65 (ng/l) in the samples was then determined by comparing the O.D. of the samples to the standard curve.

2.11. Western blotting

Concentrations of phosphorylated IKKβ, IκBα, phosphorylated IκBα, and NF-κB were determined using western blot assays. Cytoplasmic and nuclear proteins were extracted from frozen lung tissue using the nuclear and cytoplasmic protein extraction kit (Beyotime Institute of Biotechnology) according to the manufacturer's instructions. Protein concentrations were determined using the bicinchoninic acid (BCA) protein assay kit (Beyotime Institute of Biotechnology). Proteins were separated using 12% sodium dodecyl sulfate -polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were incubated overnight with primary antibodies at 4°C. The following primary antibodies were used: anti-phosphorylated IKKβ (1:1000, ImmunoWay Biotechnology Company, Newark, USA), anti-IκBα (1:200, Santa Cruz Biotechnology, Santa Cruz, CA), anti-psphorylated IκBα (1:1000, Cell Signaling Technology, Danvers, MA), and anti-NF-κB (1:1000, ImmunoWay Biotechnology Company, Newark, USA). After washing, the membranes were incubated with HRP-conjugated secondary antibodies (1:5000) for 45 min at 37°C. Proteins were visualized using the enhanced chemiluminescence (ECL) plus system (Beyotime Biotechnology) according to the manufacturer's instructions and the results were quantified using the ImageJ 1.34s software. Results were presented as the ratio of target protein levels to β-actin or Histone H3 levels (internal control).

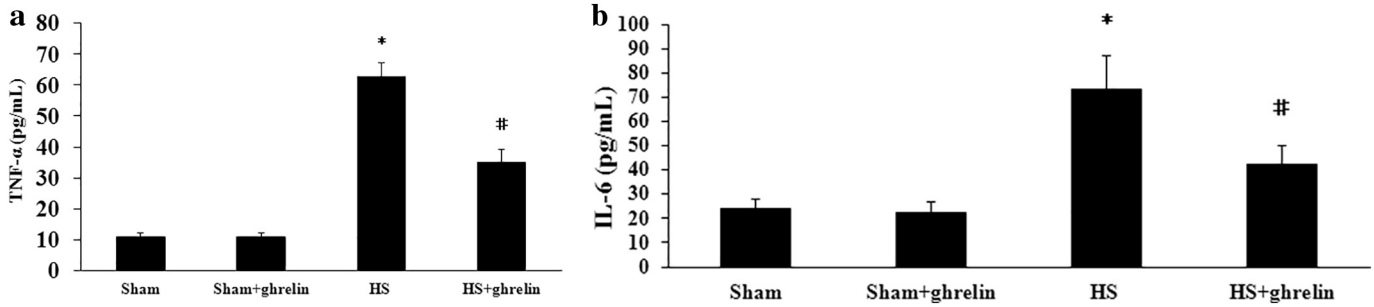

Fig. 6. Effect of ghrelin on plasma levels of inflammatory after hemorrhagic shock (HS)-induced acute lung injury in rats.
After HS, TNF-α (a) and IL-6 (b) levels were measured in all groups; *$P < 0.01$ versus the sham and sham + ghrelin groups; #$P < 0.01$ versus the HS group. Data are presented as the mean ± SD (n = 8) and were compared by one-way ANOVA and the Student-Newman-Keuls test.

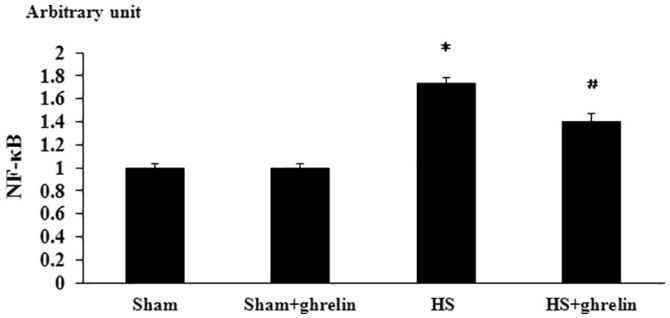

Fig. 7. Effect of ghrelin on NF-κB activity in lung tissue after hemorrhagic shock (HS)-induced acute lung injury in rats.
NF-κB activity was measured based on the nuclear translocation and DNA binding of the p65 subunit in nuclear extracts from lung tissues of all groups. *$P < 0.01$ versus the sham and sham + ghrelin groups; #$P < 0.01$ versus the HS group. Data are presented as the mean ± SD (n = 8) and were compared by one-way ANOVA and the Student-Newman-Keuls test.

