Protective effect of taurine on sepsis-induced lung injury via inhibiting the p38/MAPK signaling pathway

JIAO CHEN[1*], XIANG XUE[1*], JIANQIN CAI[1], LING JIA[1], BAODI SUN[2] and WEI ZHAO[1]

Departments of [1]Critical Care Medicine and [2]Emergency Medicine, Sir Run Run Hospital,
Nanjing Medical University, Nanjing, Jiangsu 211100, P.R. China

Abstract. Sepsis, a leading cause of acute lung injury (ALI), is characterized by an overwhelming systemic inflammatory response and widespread organ injury, particularly in the lungs. Taurine, an intracellular free amino acid, has been used for the treatment of various diseases, including lung injury; however, the underlying mechanisms are unclear. The present study aimed to investigate the protective effect of taurine on septic ALI and the underlying mechanism. A septic ALI model was established by performing cecal ligation and puncture (CLP) surgery on Sprague Dawley rats. Following successful model establishment, rats were treated with taurine. The results of hematoxylin and eosin, respiratory function detection, malondialdehyde level and superoxide dismutase activity determination and ELSIA demonstrated that taurine significantly alleviated lung injury, restored respiratory function, reduced oxidation and decreased the concentrations of inflammatory factors in CLP-induced septic ALI model rats. In addition, compared with that in the ALI group, western blotting results indicated that taurine ameliorated lung epithelial injury by significantly increasing the expression levels of lung epithelial markers, E-cadherin and occludin. The western blotting results demonstrated that, compared with the control group, the p38/MAPK and NF-κB signaling pathways were significantly activated in CLP-induced septic ALI model rats, but taurine significantly suppressed ALI-mediated signaling pathway activation. To investigate the mechanism underlying taurine in the treatment of septic ALI, CLP-induced septic ALI model rats were treated with an antagonist of the p38/MAPK signaling pathway (SB203580). The effects of SB203580 on CLP-induced septic ALI model rats were similar to those of taurine. SB203580 significantly attenuated sepsis-induced lung injury and increases in IL-1β and TNF-α concentrations in the lung tissue. In addition, SB203580 promoted restoration of the injured lung tissue and respiratory function in CLP-induced septic ALI model rats. The western blotting results indicated that SB203580 significantly decreased the ratios of phosphorylated (p)-p38/p38 and p-p65/065, and increased the protein expression levels of E-cadherin and occludin compared with those in the ALI group. In summary, the present study demonstrated that taurine alleviated sepsis-induced lung injury, which was associated with suppression of the inflammatory response and oxidative stress via inhibiting the p38/MAPK signaling pathway. Therefore, the p38/MAPK signaling pathway may serve as a potential therapeutic target for the treatment of sepsis-induced ALI.

Introduction

Sepsis results in life-threatening organ dysfunction that is characterized by overwhelming systemic inflammation (1). Lung inflammation, as a type of acute lung injury (ALI), is often induced in severe sepsis (2). Despite improvements in intensive care management and multidisciplinary treatments, the mortality rate of sepsis was 17% and was 26% for severe sepsis during 1979-2015 worldwide (3-7). Therefore, the pathogenesis of sepsis-induced lung injury requires further investigation to identify new effective therapeutics.

Previous studies have indicated that sepsis-induced lung injury is primarily associated with systemic inflammatory response syndrome, including the activation of inflammatory cytokines and inflammatory signaling pathways (8-10). Numerous inflammatory signaling pathways serve an important role in the pathogenesis of sepsis-induced lung injury, including the MAPK and NF-κB signaling pathways. The MAPK and NF-κB signaling pathways induce the excessive release of inflammatory cytokines, including TNF-α, IL-1β and other associated cytokines, which contribute to lung

Correspondence to: Professor Wei Zhao, Department of Critical Care Medicine, Sir Run Run Hospital, Nanjing Medical University, 109 Longmian Road, Nanjing, Jiangsu 211100, P.R. China
E-mail: 13605158009@163.com

Dr Baodi Sun, Department of Emergency Medicine, Sir Run Run Hospital, Nanjing Medical University, 109 Longmian Road, Nanjing, Jiangsu 211100, P.R. China
E-mail: sunbaodi321@163.com

*Contributed equally

dysfunction and mortality in sepsis-induced lung injury (11-13). p38/MAPK is a key member of the MAPK signaling pathway that serves an essential role in cell proliferation and survival, and can be activated by various inflammatory mediators and environmental stress (14). Previous studies have reported that activation of the p38/MAPK signaling pathway is involved in the development of ALI/acute respiratory distress syndrome (ARDS). These studies indicated that pharmacological targeting of the p38/MAPK signaling pathway could decrease the expression levels of inflammation factors and attenuate ALI/ARDS (15,16).

