Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2026**; 31(1): 45524
https://doi.org/10.31083/FBL45524

Original Research

Berberine Alleviates Lipopolysaccharide-Induced Acute Lung Injury by Modulating the AMPK-HMGB1-NF-κB Signaling Axis

Tingxia Lv[1], Chunpan Zhang[1], Yawei Zeng[1], Lan Hu[1], Guangwei Huang[1], Chao Wang[1,*], Wenjie Qi[1,*]

[1]Department of Infectious Disease, Beijing Friendship Hospital, Capital Medical University, 100050 Beijing, China
*Correspondence: chaowang30@ccmu.edu.cn (Chao Wang); qi_wenjie@ccmu.edu.cn (Wenjie Qi)

Academic Editors: Srinivasa Reddy Bonam and Eun Sook Hwang
Submitted: 4 August 2025 Revised: 18 September 2025 Accepted: 29 September 2025 Published: 23 January 2026

Abstract

Background: The activation of adenosine-monophosphate-activated protein kinase (AMPK) by berberine (BBR) benefits various inflammatory diseases. Conversely, high mobility group box-1 (HMGB1), a prototypical damage-associated molecular pattern (DAMP), typically exerts opposing effects. This research aims to investigate the relationship between AMPK and HMGB1, elucidating the functions and underlying mechanisms by which BBR alleviates acute lung injury (ALI) caused by lipopolysaccharide (LPS). **Methods**: Male C57BL/6J mice were intragastrically administered BBR twice daily for three days with a total of 25 and 100 mg/kg/day. On day four, an intraperitoneal injection of 10 mg/kg LPS was administered, and BBR was given two hours before and six hours after this injection, respectively. Eighteen hours post-LPS administration, lung tissues and serum samples were collected to assess indicators of lung tissue injury, inflammation, oxidative stress, and apoptosis. The relationship between AMPK activation, HMGB1 release, and inflammatory activation was investigated in both mice and RAW264.7 cells using protein expression analysis, AMPK silencing, and exogenous HMGB1 introduction. **Results**: Our findings demonstrate that BBR activates AMPK and inhibits HMGB1 expression, translocation, and release in LPS-induced ALI, resulting in reduced histopathological lung injuries, decreased expression of inflammatory cytokine genes, and diminished oxidative stress and apoptosis. Mechanistic studies revealed that BBR decreases extracellular HMGB1 in LPS-stimulated RAW264.7 cells and inhibits HMGB1-stimulated nuclear factor Kappa B (NF-κB) activation. Concurrently, silencing the activation of AMPK by siRNA and compound C reversed the BBR-reduced extracellular HMGB1 level in LPS-stimulated RAW264.7 cells. **Conclusions**: Based on these findings, we conclude that BBR effectively inhibits inflammation, oxidative stress, and apoptosis in LPS-induced ALI by modulating the AMPK-HMGB1-NF-κB axis. Consequently, BBR and other AMPK activators may represent promising therapeutic options for managing systemic inflammation and injury during sepsis.

Keywords: berberine; sepsis; acute lung injury; AMP-activated protein kinase; HMGB1 protein

1. Introduction

Sepsis is a life-threatening impairment of organ function that arises from an unregulated systemic immune and inflammatory response by the host to an infection [1]. Among the various types of organ dysfunction, lung tissue injury is a pivotal contributor to the morbidity and mortality associated with sepsis [2,3]. To date, traditional management strategies, such as early antibiotic administration and fluid resuscitation, have shown efficacy in mitigating early-stage sepsis to some degree. However, due to the significant host-mediated systemic inflammatory responses observed during sepsis, therapeutic approaches targeting specific signaling pathways and molecules warrant greater attention [1]. High mobility group box-1 (HMGB1), a multifunctional damage-associated molecular pattern (DAMP) and signaling molecule, has been identified as a crucial late-phase inflammatory mediator that contributes to endotoxin lethality in sepsis. Upon infection, the interaction between HMGB1 and TLR4/MD2, as well as the formation of HMGB1-lipopolysaccharide (LPS) complexes, plays a key role in triggering downstream inflammatory pathways, oxidative stress, apoptosis, and pyroptosis [4–6]. Consequently, targeting HMGB1 may lead to organ protection and potentially improve survival outcomes [1].

Berberine (BBR, Fig. 1A), a benzylisoquinoline alkaloid, is an active constituent present in various medicinal plants and exhibits multiple pharmacological properties, including antimicrobial, antiprotozoal, antidiarrheal, and antitrachoma activities. Recently, BBR has also been reported to possess therapeutic potential for metabolic, neurological, and cardiological diseases [7–11]. Among the various targets of BBR, adenosine-monophosphate-activated protein kinase (AMPK) activation is considered a pivotal target of BBR, mediating its diverse pharmacological effects. Previous studies have indicated that AMPK activation benefits the balance between oxidants and antioxidants during lung injury, whereas the release of HMGB1 triggers inflammation and subsequent apoptosis [4,5,12].

Predictably, BBR may also activate AMPK during acute lung injury (ALI). Nonetheless, the involvement of BBR and the activation of AMPK in the regulation of lung injury, inflammation, oxidative stress, and apoptosis in the

Copyright: © 2026 The Author(s). Published by IMR Press.
This is an open access article under the CC BY 4.0 license.

Publisher's Note: IMR Press stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

context of sepsis, along with the role of HMGB1 in this process, remains inadequately understood. This research aims to evaluate the impact of BBR on lung damage, inflammatory responses, oxidative stress levels, and apoptosis in mice subjected to LPS-induced sepsis. Furthermore, we elucidated the effect of BBR on AMPK activation and HMGB1 levels during ALI, and the correlation between BBR-induced AMPK activation and HMGB1 release in LPS-stimulated mouse monocyte/macrophage cells, RAW264.7. Our results highlight the effectiveness of BBR in suppressing inflammation, oxidative stress, and apoptosis in LPS-induced ALI, mediated *via* modulation of the AMPK-HMGB1-NF-κB signaling pathway.

2. Materials and Methods

2.1 Animal Experiments

Male C57BL/6J mice (6–8 weeks, 18–20 g) were sourced from SPF Biotechnology Co., Ltd. (Beijing, China) and housed for one week under a 12-h light/dark cycle in specific pathogen-free conditions before the experiment. To evaluate the effect of BBR (A75865, InnoChem, Beijing, China; purity $\geq$97%) on LPS-induced ALI in septic mice, the mice were randomly allocated into four groups: Control, LPS, LPS + BBR (25 mg/kg/day), and LPS + BBR (100 mg/kg/day), with six mice in each group. The LPS-induced ALI model was modified based on previously described methods [13,14]. The mice were administered a single intraperitoneal injection of LPS at a dosage of 10 mg/kg of body weight without the use of analgesia, as previously used [15–17]. This minimally invasive procedure did not induce signs of distress and adhered to the 3R principles of animal welfare. Briefly, following intragastric administration of BBR dissolved in 0.5% carboxymethyl cellulose sodium (CMC-Na) or a control solvent twice daily for three days, LPS (L8880, Solarbio, Beijing, China) dissolved in normal saline was injected intraperitoneally at a dosage of 10 mg/kg body weight two hours following BBR treatment on day 4. At six hours post-LPS injection, the mice underwent a final treatment with either BBR or the control solvent. Eighteen hours after LPS injection, the mice were subjected to terminal anesthesia using 5% isoflurane (I8000, Solarbio, Beijing, China). After confirming that the mice were in a state of deep anesthesia and the absence of pain reflexes, their abdominal cavities were opened. Blood samples were obtained from the inferior vena cava with a 1-mL syringe, and the mice were euthanized via exsanguination, following which lung tissues were collected. The harvested lung tissues were either preserved at –80 °C for further analysis or fixed in 10% neutral formaldehyde for histological examination. All animal-related procedures received approval from the Ethics Committee of Beijing Friendship Hospital, Capital Medical University (Approval number #24-2034) and were performed in accordance with the National Guidelines for the Housing and Care of Laboratory Animals.

2.2 Biochemical Analysis

Blood samples underwent centrifugation at 2500 g for a duration of 10 min to obtain serum. The levels of HMGB1 in serum and cell culture supernatants were measured using a mouse HMGB1 enzyme-linked immunosorbent assay (ELISA) kit (SEKM-0145, Solarbio, Beijing, China). Additionally, mouse lung tissues were homogenized, and commercial assay kits were utilized to quantify malondialdehyde (MDA) (S0131, Beyotime Biotechnology, shanghai, China), superoxide dismutase (SOD) (S0101, Beyotime Biotechnology, shanghai, China), and myeloperoxidase (MPO) (A044-1-1, Nanjing Jiancheng Biotechnology Co., Ltd., Nanjing, China) to assess oxidative stress in the lungs and infiltration of neutrophils.