2.12. Statistical analysis

SPSS 17.0 software was used for all statistical analyses. All data are presented as mean ± standard deviation. Analysis of variance (ANOVA) and the Student–Newman–Keuls q-test were used to compare values among all groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Exogenous ghrelin alleviates the decrease in MAP in a rat model of hemorrhagic shock

The MAP in the HS group was significantly lower than that in the sham and sham + ghrelin group after the induction of shock ($P < 0.01$, Fig. 1). However, ghrelin-treated rats had markedly higher MAP compared to that in HS group rats at both time points following resuscitation ($P < 0.01$, Fig. 1).

3.2. Exogenous ghrelin alleviates acute lung injury in a rat model of HS

Lung histological changes in HS group rats were characterized by alveolar disruption, interstitial edema, and the infiltration of inflammatory cells, when compared with the sham and sham + ghrelin groups. In contrast, these changes were attenuated in the HS + ghrelin group (Fig. 2a-h). No gross inflammatory changes were observed in the sham and sham + ghrelin groups. As indicated in Figure 2i, the lung injury score was increased significantly after HS compared to that in the sham-operated animals ($P < 0.01$). Administration of ghrelin after HS markedly reduced the lung injury score ($P < 0.01$).

HS also resulted in an increase in pulmonary edema as measured by the W/D ratio (4.79 ± 0.15 vs. 4.20 ± 0.08 and 4.21 ± 0.06; $P < 0.01$ vs. the sham and sham + ghrelin groups, respectively; Fig. 3). However, the administration of ghrelin attenuated lung edema induced by HS (HS + ghrelin group, 4.44 ± 0.09; $P < 0.01$ vs. the HS group; Fig. 3).

Protein content in the bronchoalveolar lavage fluid (BALF), an indicator of the severity of lung vascular injury, increased significantly after HS when compared to that in the sham and sham + ghrelin groups (712.09 ± 155.38 μg/mL vs. 146.94 ± 38.69 μg/mL and 134.13 ± 12.37 μg/mL, respectively; $P < 0.01$; Fig. 4), whereas this was considerably reduced in the HS + ghrelin group (HS + ghrelin, 316.63 ± 61.09 μg/mL; $P < 0.01$ vs. the HS group; Fig. 4).

3.3. Exogenous ghrelin reduces pulmonary neutrophil infiltration in a rat model of HS

The HS group exhibited a significant increase MPO activity in rat lungs compared to that in the sham and sham + ghrelin groups (2.23 ± 0.2 U/g vs. 1.51 ± 0.15 U/g and 1.44 ± 0.09 U/g, respectively; $P < 0.01$; Fig. 5). However, MPO activity in the HS + ghrelin group was lower than that in the HS group (HS + ghrelin, 1.87 ± 0.22 U/g; $P < 0.01$ vs. the HS group; Fig. 5).

3.4. Exogenous ghrelin decreases the expression of inflammatory mediators after HS

Plasma IL-6 and TNF-α levels were measured after HS. In the HS group, IL-6 and TNF-α levels were significantly increased compared to those in the sham and sham + ghrelin groups. In contrast, ghrelin treatment attenuated this increase in plasma cytokine levels following HS (Fig. 6a and b).

3.5. Exogenous ghrelin treatment inhibits IKK/IκBα/NF-κB signaling in the lung after HS

HS was found to significantly enhance NF-κB activity in the nuclear fraction of lung tissue compared to that in the sham and sham + ghrelin groups. However, NF-κB activity was notably decreased after ghrelin administration ($P < 0.01$ vs. the HS group; Fig. 7).

Next, we examined the expression of IKK, IκB, and NF-κB, three factors involved in NF-κB signaling by western blotting. Phosphorylated IKK, phosphorylated IκB, and NF-κB all increased after HS ($P < 0.01$, Fig. 8a, b, and d); however, ghrelin administration down-regulated these markers. ($P < 0.01$, Fig. 8a, b, and d). The degradation of IκB increased in the HS group, that is, the expression of IκB was decreased. ($P < 0.01$, Fig. 8c). However, ghrelin administration reduced the degradation of IκB ($P < 0.01$, Fig. 8c).