Taurine (2-aminoethanesulfonic acid) is a type of intracellular free amino acid. As a cysteine derivative, taurine is highly expressed in all mammalian tissues and has important physiological effects on all cells in the body, including endogenous antioxidant, detoxification, osmoregulation, anti-inflammatory and membrane stability effects (17-19). In addition, previous studies have indicated that taurine could protect against lung injury by modulating oxidative stress, reducing cell apoptosis and decreasing the expression levels of inflammatory factors (20-22). Therefore, taurine may serve as a novel candidate for treating sepsis-induced ALI; however, the underlying molecular mechanism remains unclear.

The aim of the present study was to investigate the protective effects of taurine on sepsis-induced ALI, as well as the underlying molecular mechanism. In particular, the effects of taurine on lung tissue pathological changes, respiratory function, malondialdehyde (MDA) levels, superoxide dismutase (SOD) activity and inflammatory factor concentrations were evaluated in a Sprague Dawley (SD) cecal ligation and puncture (CLP)-induced septic ALI rat model. Furthermore, the roles of the p38/MAPK and NF-κB signaling pathways in the pathogenesis of sepsis-induced ALI were investigated.

Materials and methods

Ethics statement. The present study was conducted according to the Guide for the Care and Use of Experimental Animals established by the National Society for Medical Research (23). The present study was approved by the Ethics Committee of Nanjing Medical University (approval no. SYXK 2019-0042). All surgeries on the rats were conducted under anesthesia (sodium pentobarbital), and every effort was made to alleviate pain.

Animal model and study design. In total, 168 SD rats (male; age, 8-10 weeks; weight, 220-250 g) were provided by the Laboratory Animal Centre of Nanjing Medical University. All rats were acclimatized for 7 days prior to the experiment, fed with standard chow (Global Diet; Shanghai, China) and provided with *ad libitum* access to water in standard shoebox cages. The rats were housed in a normal atmosphere under 12-h light/dark cycles at 20-25°C with 50-60% humidity. The surgical procedure to establish the CLP-induced septic rat model was performed as previously described (24). Briefly, all rats were fasted and anesthetized by the intraperitoneal injection of 50 mg/kg sodium pentobarbital (Sigma-Aldrich; Merck KGaA). Subsequently, the middle abdominal incision site was shaved and the cecum was exposed via a 2-cm

abdominal midline incision. The cecum was mobilized, ligated and punctured with a 21-gauge needle. Some of the fecal matter was squeezed through the puncture wound, then the bowel was repositioned and the abdomen was sutured. The animals were resuscitated with a subcutaneous sterile saline immediately after CLP surgery. The sham-operated control rats underwent the same procedure, but without ligation or puncture of the cecum. No mortality was observed in the rats after CLP was induced. At 3 days post-CLP, rats were anesthetized by the intraperitoneal injection of 50 mg/kg sodium pentobarbital (Sigma-Aldrich; Merck KGaA) and blood samples were collected via cardiac puncture using a 1.5 ml tube. Subsequently, the anesthetized rats were sacrificed by cervical dislocation. The lung tissue samples were collected for further analysis. Death was confirmed by monitoring cessation of the heartbeat and pupil dilation.

To investigate the protective effects of taurine on sepsis-induced lung injury, SD rats were randomly assigned to the following three groups (n=24 per group): i) Control, rats received an intraperitoneal injection of normal saline (1 ml/kg; 200 µl); ii) ALI, CLP-induced septic ALI model rats received an intraperitoneal injection of normal saline (1 ml/kg; 200 µl); iii) ALI + taurine, CLP-induced septic ALI model rats received an intraperitoneal injection of taurine for 3 days (200 mg/kg/day in 200 µl normal saline; Sigma-Aldrich; Merck KGaA). To clarify the molecular mechanisms underlying taurine-mediated protection of lung tissue following CLP induction, rats were randomly assigned to the following four groups (n=24 per group): i) Control, rats received an intraperitoneal injection of normal saline (1 ml/kg; 200 µl); ii) ALI, CLP-induced septic ALI model rats received an intraperitoneal injection of normal saline (1 ml/kg; 200 µl); iii) ALI + taurine, CLP-induced septic ALI model rats received an intraperitoneal injection of taurine for 3 days (200 mg/kg/day in 200 µl normal saline; Sigma-Aldrich; Merck KGaA); and iv) ALI + SB203580, CLP-induced septic ALI model rats received an intraperitoneal injection of SB203580 (10 mg/kg in 200 µl normal saline; Sigma-Aldrich; Merck KGaA). The dose selection and route of administration of taurine was based on our previous study (14).