2.3 Histological Analysis

Lung tissues that were fixed in paraffin, and sections measuring 5 µm were subjected to standard hematoxylin and eosin (H&E) staining to assess histopathological alterations using a light microscope. A semi-quantitative scoring system was utilized to evaluate the infiltration of neutrophils, the presence of pulmonary edema, disorganization within the lung parenchyma, and any hemorrhagic changes in the lung tissue. The scoring criteria were defined as follows: 0, no pathological changes observed in the visual field; 1, 2, 3, and 4 represent pathological changes present in less than 25%, 25%–50%, 51%–75%, and greater than 75% of the total visual field, respectively [18]. Apoptotic characteristics in lung tissues preserved with formaldehyde were identified using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining, which employed the DAB (SA-HRP) (PR30010, Proteintech, Wuhan, China) TUNEL Cell Apoptosis Detection Kit (G1507, Servicebio, Wuhan, China). The proportion of TUNEL-positive cells was quantified using ImageJ software (NIH, Bethesda, MD, USA). Additionally, immunofluorescence was performed to detect HMGB1 in lung tissue, and its expression and intracellular distribution were evaluated.

2.4 Cytoplasmic and Mitochondrial Protein Isolation

Mitochondria were isolated from lung tissue using a mitochondrial isolation kit (C3606, Beyotime Biotechnology, Shanghai, China). In summary, the lung tissue underwent homogenization followed by centrifugation at 4 °C and 600 g for 10 min to eliminate cell nuclei. The resulting supernatant was centrifuged a second time at 11,000 g and 4 °C for 10 minutes to collect crude mitochondrial pellets. Next, the supernatant underwent centrifugation at 12,000 g for 10 minutes to separate cytoplasmic proteins. The concentration of proteins was assessed using a BCA protein assay kit (#23225, Thermo Fisher Scientific, Waltham, MA, USA), after which the proteins were heated and analyzed via Western blot.

2.5 RNA Extraction and Quantitative Real-Time Reverse-Transcription PCR (qRT-PCR)

RNA was extracted from the cells using the RaPure total RNA kit (R4011, Magen, Shanghai, China), in accordance with the instructions provided by the manufacturer. The mRNA expression levels of the inflammatory cytokine genes *Tnfα* and *Il6*, along with the internal control gene glyceraldehyde 3-phosphate dehydrogenase (Gapdh), were amplified using specific primers: *Gapdh* (5′-CTCTGGAAAGCTGTGGCGTGATG-3′ and 5′-ATGCCAGTGAGCTTCCCGTTCAG-3′), *Tnfα* (5′-CCAAAGGGATGAGAAGTTCC-3′ and 5′-CTCCACTTGGTGGTTTGCTA-3′), and *Il6* (5′-CCATCCAGTTGCCTTCTTGG-3′ and 5′-TGCAAGTGCATCATCGTTGT-3′) [14,19]. Quantification was performed using one-step qRT-PCR with the HiScript II One Step qRT-PCR SYBR Green Kit (Q221-01, Vazyme). The comparative Ct method was employed to calculate relative mRNA levels, following normalization to Gapdh.

2.6 Western Blot

Lung homogenates or cell lysates were collected utilizing a protein extraction reagent (78510, Thermo Fisher Scientific, Waltham, MA, USA) combined with inhibitor cocktails for proteases and phosphatases (C0001 and C0004, TargetMol, Boston, MA, USA). The concentration of protein was assessed using a BCA protein assay kit. For Western blotting, protein samples were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and subsequently transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were blocked with 5% fat-free milk and incubated overnight at 4 °C with antibodies against ACTB (GB15001, Servicebio, Wuhan, China), HMGB1 (ET1601-2, HUABIO, Hangzhou, China), AMPKα1 (ET1608-40, HUABIO, Hangzhou, China), p-AMPKα1 (2535, CST, Danvers, MA, USA), NF-κB p65 (4764, CST, Danvers, MA, USA), p-NF-κB p65 (3033, CST, Danvers, MA, USA), cleaved Caspase-3 (WL02117, WanleiBio, Shenyang, China), Cleaved Caspase-9 (WL01838a, Wanleibio, Shenyang, China), Bax (WL01637, Wanleibio, Shenyang, China), Bcl-2 (WL015556, Wanleibio, Shenyang, China), and histone H3 (9715, CST, Danvers, MA, USA). The horseradish-peroxidase-conjugated secondary antibody was used to incubate the PVDF membrane, and the signals of the target proteins were assessed with Enhanced Chemiluminescence (ECL) chemiluminescence detection kits (E412, Vazyme, Nanjing, China) utilizing the ChemiDoc MP imaging system (Bio-Rad, Hercules, CA, USA). Signal intensity was measured using the Gel-Pro Analyzer (Thermo Fisher Scientific, Waltham, MA, USA), calculating the ratio of target proteins to the internal control protein ACTB, which was then normalized to 1.0 for the control group.

2.7 Cell Treatment and Cytotoxicity Assay

Mouse monocyte/macrophage cells RAW264.7 (CL-0190, Procell Life Science & Technology, Wuhan, China) were maintained in a 5% CO_2 atmosphere at 37 °C and cultured in DMEM supplemented with 10% FBS, 100 IU/mL penicillin, and 100 µg/mL streptomycin. RAW264.7 cells were validated by STR profiling and tested negative for mycoplasma (D101, Vazyme, Nanjing, China). To investigate the critical role of HMGB1 in BBR-mediated NF-κB inhibition, RAW264.7 cells were seeded in 6-well plates until they reached a confluence of 70%. After a pretreatment period of 2 hours with either 2 or 10 µM BBR, the cells were treated with 0.5 µg/mL LPS for 24 h. Subsequently, total protein and RNA were extracted to assess the levels of p-NF-κB and NF-κB proteins, as well as the mRNA expression of the inflammatory genes *Tnfα* and *Il6*. The supernatant HMGB1 level in cell culture was quantified using the ELISA method. Similarly, RAW264.7 cells were pretreated with 2 and 10 µM BBR and then simultaneously treated with 1 µg/mL recombinant mouse HMGB1 (rmHMGB1, 50913-M01H, Sino Biological, Beijing, China) protein for 24 h to evaluate NF-κB activation and the inflammatory response.

To investigate whether AMPK activation by BBR contributes to the reduction of extracellular HMGB1 levels in LPS-stimulated RAW264.7 cells, we inhibited AMPK activation using RNA silencing or the inhibitor compound C (#T1977, TargetMol, Boston, MA, USA). Briefly, either 50 nM negative control siRNA or siRNA targeting AMPKα 1 (target sequence: 5′-ATGATGTCAGATGGTGAATTT-3′), synthesized by Sangon Biotech (Shanghai, China) [20,21] was transiently transfected into RAW264.7 cells via Lipofectamine RNAiMAX reagent (13778, Invitrogen, Carlsbad, CA, USA) and incubated for an additional 24 h. Subsequently, 0.5 µg/mL LPS, with or without 10 µM BBR treatment, was added to the culture medium for another 24 h. The protein expressions of AMPKα1 and p-AMPKα1 were detected by Western blot, while the extracellular HMGB1 content was measured using the ELISA method. Additionally, RAW264.7 cells were pretreated with 10 µM BBR and BBR plus 5 µM Compound C for 2 hours, followed by induction with 0.5 µg/mL LPS for 24 hours, after which AMPK activation and extracellular HMGB1 levels were also assessed. To evaluate potential cytotoxicity in these experiments, the corresponding treatments were conducted in 96-well plates, and cell viability was assessed according to the cell counting kit-8 method with commercial kits (C0005, TargetMol, Boston, MA, USA).