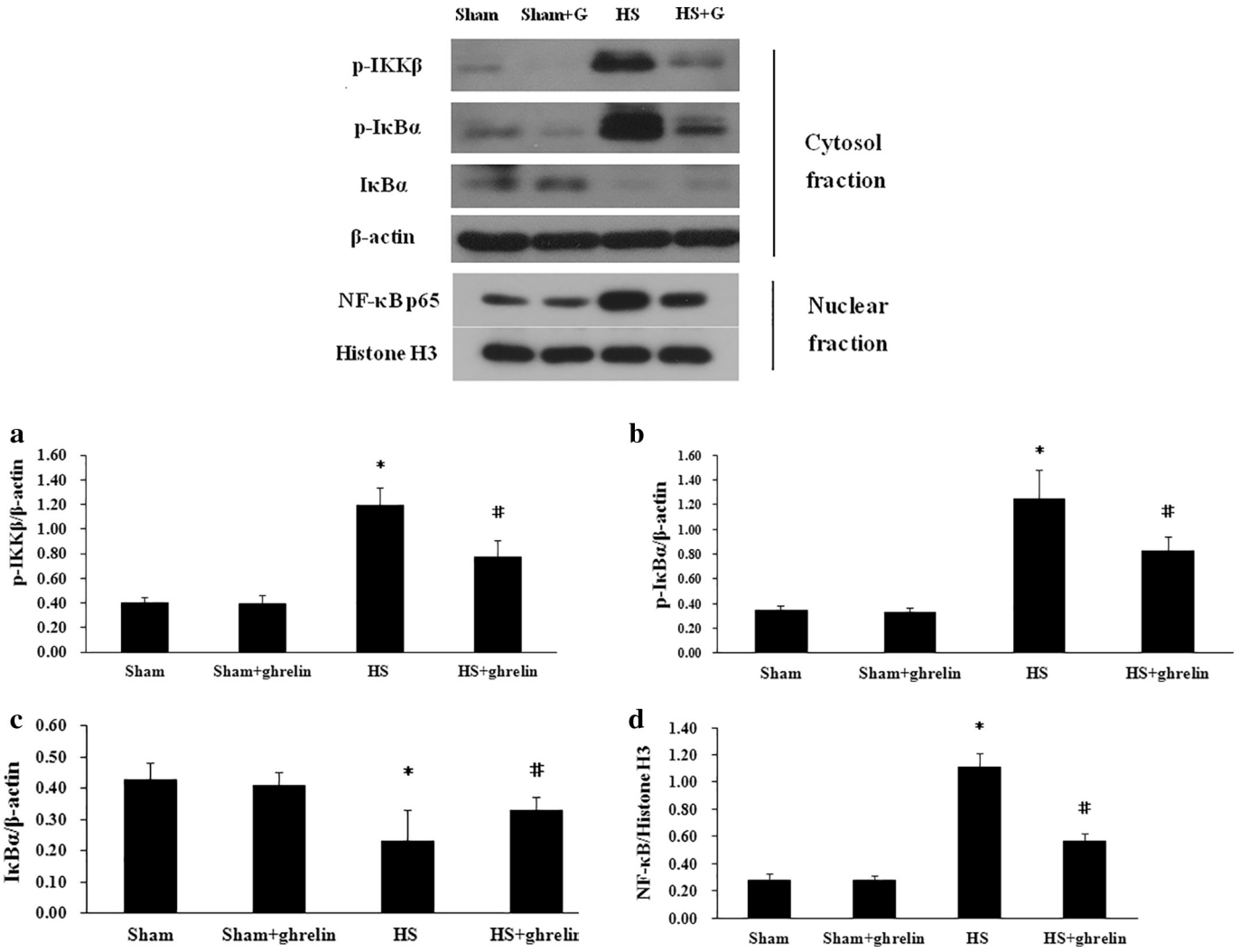

Fig. 8. Effect of ghrelin on the expression of p-IKKβ, IκBα, p-IκBα, and nuclear NF-κB after hemorrhagic shock (HS)-induced acute lung injury in rats. Levels of these markers were assessed in the four groups by western blotting; $*P < 0.01$ versus the Sham and Sham + ghrelin groups; $\#P < 0.01$ versus the HS group.

4. Discussion

In this study, we demonstrated the protective effects of ghrelin against ALI following HS. Our results showed that ghrelin significantly attenuated histological injury, reduced the lung W/D ratio, suppressed protein levels in the BALF and neutrophil infiltration, inhibited the pro-inflammatory response (plasma TNF-α and IL-6 levels) and IKK/IκBα/NF-κB pathway activation, and restored decreased MAP after resuscitation as compared to those in the HS group. To the best of our knowledge, this is the first study evaluating the effect of ghrelin on ALI in a rat model of HS.

Ghrelin, a gastrointestinal hormone, was initially discovered in 1999 as a novel endogenous ligand for the growth hormone secretagogue receptor [10]. In addition to its growth hormone releasing property, the roles of ghrelin have also been indicated in the endocrine, gastrointestinal, cardiovascular, nervous and immune systems [15,16]. Previous studies reported that ghrelin acts via GHS-R to specifically inhibit the expression of proinflammatory cytokines in human monocytes and T cells [17]. Talat et al. [18] demonstrated that ghrelin and its receptor GHS-R were present in macrophages and that the effects of ghrelin on pro-inflammatory cytokine production were explicitly mediated by GHS-R and not indirectly. Moreover, ghrelin was found to function through the central GHS-R to inhibit peripheral sympathetic nerve activity, resulting in the suppression of inflammation in sepsis-associated organ injury [19,20]. GHS-R mRNA was also found to be expressed in lung tissue [21,22]. Therefore, an understanding of GHS-R distribution is essential to understand the anti-inflammatory mechanisms of ghrelin in the lungs.