Respiratory function detection. Prior to sacrifice, arterial blood gas content was examined using i-STAT® G7+ cartridges and an i-STAT portable clinical analyzer (Abbott Point of Care Inc.). The cartridges were recovered immediately before use to avoid compression. For arterial blood gas analysis, arterial blood gas was sampled from the left ventricle of each rat, added into the sample well and allowed to fill by passive movement to the indicated level (volume, 80-100 ml). The cap on the sample well was closed and the cartridge was inserted into the analyzer. The values of partial pressure (Pa) of oxygen (PaO_2) and carbon dioxide ($PaCO_2$) were recorded after the calibration and analysis cycle.

ELISA. Part of right lung tissue was cut into pieces and mechanically disrupted into a homogenate with RIPA buffer (Beyotime Institute of Biotechnology) containing protease inhibitor cocktail (Roche Diagnostics GmbH). Then, the samples were centrifuged at 12,000 x g for 30 min at 4°C

and the supernatants were collected. The concentrations of IL-1β and TNF-α in the supernatants were measured using commercially available ELISA kits according to the manufacturer's instructions. The IL-1β (cat. no. ELR-IL1b) and TNF-α (cat. no. ELR-TNFa1) ELISA kits were purchased from Ray Biotech, Inc.

MDA level and SOD activity detection. The level of MDA is an index of membrane lipid peroxidation, and its level reflects the lipid peroxidation rate and strength. Moreover, MDA levels can be used as a marker to indicate the degree of tissue peroxidation damage. SOD serves an important role in the balance of oxidation and antioxidation. SOD can remove superoxide anion free radicals and protect cells from damage (25). The activity of SOD and the levels of MDA were measured in the lung tissue sample supernatants as previously described (25). The SOD (cat. no. A001-1-2) and MDA (cat. no. A003-1-1) kits were purchased from Nanjing Jiancheng Bioengineering Institute, and were used according to the manufacturer's protocol. Briefly, the right lung tissue of each rat was dissected and homogenized with three volumes of ice-cold 1.15% potassium chloride (ratio of tissue to potassium chloride was 1:3). To determine MDA levels, the reaction mixture (0.2 ml 8.0% SDS, 1.5 ml 20% acetic acid and 1.5 ml 0.8% aqueous thiobarbituric acid) was added to 0.1 ml homogenized tissue and adjusted to pH 3.5. Then, the final volume was adjusted to 4.0 ml with distilled water. Subsequently, 5.0 ml N-butanol and pyridine mixture [15:1 (v/v)] was added. The mixture was shaken vigorously and centrifuged at 1,200 x g for 10 min at 4˚C. Finally, the absorbance of the organic layer was measured at a wavelength of 532 nm using a Spectronic UV 120 spectrophotometer (Thermo Fisher Scientific, Inc.). The MDA levels are presented as nmol/mg protein.

Xanthine and xanthine oxidase are used to generate superoxide radicals that react with p-iodonitrotetrazlium violet (INT) to form a red formazan dye, which can be measured with a Spectronic UV 120 spectrophotometer (Thermo Fisher Scientific, Inc.) at a wavelength of 505 nm to determine SOD activity (26). SOD activity is presented as U/mg protein. The assay mixture contained 0.01 M phosphate buffer, 3-cyclohexilamino-1-propanesulfonic acid (CAPS) buffer solution (50 mM CAPS and 0.94 mM EDTA in a saturated solution of sodium hydroxide; pH 10.2), substrate solution (0.05 mM xanthine and 0.025 mM INT) and 80 μl xanthine oxidase.