2.8 Statistical Analyses

The data in this article are presented as the mean ± SD or as representative figures. Statistical analyses were performed using SPSS 17.0 (IBM, Armonk, NY, USA) or GraphPad Prism 8 (GraphPad Prism Software Inc. La Jolla, CA, USA) and analyzed by Student's *t*-test or One-way

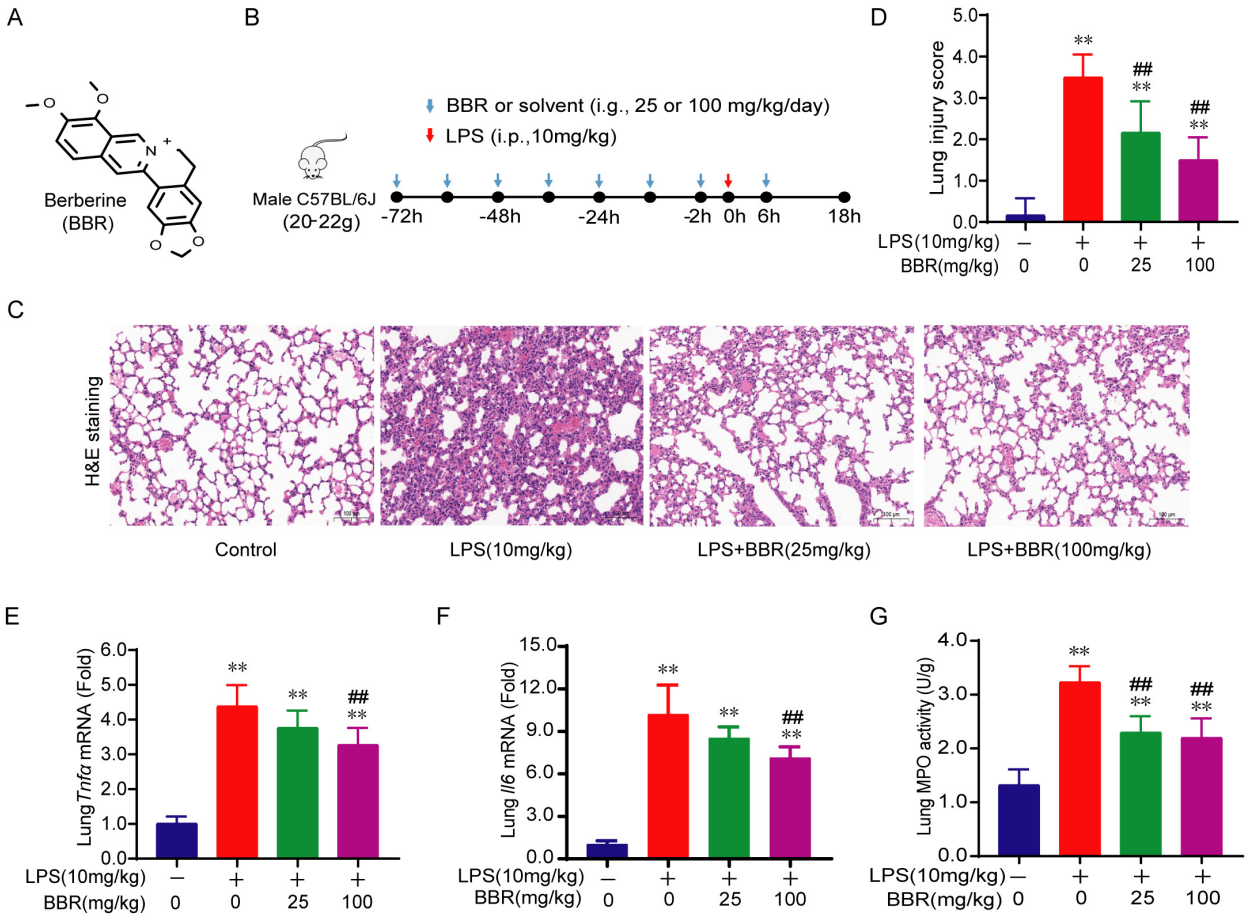

Fig. 1. BBR ameliorates LPS-induced ALI and inflammatory response in mice. (A,B) Chemical structure of BBR (A) and experimental scheme (B). (C) H&E staining of lung tissues at 18 h after LPS stimulation (200×, scale bar = 100 μm). (D) lung injury score. (E,F) mRNA levels of inflammatory cytokine genes *Tnfα* and *Il6* in lung tissues determined by quantitative PCR. (G) MPO level in the lung tissues of mice. Data are presented as the mean ± standard deviation (SD) (n = 6 for every group) or the representative figures. One-way analysis of variance (ANOVA), $^{**}p < 0.01$ *vs.* the control group. $^{\#\#}p < 0.01$ *vs.* the LPS group. BBR, berberine; LPS, lipopolysaccharide; ALI, acute lung injury; H&E, hematoxylin and eosin; *Tnfα*, tumor necrosis factor-α; *Il6*, interleukin 6; PCR, polymerase chain reaction; MPO, myeloperoxidase.

analysis of variance followed by Student-Newman-Keuls post hoc tests. The threshold for statistical significance was established at $p < 0.05$ or 0.01.

3. Results

3.1 BBR Ameliorates LPS-Induced ALI and Inflammatory Response in Mice

To evaluate the effects of BBR (Fig. 1A) on LPS-induced ALI, mice were administered BBR twice daily (a total of 25 or 100 mg/kg/day) for 3 days before LPS induction, with an additional treatment at 6 hours post-LPS injection (Fig. 1B). 18 h following LPS stimulation, lung tissues stained with H&E from the group treated solely with LPS displayed marked infiltration of inflammatory cells, edema, increased interstitial congestion, and thickened alveolar walls. Conversely, BBR treatment effectively alleviated these pathological changes (Fig. 1C), with the lung injury score corroborating the protective effect of BBR in ALI (Fig. 1D). Inflammatory mediators play a vital role in the development of ALI triggered by LPS. Additionally, our findings indicated that BBR markedly reduced the overexpression of the inflammatory genes *Tnfα* and Il6 in the lungs of mice subjected to LPS (Fig. 1E,F). Neutrophil infiltration in lung tissues occurs during ALI, with MPO activity serving as a key indicator. As depicted in Fig. 1G, LPS significantly elevated MPO activity, whereas BBR treatment markedly reduced MPO activity, further confirming the protective effects of BBR against lung injury and inflammation. These findings suggest that BBR notably reduces the inflammatory response and alleviates LPS-induced ALI in mice.

3.2 BBR Inhibits Oxidative Stress and Apoptosis During LPS-Induced ALI in Mice

ALI is characterized by an excessive inflammatory response that often exacerbates oxidative stress and apop-