Our study showed that ghrelin treatment restores the significant reduction in MAP induced by HS during the resuscitation phase, but had no effects on sham rats. Ghrelin has been shown to increase cardiac contractility and improve vascular function after severe sepsis [23]. The inflammatory response induced by HS is similar to that observed in sepsis [24]. Therefore, the possible mechanisms responsible for the hemodynamic effects of ghrelin in HS rats might be linked to the anti-inflammatory property of this agent. It has been shown that intravenous infusion of high doses of ghrelin decreases peripheral artery resistance, leading to a drop in blood pressure in humans [25,26]. However, the high doses of human ghrelin required to obtain a blood pressure reduction might be different when compared with that used in our rat model. Hence, the different effects of ghrelin on hemodynamics require further exploration.

There is considerable experimental and clinical evidence that pro-inflammatory cytokines such as TNF-α and IL-6 play a major role in the pathogenesis of ALI after HS [27]. TNF-α signaling via the TNF receptor in a wide range of cells results in the rapid activation of IKK, the

degradation of IκB, and enhanced transcriptional activity of NF-κB. Through the activation of NF-κB, TNF signaling directly regulates the expression of a variety of other cytokines such as IL-6 [6,28]. Circulating levels of IL-6 have been shown to be excellent predictors of the severity of the inflammatory response [29]. Ghrelin has been reported to downregulate brain levels of TNF-α and IL-6 after traumatic brain injury and HS in rats [30]. Further, activated neutrophils play a central role in the development of ALI and in the activation of the NF-κB [31]. In this study, we showed that ghrelin attenuates the TNF-α and IL-6 expression and neutrophil recruitment, similar to that observed in previous reports, demonstrating the anti-inflammatory effects of ghrelin.

The NF-κB signaling pathway plays a central role in regulating the transcription of cytokine-encoding genes and has been considered a promising therapeutic target for inflammatory lung diseases [32,33]. Under inactivating conditions, NF-κB dimers are bound to IκB-inhibitory proteins, which sequester NF-κB in the cytoplasm. Once activated, the IκB kinase (IKK) complex phosphorylates IκB proteins, triggering IκB ubiquitination and proteasomal degradation, releasing NF-κB dimers to translocate to the nucleus to induce gene expression [34]. It has been demonstrated that activation of the IKK/IκB/NF-κB pathway plays a significant role in the development of ALI during the inflammatory response following HS [35]. Peng et al. [36] reported that ghrelin inhibits NF-κB activity by modulating the expression of an upstream activator, nucleotide-binding oligomerization domain2 (NOD2), which transmits signals to receptor interacting protein (Rip2) to activate NF-κB. Ghrelin has also been reported to down-regulate TLR-4 and NF-κB expression in ventilator-induced lung injury in rats [37]. Our study provides in vivo evidence showing that ghrelin inhibits the degradation of IκBα and prevents the translocation of NF-κB into the nucleus in lungs after HS.

The IKK complex consists of two catalytically active kinases, namely IKKα and IKKβ, and the regulatory subunit, IKKγ (NEMO) [38]. Both IKKα and IKKβ can phosphorylate IκB, but IKKβ exhibits a higher kinase activity [39]. Thus, IKKβ was chosen as a marker for our study. A recent study by Slomiany et al. [40] reported that although ghrelin enhances lipopolysaccharide-induced extracellular signal-regulated kinase (ERK) and IKK-β phosphorylation in rat gastric mucosal cells, it inhibits IKK-β activation through up-regulation of Src/Akt-dependent *S*-nitrosylation, leading to suppression of IκBα degradation and NF-κB nuclear translocation. Nevertheless, our results using an in vivo animal model suggest that ghrelin protects the lungs from HS injury through inhibition of IKK-β phosphorylation. This indicates that the effects of ghrelin on IKK-β might differ depending on the cell type and the signaling pathway. The present study demonstrated for the first time that ghrelin suppresses IKK activation in lung tissue in a model of HS. However, the mechanisms associated with IKK suppression by ghrelin remains to be elucidated.

5. Conclusion

In summary, the present study demonstrated for the first time that exogenous ghrelin significantly attenuated the inflammatory response and ALI in a rat HS model. This protective effect was associated with inhibition of IKKβ phosphorylation, IκBα degradation, and NF-κB activation and nuclear translocation. Therefore, exogenous ghrelin might attenuate ALI after HS by inhibiting the activation of the IKK/IκBα/NF-κB signaling pathway. In future studies, we will optimize the lung-protective effects of ghrelin considering longer time points after HS and resuscitation, and will further determine the mechanism through which ghrelin modulates IKK signaling.

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Conflicts of interest

The authors declare that there are no potential conflicts of interest.

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