Histological examination. After fixing with 4% neutral phosphate-buffered paraformaldehyde for 16 h at room temperature, the lower left lung was embedded in paraffin and sectioned into 5-μm thick slices. To determine the integrity of the histology structure and the collagen deposition condition, the slices were stained with hematoxylin and eosin (H&E; Beyotime Institute of Biotechnology) according to the manufacturer's instructions. Stained sections were observed using a light microscope (Olympus Corporation) and scored by two pathologists who were blinded to the samples and had expertise in lung pathology. The lung injury score was determined using the Tanino Method (27). In brief, morphological alterations in the lungs were scored based on the extent of pathology on a scale of 0-4 by assessing interstitial edema, alveolar edema, hemorrhage and neutrophil infiltration. The

following scoring values were used: 0, no reaction in the alveolar walls; 1, diffuse reaction in the alveolar walls, but without thickening of the interstitium; 2, diffuse presence of the inflammatory cells in the alveolar walls with a slight thickening of the interstitium; 3, moderate interstitial thickening accompanied by inflammatory cell infiltrates; and 4, interstitial thickening involving >1/2 of the microscopic field. The results are expressed as the average of the values from 30 microscopic fields.

Lung wet/dry ratio determination. After sacrifice, the left upper lung tissue (~1 g) was dissected, cleansed of blood or liquid with absorbent paper, weighed to obtain the 'wet' weight and then heated in an 80˚C thermostatic oven for 48 h. Subsequently, the tissue was weighed again to obtain the 'dry' weight. The ratio of wet to dry lung was calculated to assess tissue edema.

Western blotting. Western blotting was performed as previously described (28). Proteins were extracted from lung tissues using RIPA (Beyotime Institute of Biotechnology) containing protease inhibitor cocktail (Roche Diagnostics GmbH) for 30 min on ice. Protein concentrations were determined using the BCA Protein Assay Kit (Beyotime Institute of Biotechnology). All the protein samples were boiled for 10 min in equal volumes of 5X SDS buffer. Subsequently, proteins (20 μg) were separated via 12% SDS-PAGE and transferred to PVDF membranes (Roche Diagnostics GmbH) using a semi-dry transfer apparatus (Bio-Rad Laboratories, Inc.). After blocking with 5% skimmed milk in PBS-Tween-20 (PBST; PBS with 0.5% Tween-20) at room temperature for 1 h and washing three times with PBST, the membranes were incubated overnight at 4˚C with the following primary antibodies (all purchased from Abcam): Rabbit anti-occludin (1:1,000; cat. no. ab216327), rabbit anti-E-cadherin (1:10,000; cat. no. ab76319), rabbit anti-p-p65 (1:1,000; cat. no. ab194726), rabbit anti-p65 (1:1,000; cat. no. ab16502), rabbit anti-p-p38 (1:1,000; cat. no. ab47363), rabbit anti-p38 (1:3,000; cat. no. ab170099) and mouse anti-β-actin (1:3,000; cat. no. ab7817). After three washes with PBST, the membranes were incubated with a horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse secondary antibody (1:5,000; cat. nos. BM2006 or BA1050, respectively; Wuhan Boster Biological Technology, Ltd.) for 1 h at 37˚C. Immunocomplexes were visualized using an enhanced chemiluminescence system (Thermo Fisher Scientific, Inc.) and an Odyssey Scanning System (LI-COR Biosciences). Protein expression was semi-quantified using ImageJ software (version 3.0; National Institutes of Health) with β-actin as the loading control.