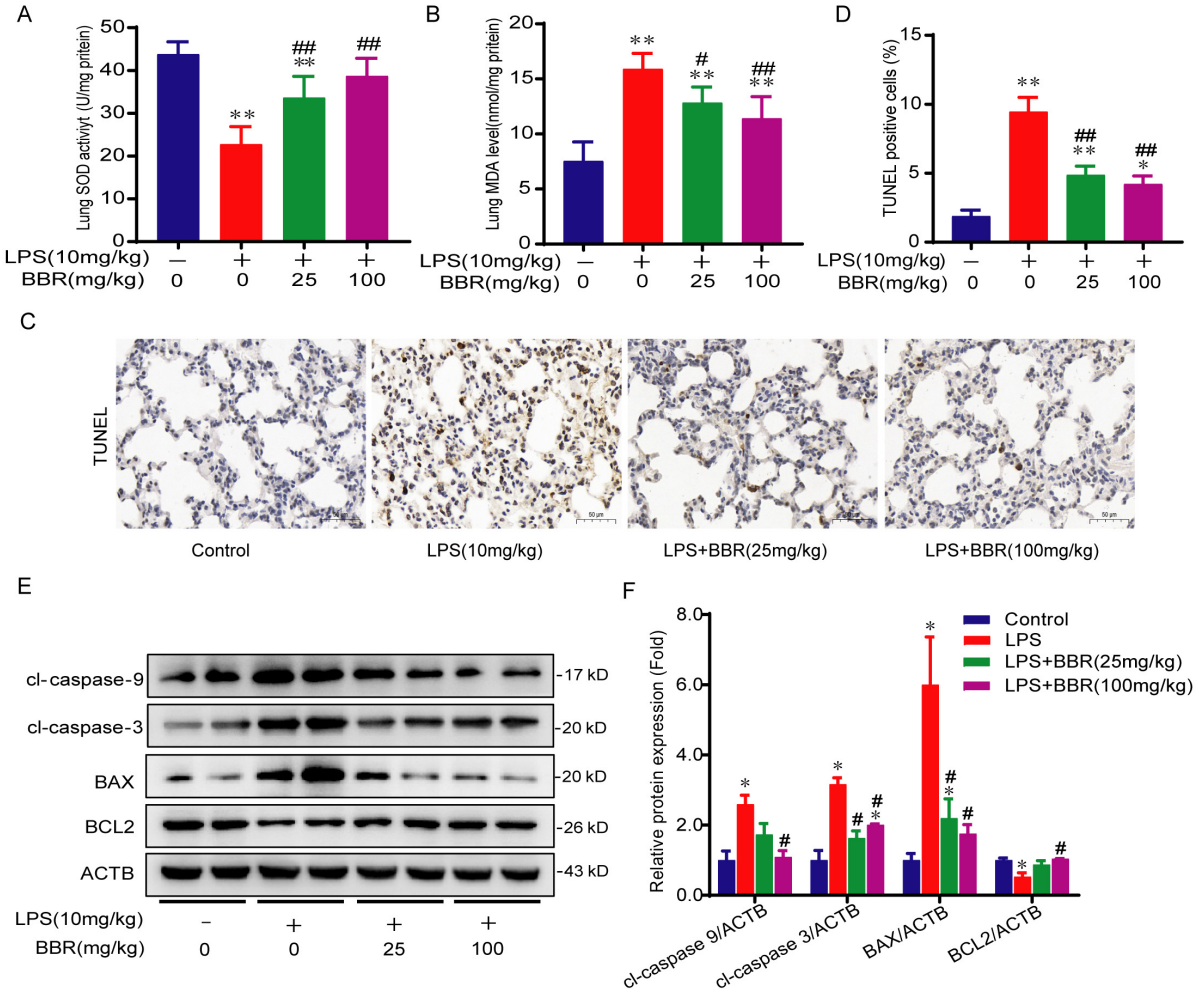

Fig. 2. BBR inhibits oxidative stress and apoptosis during LPS-induced ALI in mice. (A,B) MDA levels and SOD activity in lung tissues were assessed as oxidative stress indicators at 18 h after LPS and/or BBR treatment. (C,D) Representative staining (200×, scale bar = 50 μm) and positive-cell quantification of the TUNEL assay in mouse lung tissues. (E,F) Western blot analysis and quantification of apoptosis-associated proteins in mouse lung tissues. The results are shown as means ± standard deviation (n = 6 for every group) or as representative figures. One-way analysis of variance (ANOVA) was conducted. $^{*}p < 0.05$ and $^{**}p < 0.01$ *vs.* the control group. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs.* the LPS group. MDA, malondialdehyde; SOD, superoxide dismutase; TUNEL, transferase dUTP nick end labeling; BCL2, B cell lymphoma 2; BAX, BCL2-Associated X protein.

tosis [22]. To investigate the effects of BBR on oxidative stress injury, we measured the activity of the antioxidant enzyme SOD and the levels of MDA in lung tissues. The results indicate that LPS significantly reduced SOD activity and increased MDA levels in lung tissues, whereas BBR treatment significantly reversed these alterations (Fig. 2A,B), emphasizing its potent antioxidant properties. To evaluate the effect of BBR on apoptosis, we conducted the TUNEL assay on lung tissues and analyzed the expression of apoptosis-related proteins. Our findings revealed a marked increase in the proportion of TUNEL-positive cells following LPS treatment, which was notably reduced by BBR (Fig. 2C,D). Additionally, BBR markedly suppressed the levels of apoptosis-related proteins, specifically cleaved caspase-3 and cleaved caspase-9, induced by LPS (Fig. 2E,F). Compared with the control group, the LPS group exhibited lower B cell lymphoma 2 (BCL2), levels and higher BCL2-Associated X protein (BAX) levels, and these abnormalities were also significantly attenuated by BBR treatment (Fig. 2E,F). The findings collectively affirm the antioxidative stress and anti-apoptotic properties of BBR in LPS-induced ALI, which correspond to its anti-inflammatory function.

3.3 BBR Activates AMPK and Suppresses HMGB1-Mediated NF-κB Activation in LPS-Induced ALI in Mice

BBR has been widely reported to activate AMPK, which in turn contributes to the maintenance of oxidant-antioxidant balance in various inflammatory conditions [12,23]. Conversely, excessive HMGB1 release triggers inflammatory responses and subsequent apoptosis in sep-

sis [4,5]. Consequently, we chose to examine the roles of AMPK and HMGB1 in mediating the protective effects of BBR against sepsis-induced inflammatory activation, a critical mechanism underpinning ALI. The results indicate that LPS significantly reduced phosphorylated AMPK (p-AMPK) levels and increased total HMGB1 protein levels, resulting in NF-κB activation in mouse lung tissues (Fig. 3A,B). In contrast, BBR treatment partially reversed these effects without affecting the total AMPK levels (Fig. 3A,B). The regulatory effect of BBR on AMPK aligns with previous reports on its effects in other inflammation-related models [23,24]. Furthermore, our results demonstrated that BBR inhibits the nuclear translocation of HMGB1, as evidenced by increased nuclear and decreased cytoplasmic HMGB1 protein levels compared with the LPS-induced ALI model (Fig. 3C,D). Immunofluorescence staining of HMGB1 in mouse lungs also showed increased total and cytoplasmic HMGB1 levels following LPS induction, which were mitigated by BBR treatment (Fig. 3E). The function of HMGB1 depends on its release into circulating fluids, where it acts as a DAMP by binding to cell surface receptors, ultimately triggering the inflammatory response. Consequently, we measured the serum levels of HMGB1 in the ALI model induced by LPS. As anticipated, LPS resulted in a marked increase in serum concentrations of HMGB1, while treatment with BBR significantly lowered these levels in a dose-dependent way (Fig. 3F). Consequently, BBR exerts inhibitory effects on HMGB1-mediated NF-κB activation in LPS-induced ALI, and this mechanism may be related to the activation of AMPK during this process.

3.4 BBR Decreases Extracellular HMGB1 in LPS-Induced RAW264.7 Cells and Inhibits HMGB1-Stimulated NF-κB Activation

The primary mechanism underlying sepsis is immune dysfunction, with macrophages playing a pivotal role [25]. In this study, we utilized the mouse macrophage cell line RAW264.7 to elucidate the role of HMGB1 in BBR-mediated inhibition of NF-κB activation during sepsis. After 24 hours of treatment with LPS and BBR, LPS significantly induced the activation of NF-κB (Fig. 4A), increased the extracellular HMGB1 level (Fig. 4B), and was accompanied by excessive expression of *Tnf*α (Fig. 4C) and Il6 (Fig. 4D) mRNA. In contrast, BBR reversed the increased extracellular HMGB1 level and inflammatory activations induced by LPS (Fig. 4A–D). HMGB1 typically acts as a potent inflammation inducer by binding to multiple cell-surface receptors [26] or by binding with LPS to amplify its pro-inflammatory activity [27,28]. To further verify the role of HMGB1 in the protective effect of BBR during sepsis, we evaluated the anti-inflammatory effect of BBR in HMGB1-stimulated RAW264.7 cells. The results indicated that HMGB1 significantly promoted NF-κB activation and upregulated the inflammatory genes *Tnf*α and *Il6* expression, whereas BBR partially mitigated this effect (Fig. 4E–G). Therefore, the evidence that BBR decreases extracellular HMGB1 in LPS-induced RAW264.7 cells and inhibits HMGB1-induced NF-κB activation suggests that BBR alleviates sepsis-related inflammatory injury, at least partially, through decreasing extracellular HMGB1, which is consistent with the results derived from animal experiments (Fig. 3).

3.5 Silencing the Activation of AMPK Reverses BBR-Reduced Extracellular HMGB1 Level in LPS-Stimulated RAW264.7 Cells

As extracellular HMGB1 serves as a final effector in the inflammatory activation process, we further assessed whether BBR-induced decreases in extracellular HMGB1 levels are mediated through AMPK activation in LPS-stimulated RAW264.7 cells. For this, we utilized AMPKα1-specific small interfering RNA (siRNA) and the pharmacological p-AMPK inhibitor compound C to knock down or inhibit AMPK activation. Results indicate that, under non-cytotoxic conditions in RAW264.7 cells (Fig. 5A), AMPK protein expression was inhibited after transfection with siAMPK, leading to subsequently lower p-AMPK levels (Fig. 5B). Compared with the control group, LPS reduced p-AMPK levels, whereas BBR enhanced its level. However, by knockdown of AMPKα1, the p-AMPK level remains lower even in the presence of BBR (Fig. 5B). Correspondingly, the inhibition of supernatant HMGB1 level by BBR in LPS-induced RAW264.7 cells was reversed by AMPK silencing (Fig. 5C). Similarly, at a non-cytotoxic concentration (Fig. 5D), Compound C also reversed BBR's effects on p-AMPK activation and extracellular HMGB1 levels (Fig. 5E,F). The findings indicate that the reduction in extracellular HMGB1 levels caused by BBR is at least partially facilitated through the enhancement of p-AMPK level in RAW264.7 cells stimulated by LPS.

4. Discussion

Targeting the signaling pathways and molecules that mediate systemic inflammation represents a critical therapeutic strategy for alleviating organ injury during sepsis [1]. We previously reviewed the protective effects of BBR against multiple organ injuries, encompassing the intestine, liver, kidney, and lung [29]. In this study, we further revealed that BBR significantly mitigates LPS-ALI, as evidenced by reductions in histopathological damage, inflammatory responses, oxidative stress, and apoptosis. Mechanistic insights reveal that BBR activates AMPK, which subsequently diminishes serum and extracellular HMGB1 levels, ultimately inhibiting HMGB1-mediated NF-κB activation.

To date, there remains a lack of effective treatments for irreversible septic shock and multiple organ dysfunction syndromes, although extensive research has been conducted on dysregulated signaling pathways and molecules

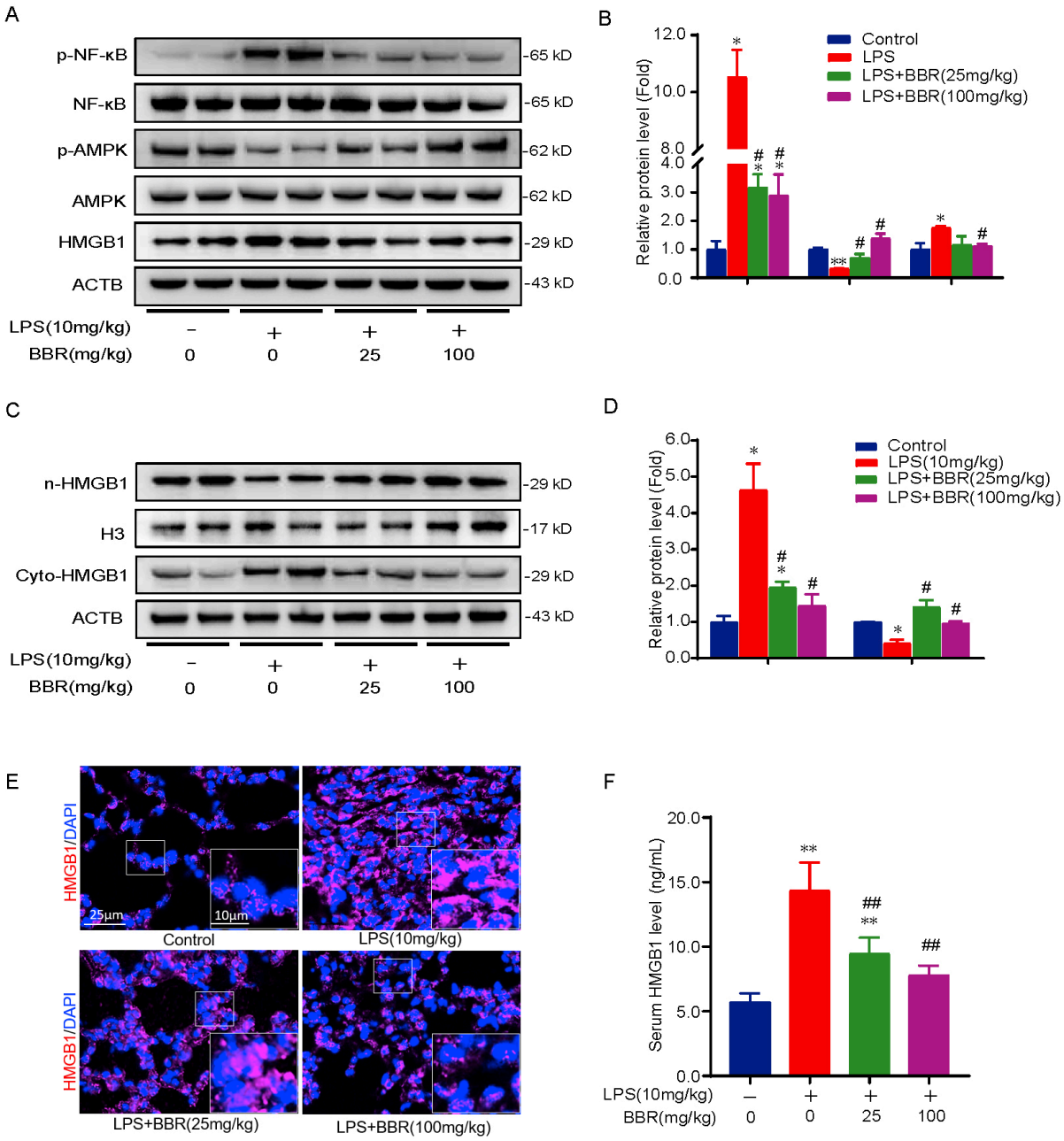

Fig. 3. BBR activates AMPK and suppresses HMGB1-mediated NF-κB activation in LPS-induced ALI in mice. (A,B) Western blot analysis and quantification of p-AMPK, AMPK, p-NF-κB, NF-κB, and HMGB1 in mouse lung tissue. (C,D) Western blot analysis and quantification of HMGB1 in the nuclei and cytoplasm. (E) Immunofluorescence analysis of HMGB1 protein expression in mouse lung tissue (scale bar = 25 μm; enlarge image scale bar = 10 μm). (F) ELISA analysis of serum HMGB1 levels. Data are presented as the mean ± SD (n = 6 for every group) or the representative figures. One-way analysis of variance (ANOVA), $*p < 0.05$ and $**p < 0.01$ *vs.* the control group. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs.* the LPS group. AMPK, adenosine-monophosphate-activated protein kinase; HMGB1, high mobility group box-1; NF-κB, nuclear factor Kappa B; ELISA, enzyme-linked immunosorbent assay.

underlying sepsis pathogenesis [1]. Regarding signaling- and molecule-targeted anti-sepsis strategies, BBR could alleviate LPS-induced lung injury through mechanisms including inhibition of TNF-α production and the expression and activation of cytosolic phospholipase A2 [30], activation of the PERK-mediated Nrf2/HO-1 signaling axis [31], and suppression of NF-κB and IL-6-mediated STAT3 activation [32]. In line with these findings, our research also demonstrates that BBR significantly reduces lung injury, inflammation, oxidative stress, and apoptosis. Consider-

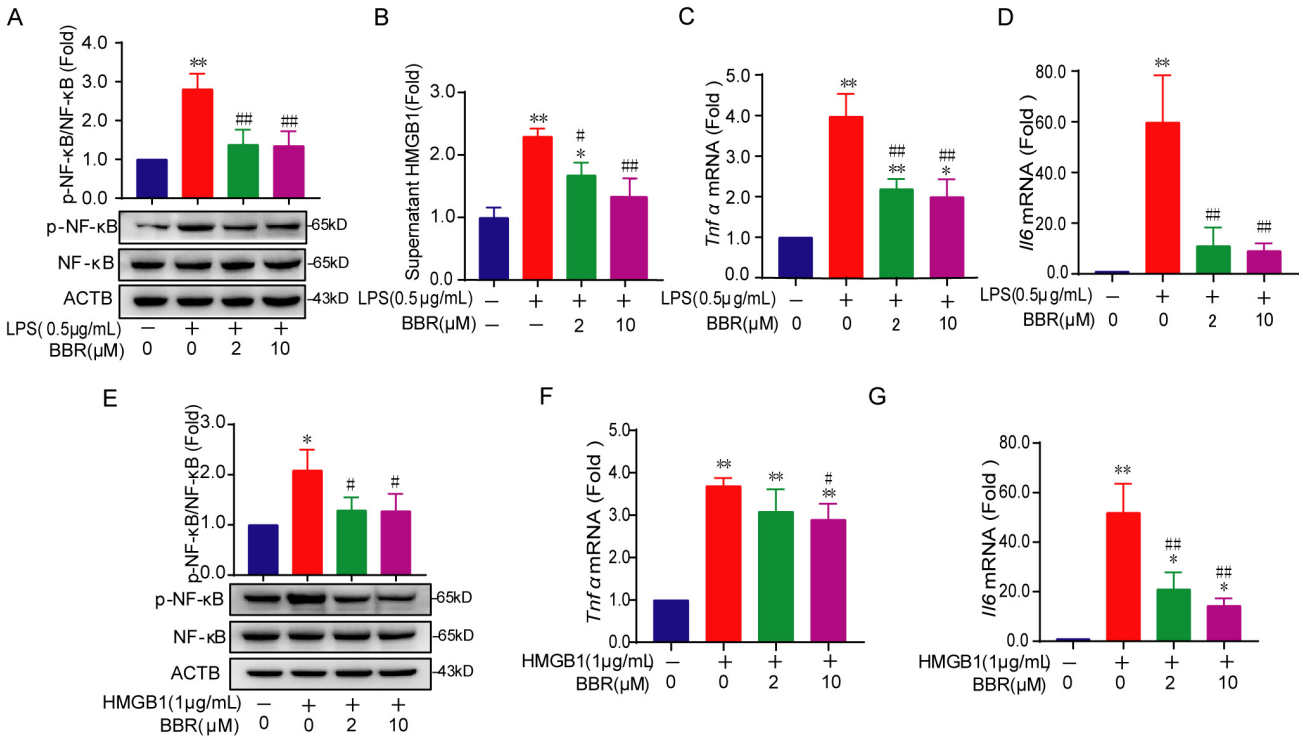

Fig. 4. BBR decreases extracellular HMGB1 in LPS-induced RAW264.7 cells and inhibits HMGB1-stimulated NF-κB activation. (A,B) The p-NF-κB and NF-κB expression (A), and the extracellular HMGB1 level (B) after treatment with BBR in LPS-induced RAW264.7 cells. (C,D,F,G) The mRNA expression of inflammatory gene *Tnfα* and *Il6* detected by qRT-PCR. (E) The p-NF-κB and NF-κB expression after treatment by rmHMGB1 and/or BBR in RAW264.7 cells. Data are presented as mean ± SD (n = 3). One-way analysis of variance (ANOVA), $*p < 0.05$, $**p < 0.01$ *vs*. the control group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ *vs*. the LPS or rmHMGB1 treated group.