Statistical analysis. All experiments were repeated at least three times with nearly identical results. Statistical analyses were performed using SPSS software (version 21.0; IBM Corp.). Data are presented as the mean ± standard deviation. Parametric data were analyzed using one-way ANOVA followed by Tukey's post hoc test. Data with a non-parametric distribution were analyzed using the Kruskal-Wallis test followed by Dunn's post hoc test. P<0.05 was considered to indicate a significant difference.
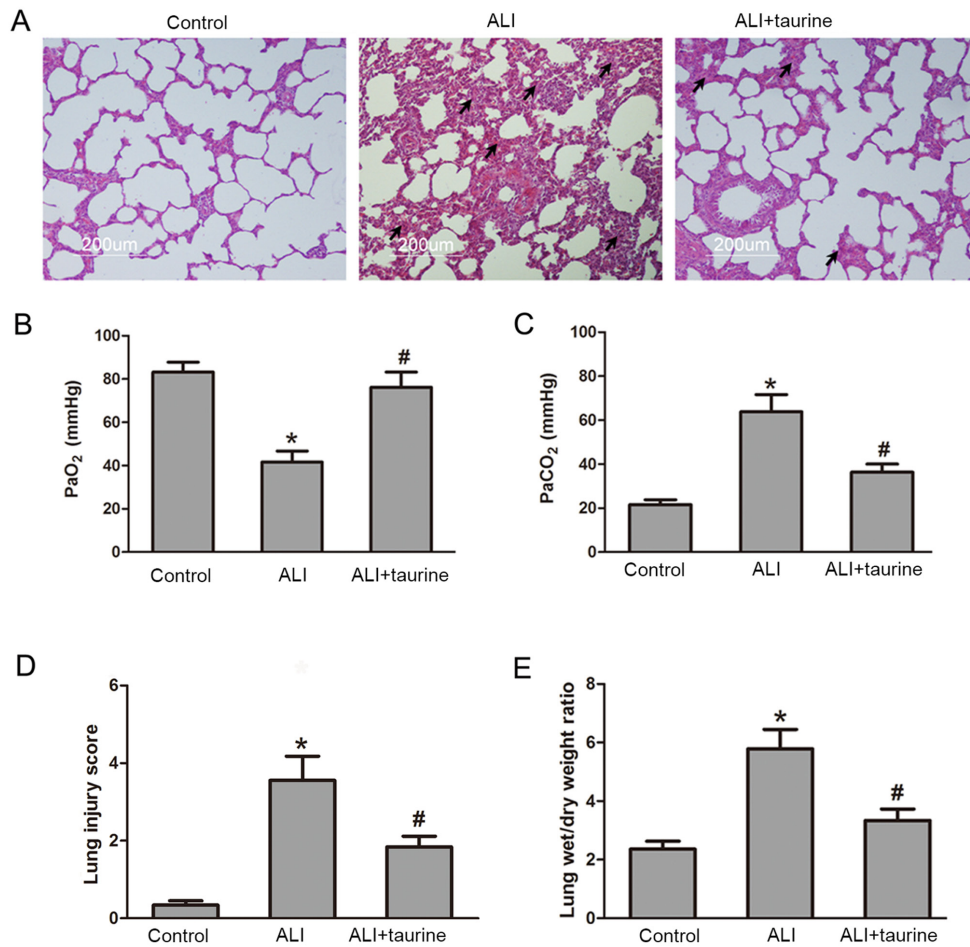
Figure 1. Taurine suppressed lung injury in CLP-induced septic ALI model rats. Acute lung injury was induced in rats by sepsis. At day 3 post-CLP, analyses were performed. CLP-induced septic ALI model rats were treated with or without taurine, and then lung tissues in each group were analyzed by performing H&E staining, respiratory function detection tests and lung injury score examinations. (A) Representative images of H&E staining in each group (magnification, x100). Black arrows indicate neutrophil infiltration. Content of (B) O_2 and (C) CO_2 in arterial blood. (D) Lung injury score in each group. (E) Lung wet/dry weight ratio of the lung tissues in each group. Data are presented as the mean ± standard deviation. *P<0.05 vs. control; #P<0.05 vs. ALI. CLP, cecal ligation and puncture; H&E, hematoxylin and eosin; Pa, partial pressure; ALI, acute lung injury.

Results

Taurine suppresses sepsis-induced ALI in rats. The lung is particularly susceptible to acute injury in CLP-induced polymicrobial sepsis (29). To determine the protective effect of taurine on sepsis-induced lung injury, a series of lung sections obtained from the rats in each group were stained with H&E (Fig. 1A). The tissue sections obtained from the control rats were histologically normal and displayed no inflammation or epithelial damage. After CLP-induced sepsis, the lung tissue sections from rats in the ALI group displayed extensive cellular thickening of the interalveolar septa and interstitial edema, and infiltration of inflammatory cells. However, inflammatory cell infiltration and edema were notably ameliorated in the lungs following treatment with taurine in ALI rats. As shown in Fig. 1B and C, the respiratory function test results indicated that PaO_2 levels in the ALI group were significantly decreased, but $PaCO_2$ levels were significantly increased compared with those in the control group. Taurine treatment significantly increased PaO_2 levels and decreased $PaCO_2$ levels in CLP-induced septic ALI model rats. The lung injury score was also evaluated using histological sections and a semi-quantitative scale (Fig. 1D). The lung injury score

following CLP was significantly increased compared with that in the control group, whereas the score was significantly decreased in CLP-induced septic ALI model rats following treatment with taurine. In addition, after lung injury, sepsis resulted in an increase in lung weight owing to inflammatory and fibrotic components. Compared with that in the control group, the lung wet/dry ratio in the ALI group was significantly increased (Fig. 1E). By contrast, the lung wet/dry ratio was significantly decreased in the ALI + taurine group compared with that in the ALI group. These results indicated that taurine protected the lung tissue and attenuated sepsis-induced injury.