ing BBR's ability to activate AMPK across diverse disease models, HMGB1's role as a DAMP in inflammatory diseases, and BBR's protective role for ischemia-reperfusion injury by inhibiting the HMGB1-NF-κB pathway [33,34], we posit a potential causal link between BBR-mediated AMPK activation and HMGB1-mediated inflammatory injury in sepsis.

Serum HMGB1 concentrations have been closely linked with mortality in animal sepsis models [35,36]. Our study in a murine septic model revealed that BBR reduced serum HMGB1 concentrations, potentially by suppressing the total protein expression, nucleus-to-cytoplasm translocation, and extracellular release of HMGB1 (Fig. 3). These results reinforce the concept that HMGB1 translocation from the nucleus to the cytoplasm is a pivotal step preceding its extracellular release [37]. Although the reduced HMGB1 release might also be attributed to the enhanced HMGB1 deacetylation by SIRT3 or SIRT1, which are potentially regulated by BBR [10,38–40], it might also stem from BBR's potential modulation of the AMPK/mTOR and Nrf2/HO-1 signaling pathways [41,42]. Collectively, the decrease in extracellular HMGB1—the functional form of HMGB1—contributes to the multifaceted regulation of inflammatory diseases by BBR, thereby reinforcing the idea that BBR is a multifunctional compound with diverse pharmacological activities [43].

Multiple cell types, such as alveolar epithelial cells, neutrophils, monocytes, and macrophages, participate in the progression and prognosis of sepsis-related lung injury. Among them, although alveolar epithelial cells initiate inflammation *via* barrier damage, neutrophils drive injury through NETosis and oxidative stress, and monocytes through infiltration and differentiation, macrophages serve as central orchestrators by polarizing into pro-inflammatory (M1) or reparative (M2) phenotypes, ultimately influencing the progression or resolution of sepsis-induced lung injury across all phases [1,44,45]. Consequently, we investigated the anti-inflammatory mechanisms specifically in RAW264.7 macrophages derived from mice. Exogenous HMGB1 accumulates on the surface of macrophages, forming a complex with its receptors, which subsequently activates the inflammatory pathway. As expected, our findings revealed that BBR's inhibition of NF-κB activation is partly mediated by decreased extracellular HMGB1 levels, which depend on AMPK activation by BBR. Notably, AMPK can also suppress NF-κB activation *via* alternative pathways, such as MAPK (P38, ERK, and JNK) [46].

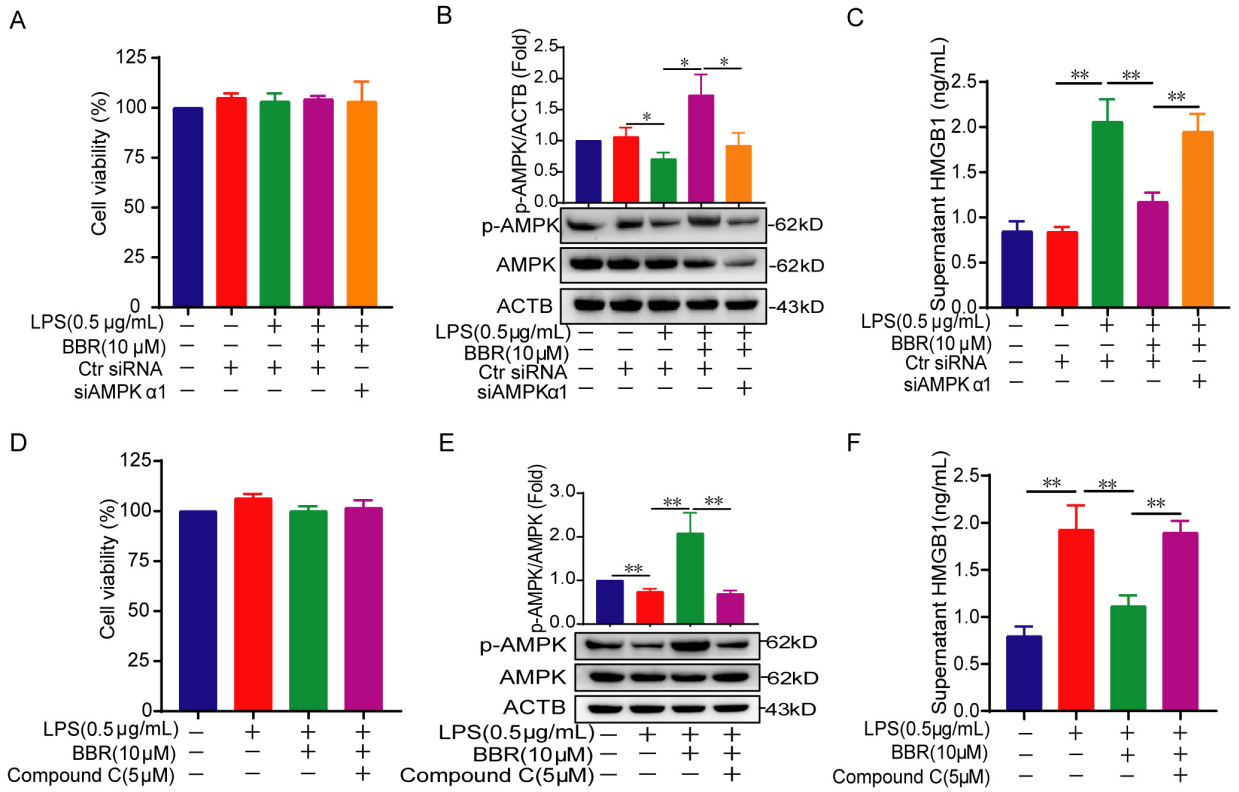

Fig. 5. Silencing the activation of AMPK reverses BBR-reduced extracellular HMGB1 level in LPS-stimulated RAW264.7 cells. (A,D) Cytotoxicity detection in RAW264.7 cells after 24 h or 48 h treatment with LPS, BBR, siAMPK, or compound C. (B,E) Western blot analysis of p-AMPK and AMPK protein levels. (C,F) HMGB1 levels in cell culture supernatants detected *via* an ELISA method. Data are presented as the mean ± SD (n = 3). Student's *t*-test and One-way analysis of variance (ANOVA), $*p < 0.05$, $**p < 0.01$ *vs.* the corresponding control group.

Nonetheless, our study is subject to some limitations. For instance, we primarily explored the causal or upstream-downstream relationship by which BBR activates AMPK, reduces the release of HMGB1, and inhibits NF-κB activation. However, the direct mechanisms through which BBR modulates the AMPK-HMGB1-NF-κB pathway were not thoroughly investigated in this study. We believe the key question lies in how BBR activates AMPK. Previous studies indicated that BBR inhibits mitochondrial Complex I and modulates cellular energy metabolism, which is considered a pivotal mechanism for AMPK activation [10,23]. Moreover, given that the functional form of HMGB1 is present in the extracellular milieu, we thus mainly focus on the extracellular HMGB1. However, according to the results in the mouse model (Fig. 3), BBR could also diminish HMGB1 protein levels and impede its nuclear translocation, and thus further exploration is warranted to elucidate the precise mechanism. Furthermore, our investigation has not yet delved into the direct causation of oxidative stress and apoptosis by exaggerated inflammatory responses, as these processes are widely studied and interact as both causes and effects. Third, about the activation of AMPK by BBR, we have not set another AMPK activator as a positive control to compare its specificity and rule out potential off-target effects, though other researchers have previously demonstrated the activation of AMPK in RAW264.7 cells [24,47]. Besides, in demonstrating the mechanism of BBR in a cellular model, we did not conduct the loss function experiment of HMGB1 or establish additional control groups, such as LPS + siAMPKα1 and LPS + Compound C groups, to comprehensively compare the effects of HMGB1, AMPK, and BBR on inflammation activation.

5. Conclusions

Taken together, the current study proposes that BBR markedly inhibits inflammation, oxidative stress, and apoptosis *via* the AMPK-HMGB1-NF-κB signaling axis, unveiling a novel mechanism underlying BBR's protective effects against ALI in sepsis. Given the absence of effective treatments, BBR and other AMPK activators might serve as promising candidates for managing systemic inflammation and injury during sepsis.

Abbreviations

ANOVA, One-way analysis of variance; ALI, acute lung injury; AMPK, AMP-activated protein kinase; BBR,

Berberine; DAMP, damage-associated molecular pattern; ELISA, enzyme-linked immunosorbent assay; H&E, hematoxylin and eosin; HMGB1, High mobility group box-1; LPS, lipopolysaccharide; MDA, malondialdehyde; MPO, myeloperoxidase; p-AMPK, phosphorylated AMPK; PVDF, polyvinylidene fluoride; siRNA, small interfering RNA; SOD, superoxide dismutase; TUNEL, transferase dUTP nick end labeling.

Availability of Data and Materials

The data associated with this paper are available upon request to the corresponding author.

Author Contributions

TXL, CW, and WJQ designed the research study. TXL and CPZ extracted all data and performed analyses. YWZ, LH and GWH provided help and advice on methodology. TXL drafted the manuscript and performed the revisions. WJQ provided funding acquisition. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All animal experiments were approved by the Ethics Committee of Beijing Friendship Hospital, Capital Medical University (Approval #24-2034) and conducted following the National Guidelines for Housing and Care of Laboratory Animals.

Acknowledgment

We sincerely thank professor Bangjiang Fang for his valuable suggestions on experimental design and professor Hu Li for giving operational guidance advice in the experiment.

Funding

This work was supported by High-level Public Health Technology Talent Construction Project (Academic Leader-02-29), the National key clinical specialty construction Project (2022) & University (No. yybsh2021007), Capital Medical University Natural Science Foundation (PYZ24071).

Conflict of Interest

The authors declare no conflict of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work the authors used ChatGpt-3.5 in order to check spell and grammar. After using this tool, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

References

[1] Zhang YY, Ning BT. Signaling pathways and intervention therapies in sepsis. Signal Transduction and Targeted Therapy. 2021; 6: 407. https://doi.org/10.1038/s41392-021-00816-9.

[2] Pool R, Gomez H, Kellum JA. Mechanisms of Organ Dysfunction in Sepsis. Critical Care Clinics. 2018; 34: 63–80. https://doi.org/10.1016/j.ccc.2017.08.003.

[3] Evans CE, Zhao YY. Impact of thrombosis on pulmonary endothelial injury and repair following sepsis. American Journal of Physiology. Lung Cellular and Molecular Physiology. 2017; 312: L441–L451. https://doi.org/10.1152/ajplung.00441.2016.

[4] Yang H, Wang H, Andersson U. Targeting Inflammation Driven by HMGB1. Frontiers in Immunology. 2020; 11: 484. https://doi.org/10.3389/fimmu.2020.00484.

[5] Fang P, Dou B, Liang J, Hou W, Ma C, Zhang Q. Quercetin Reduces Oxidative Stress and Apoptosis by Inhibiting HMGB1 and Its Translocation, Thereby Alleviating Liver Injury in ACLF Rats. Evidence-based Complementary and Alternative Medicine. 2021; 2021: 2898995. https://doi.org/10.1155/2021/2898995.

[6] Irzan FI, Retinasamy T, Wen WR, Sheng ETM, Shaikh MF, Arulsamy A. The Role of HMGB1 in Infection-Related Cognitive Deficits. Frontiers in Bioscience (Landmark Edition). 2025; 30: 25544. https://doi.org/10.31083/FBL25544.

[7] Kumar A, Ekavali, Chopra K, Mukherjee M, Pottabathini R, Dhull DK. Current knowledge and pharmacological profile of berberine: An update. European Journal of Pharmacology. 2015; 761: 288–297. https://doi.org/10.1016/j.ejphar.2015.05.068.

[8] Li H, Liu NN, Li JR, Dong B, Wang MX, Tan JL, *et al*. Combined Use of Bicyclol and Berberine Alleviates Mouse Nonalcoholic Fatty Liver Disease. Frontiers in Pharmacology. 2022; 13: 843872. https://doi.org/10.3389/fphar.2022.843872.

[9] Li H, Wang XK, Tang M, Lei L, Li JR, Sun H, *et al*. *Bacteroides thetaiotaomicron* ameliorates mouse hepatic steatosis through regulating gut microbial composition, gut-liver folate and unsaturated fatty acids metabolism. Gut Microbes. 2024; 16: 2304159. https://doi.org/10.1080/19490976.2024.2304159.

[10] Wang X, Li H, Li J, Lei L, Xu J, Sun H, *et al*. Berberine dissociates mitochondrial complex I by SIRT3-dependent deacetylation of NDUFS1 to improve hepatocellular glucose and lipid metabolism. Science China. Life Sciences. 2025; 68: 2676–2696. https://doi.org/10.1007/s11427-024-2834-8.

[11] Vlasova O, Antonova I, Zenkov R, Naberezhnov D, Belitsky G, Borunova A, *et al*. Anticancer Plant Secondary Metabolites Induce Linker Histone Depletion from Chromatin. Frontiers in Bioscience (Landmark Edition). 2024; 29: 275. https://doi.org/10.31083/j.fbl2908275.

[12] Wang G, Song Y, Feng W, Liu L, Zhu Y, Xie X, *et al*. Activation of AMPK attenuates LPS-induced acute lung injury by upregulation of PGC1α and SOD1. Experimental and Therapeutic Medicine. 2016; 12: 1551–1555. https://doi.org/10.3892/etm.2016.3465.

[13] Li F, Wang HD, Lu DX, Wang YP, Qi RB, Fu YM, *et al*. Neutral sulfate berberine modulates cytokine secretion and increases survival in endotoxemic mice. Acta Pharmacologica Sinica. 2006; 27: 1199–1205. https://doi.org/10.1111/j.1745-7254.2006.00368.x.

[14] Li H, Li JR, Huang MH, Chen JH, Lv XQ, Zou LL, *et al*. Bicyclol Attenuates Liver Inflammation Induced by Infection of Hepatitis C Virus *via* Repressing ROS-Mediated Activation of MAPK/NF-κB Signaling Pathway. Frontiers in Pharmacology. 2018; 9: 1438. https://doi.org/10.3389/fphar.2018.01438.

[15] Kang L, Pang J, Zhang X, Liu Y, Wu Y, Wang J, *et al*. L-arabinose Attenuates LPS-Induced Intestinal Inflammation and Injury through Reduced M1 Macrophage Polarization. The Journal of Nutrition. 2023; 153: 3327–3340. https://doi.org/10.1016/j.tjnut.2023.09.012.

[16] Yun Y, Chen J, Wang X, Li Y, Hu Z, Yang P, *et al*. Tofacitinib Ameliorates Lipopolysaccharide-Induced Acute Kidney Injury by Blocking the JAK-STAT1/STAT3 Signaling Pathway. BioMed Research International. 2021; 2021: 8877056. https://doi.org/10.1155/2021/8877056.

[17] Liu S, Zhang S, Sun Y, Zhou W. Transcriptomics Changes in the Peritoneum of Mice with Lipopolysaccharide-Induced Peritonitis. International Journal of Molecular Sciences. 2021; 22: 13008. https://doi.org/10.3390/ijms222313008.

[18] Ye K, Lin X, Chen TZ, Wang LH, Liu SX. Heparin-Binding Protein Promotes Acute Lung Injury in Sepsis Mice by Blocking the Aryl Hydrocarbon Receptor Signaling Pathway. Journal of Inflammation Research. 2024; 17: 2927–2938. https://doi.org/10.2147/JIR.S454777.

[19] Lee SB, Lee WS, Shin JS, Jang DS, Lee KT. Xanthotoxin suppresses LPS-induced expression of iNOS, COX-2, TNF-α, and IL-6 via AP-1, NF-κB, and JAK-STAT inactivation in RAW 264.7 macrophages. International Immunopharmacology. 2017; 49: 21–29. https://doi.org/10.1016/j.intimp.2017.05.021.

[20] Tangeman L, Wyatt CN, Brown TL. Knockdown of AMP-activated protein kinase alpha 1 and alpha 2 catalytic subunits. Journal of RNAi and Gene Silencing: an International Journal of RNA and Gene Targeting Research. 2012; 8: 470–478.