MDA levels and SOD activity in lung tissues following taurine treatment. MDA levels and SOD activity in the lung tissues of each group were detected to assess the effects of taurine on oxidative stress. A significant increase in MDA levels (Fig. 2A) and a decrease in SOD activity (Fig. 2B) was observed in the lung tissue of the ALI group compared with that of the control group. The results indicated that sepsis induced membrane lipid peroxidation and superoxide toxicity in the lung tissue, whereas taurine treatment significantly reversed ALI-mediated effects, suggesting that taurine may serve a protective role against oxidative stress.
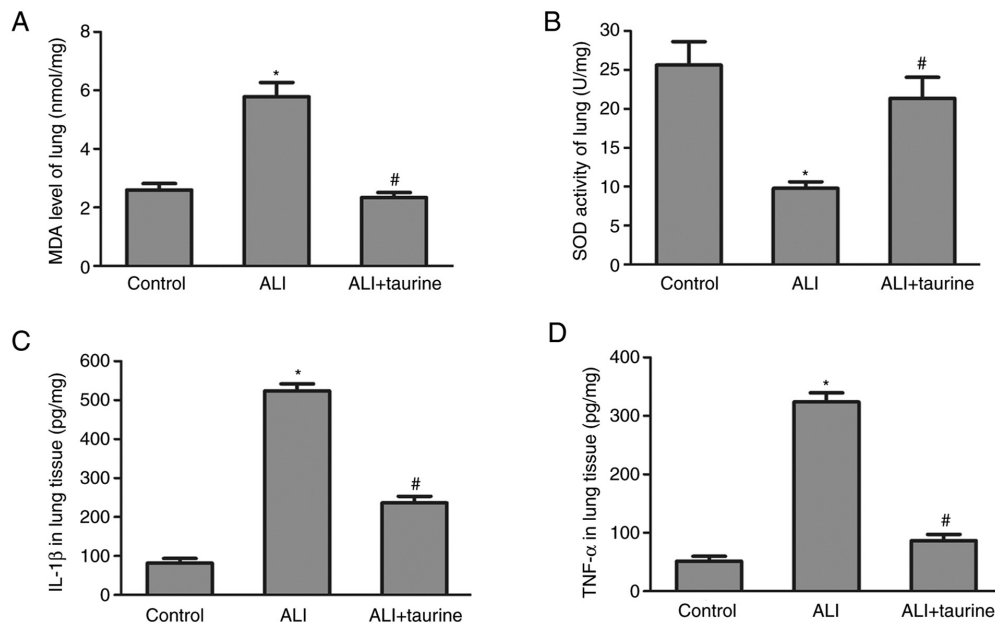
Figure 2. Taurine attenuates oxidative stress and the inflammatory response. To assess the protective effects of taurine on oxidative stress and the inflammatory response, MDA levels, SOD activity, and TNF-α and IL-1β concentrations in the lung tissues of septic rats were detected. (A) MDA levels and (B) SOD activity in the lung tissues of each group. (C) TNF-α and (D) IL-1β concentrations in the lung tissues of each group. Data are presented as the mean ± standard deviation. *P<0.05 vs. control; *P<0.05 vs. ALI. MDA, malondiadelhyde; SOD, superoxide dismutase; ALI, acute lung injury.
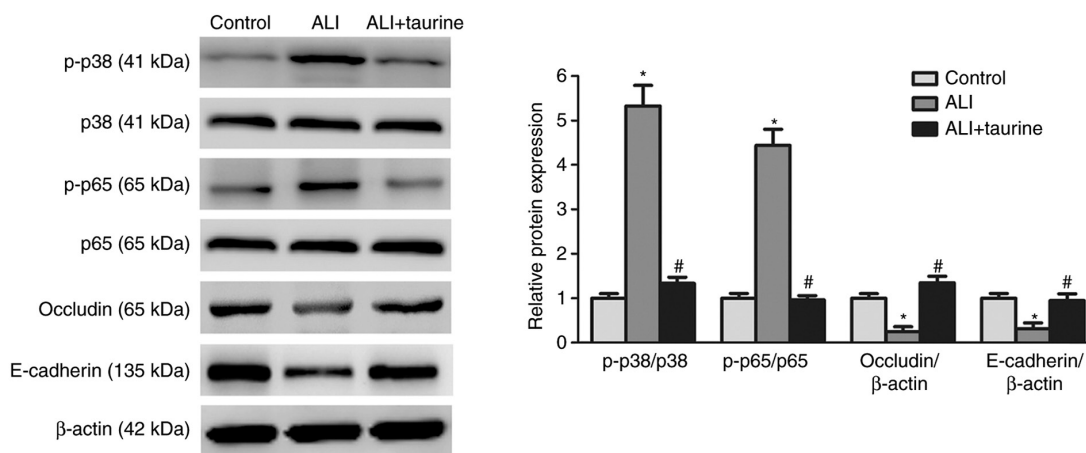