[21] Li M, Zhang L, Guan T, Huang L, Zhu Y, Wen Y, *et al*. Energy stress-activated AMPK phosphorylates Snail1 and suppresses its stability and oncogenic function. Cancer Letters. 2024; 595: 216987. https://doi.org/10.1016/j.canlet.2024.216987.

[22] Kosutova P, Mikolka P, Kolomaznik M, Balentova S, Adamkov M, Calkovska A, *et al*. Reduction of lung inflammation, oxidative stress and apoptosis by the PDE4 inhibitor roflumilast in experimental model of acute lung injury. Physiological Research. 2018; 67: S645–S654. https://doi.org/10.33549/physiolres.934047.

[23] Kong WJ, Vernieri C, Foiani M, Jiang JD. Berberine in the treatment of metabolism-related chronic diseases: A drug cloud (dCloud) effect to target multifactorial disorders. Pharmacology & Therapeutics. 2020; 209: 107496. https://doi.org/10.1016/j.pharmthera.2020.107496.

[24] Jeong HW, Hsu KC, Lee JW, Ham M, Huh JY, Shin HJ, *et al*. Berberine suppresses proinflammatory responses through AMPK activation in macrophages. American Journal of Physiology. Endocrinology and Metabolism. 2009; 296: E955–E964. https://doi.org/10.1152/ajpendo.90599.2008.

[25] Qiu P, Liu Y, Zhang J. Review: the Role and Mechanisms of Macrophage Autophagy in Sepsis. Inflammation. 2019; 42: 6–19. https://doi.org/10.1007/s10753-018-0890-8.

[26] Yuan S, Liu Z, Xu Z, Liu J, Zhang J. High mobility group box 1 (HMGB1): a pivotal regulator of hematopoietic malignancies. Journal of Hematology & Oncology. 2020; 13: 91. https://doi.org/10.1186/s13045-020-00920-3.

[27] He ZW, Qin YH, Wang ZW, Chen Y, Shen Q, Dai SM. HMGB1 acts in synergy with lipopolysaccharide in activating rheumatoid synovial fibroblasts via p38 MAPK and NF-κB signaling pathways. Mediators of Inflammation. 2013; 2013: 596716. https://doi.org/10.1155/2013/596716.

[28] Qin YH, Dai SM, Tang GS, Zhang J, Ren D, Wang ZW, *et al*. HMGB1 enhances the proinflammatory activity of lipopolysaccharide by promoting the phosphorylation of MAPK p38 through receptor for advanced glycation end products. Journal of Immunology. 2009; 183: 6244–6250. https://doi.org/10.4049/jimmunol.0900390.

[29] Lv T, Zhang C, Hu L, Wang C, Li S, Wang H, *et al*. Berberine in Sepsis: Effects, Mechanisms, and Therapeutic Strategies. Journal of Immunology Research. 2023; 2023: 4452414. https://doi.org/10.1155/2023/4452414.

[30] Zhang HQ, Wang HD, Lu DX, Qi RB, Wang YP, Yan YX, *et al*. Berberine inhibits cytosolic phospholipase A2 and protects against LPS-induced lung injury and lethality independent of the alpha2-adrenergic receptor in mice. Shock. 2008; 29: 617–622. https://doi.org/10.1097/SHK.0b013e318157ea14.

[31] Liang Y, Fan C, Yan X, Lu X, Jiang H, Di S, *et al*. Berberine ameliorates lipopolysaccharide-induced acute lung injury via the PERK-mediated Nrf2/HO-1 signaling axis. Phytotherapy Research. 2019; 33: 130–148. https://doi.org/10.1002/ptr.6206.

[32] Wang Y, Du P, Jiang D. Berberine functions as a negative regulator in lipopolysaccharide -induced sepsis by suppressing NF-κB and IL-6 mediated STAT3 activation. Pathogens and Disease. 2020; 78: ftaa047. https://doi.org/10.1093/femspd/ftaa047.

[33] Zhu JR, Lu HD, Guo C, Fang WR, Zhao HD, Zhou JS, *et al*. Berberine attenuates ischemia-reperfusion injury through inhibiting HMGB1 release and NF-κB nuclear translocation. Acta Pharmacologica Sinica. 2018; 39: 1706–1715. https://doi.org/10.1038/s41401-018-0160-1.

[34] Feng Y, Wang L, Mao Z, Wang W. HMGB1 Mediates Microglia-Astrocyte/Neuron Crosstalk and Pyroptosis by the TLR4/NF-κB Pathway in Multiple Sclerosis. Frontiers in Bioscience (Landmark Edition). 2025; 30: 37838. https://doi.org/10.31083/FBL37838.

[35] Wang H, Yang H, Czura CJ, Sama AE, Tracey KJ. HMGB1 as a late mediator of lethal systemic inflammation. American Journal of Respiratory and Critical Care Medicine. 2001; 164: 1768–1773. https://doi.org/10.1164/ajrccm.164.10.2106117.

[36] Valdés-Ferrer SI, Rosas-Ballina M, Olofsson PS, Lu B, Dancho ME, Li J, *et al*. High-mobility group box 1 mediates persistent splenocyte priming in sepsis survivors: evidence from a murine model. Shock. 2013; 40: 492–495. https://doi.org/10.1097/SHK.0000000000000050.

[37] Zhou RR, Zhao SS, Zou MX, Zhang P, Zhang BX, Dai XH, *et al*. HMGB1 cytoplasmic translocation in patients with acute liver failure. BMC Gastroenterology. 2011; 11: 21. https://doi.org/10.1186/1471-230X-11-21.

[38] Wei S, Gao Y, Dai X, Fu W, Cai S, Fang H, *et al*. SIRT1-mediated HMGB1 deacetylation suppresses sepsis-associated acute kidney injury. American Journal of Physiology. Renal Physiology. 2019; 316: F20–F31. https://doi.org/10.1152/ajprenal.00119.2018.

[39] Nie XJ, Hao BZ, Zhang BL, Li YY. GATA3 ameliorates melanocyte injuries in vitiligo through SIRT3-mediated HMGB1 deacetylation. The Journal of Dermatology. 2023; 50: 472–484. https://doi.org/10.1111/1346-8138.16634.

[40] Chen N, Wang XC, Fan LL, Zhu YH, Wang Q, Chen YB. Berberine Ameliorates Lipopolysaccharide-Induced Cognitive Impairment Through SIRT1/NRF2/NF-κB Signaling Pathway in C57BL/6J Mice. Rejuvenation Research. 2022; 25: 233–242. https://doi.org/10.1089/rej.2022.0023.

[41] Zhao M, Qin J, Shen W, Wu A. Bilobalide Enhances AMPK Activity to Improve Liver Injury and Metabolic Disorders in STZ-Induced Diabetes in Immature Rats via Regulating HMGB1/TLR4/NF-κB Signaling Pathway. BioMed Research International. 2021; 2021: 8835408. https://doi.org/10.1155/2021/8835408.

[42] Arab HH, Al-Shorbagy MY, Saad MA. Activation of autophagy and suppression of apoptosis by dapagliflozin attenuates experimental inflammatory bowel disease in rats: Targeting AMPK/mTOR, HMGB1/RAGE and Nrf2/HO-1 pathways. Chemico-Biological Interactions. 2021; 335: 109368. https://doi.org/10.1016/j.cbi.2021.109368.

[43] Chu M, Chen X, Wang J, Guo L, Wang Q, Gao Z, *et al*. Polypharmacology of Berberine Based on Multi-Target Binding Motifs. Frontiers in Pharmacology. 2018; 9: 801. https://doi.org/10.3389/fphar.2018.00801.

[44] Kumar V. Targeting macrophage immunometabolism: Dawn in the darkness of sepsis. International Immunopharmacology. 2018; 58: 173–185. https://doi.org/10.1016/j.intimp.2018.03.005.

[45] Zhou X, Dai Q, Huang X. Neutrophils in acute lung injury. Frontiers in Bioscience (Landmark Edition). 2012; 17: 2278–2283. https://doi.org/10.2741/4051.

[46] Salminen A, Hyttinen JMT, Kaarniranta K. AMP-activated protein kinase inhibits NF-κB signaling and inflammation: impact on healthspan and lifespan. Journal of Molecular Medicine. 2011; 89: 667–676. https://doi.org/10.1007/s00109-011-0748-0.

[47] Mo C, Wang L, Zhang J, Numazawa S, Tang H, Tang X, *et al*. The crosstalk between Nrf2 and AMPK signal pathways is important for the anti-inflammatory effect of berberine in LPS-stimulated macrophages and endotoxin-shocked mice. Antioxidants & Redox Signaling. 2014; 20: 574–588. https://doi.org/10.1089/ars.2012.5116.