Figure 3. Effects of taurine on the expression levels of p38/MAPK and NF-κB signaling pathway-related proteins and epithelial markers. Protein expression levels of E-cadherin, occludin, p-p38/p38 and p-p65/p65 in the lung tissues of cecal ligation and puncture-induced septic ALI model rats were determined by western blotting and semi-quantified. *P<0.05 vs. control; *P<0.05 vs. ALI. p, phosphorylated; ALI, acute lung injury.

Taurine downregulates TNF-α and IL-1β concentrations in lung tissue. The inflammatory response is induced in sepsis (20). Therefore, at 3 days post-CLP, IL-1β and TNF-α concentrations in the lung tissues of septic rats were measured by performing ELISAs. The results demonstrated that the concentrations of IL-1β and TNF-α were significantly elevated in the lung tissues of CLP-induced septic ALI model rats compared with those in the control group (Fig. 2C and D). However, the concentrations of IL-6 and TNF-α were significantly decreased in the taurine treatment group compared with those in the ALI group. The results indicated that taurine treatment inhibited the inflammatory response in sepsis-induced lung injury.

Taurine downregulates the expression levels of p38/MAPK and NF-κB signaling pathway-related proteins. The p38/MAPK

and NF-κB signaling pathways are key upstream pathways in the sepsis-induced inflammatory response (30). To further investigate the molecular mechanisms underlying the anti-inflammatory effects of taurine, whether taurine inhibited sepsis-induced activation of the p38/MAPK and NF-κB signaling pathways was investigated. Western blotting was performed to detect the protein expression levels of p-p38 and p-p65, which are key proteins of the p38/MAPK and NF-κB signaling pathways (30), respectively. Compared with those in the control group, the ratios of p-p38/p38 and p-p65/p65 were significantly increased in the lung tissues of septic rats, indicating that the p38/MAPK and NF-κB signaling pathways were activated in the injured lung tissues (Fig. 3). However, the protein expression levels of the epithelial markers, E-cadherin and occludin, were significantly decreased in the lung tissues
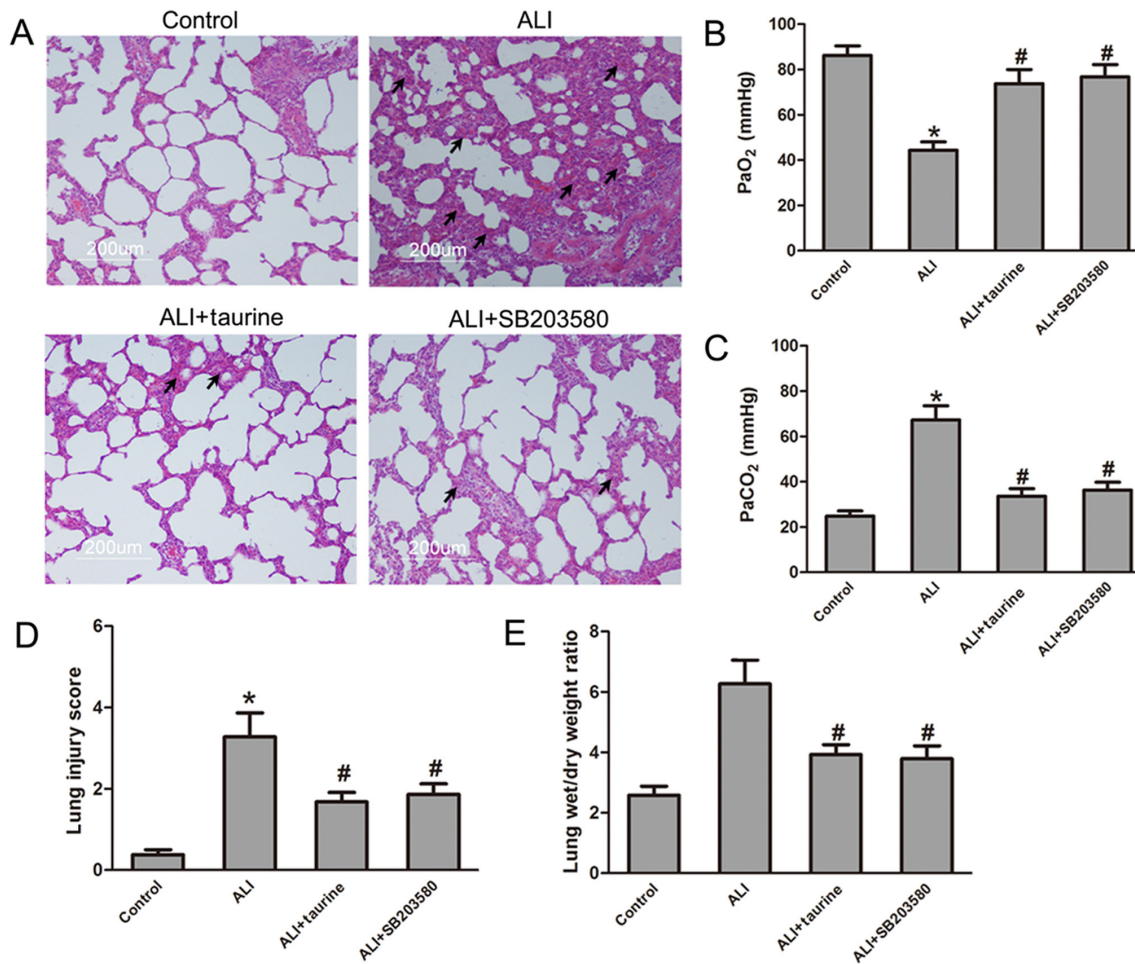
Figure 4. SB203580 protects against sepsis-induced ALI. SB203580, an inhibitor of p38/MAPK signaling, was used to treat septic rats to assess the role of the p38/MAPK signaling pathway in the pathogenesis of sepsis-induced ALI. SB203580 ameliorated lung injury and promoted lung repair by blocking p38/MAPK signaling pathway. The results displayed no significant difference between the SB203580 and taurine treatment groups. (A) Representative images of hematoxylin and eosin staining in each group (magnification, x100). Black arrows indicate neutrophil infiltration. Content of (B) O_2 and (C) CO_2 in arterial blood. (D) Lung injury score in each group. (E) Lung wet/dry weight ratio of the lung tissues in each group. Data are presented as the mean ± standard deviation. *$P<0.05$ vs. control; #$P<0.05$ vs. ALI. ALI, acute lung injury; Pa, partial pressure.

of CLP-induced septic ALI model rats compared with those in the control group. By contrast, taurine significantly decreased the protein expression levels of p-p38 and p-p65 and increased the expression levels of E-cadherin and occludin in the ALI group. Collectively, these results suggested that taurine inhibited sepsis-induced lung injury and protected lung epithelium by blocking the p38/MAPK and NF-κB signaling pathways, which were activated by sepsis.

p38/MAPK inhibitor SB203580 attenuates sepsis-induced lung injury. To identify the functions of the p38/MAPK signaling pathway in sepsis-induced ALI, CLP-induced septic ALI model rats were treated with or without the p38/MAPK inhibitor SB203580. The H&E staining results demonstrated that SB203580 markedly attenuated lung injury in CLP-induced septic ALI model rats treated with SB203580 compared with the ALI group (Fig. 4A). SB203580 treatment significantly increased PaO_2 levels and decreased $PaCO_2$ levels in the arterial blood of CLP-induced septic ALI model rats compared with those in the ALI group (Fig. 4B and C), which suggested that SB203580 suppressed sepsis-induced lung functions, a similar effect to that observed in the taurine treatment group.

Furthermore, the lung injury score and lung wet/dry ratio were determined in each group. As shown in Fig. 4D and E, the results indicated that SB203580 significantly decreased the lung injury score and lung wet/dry ratio in CLP-induced septic ALI model rats compared with those in the ALI group. Notably, the results of SB203580 treatment group were similar to those in the taurine treatment group, which suggested that the p38/MAPK signaling pathway may serve as an effective therapeutic target for sepsis-induced ALI.

SB203580 suppresses oxidative stress and the inflammatory response. To determine the effects of SB203580 on oxidative stress and the inflammatory response, MDA and SOD expression levels and IL-1β and TNF-α concentrations in the lung tissues of each group were detected. The results demonstrated that SB203580 treatment significantly decreased MDA levels and the concentrations of IL-1β and TNF-α, but restored SOD activities compared with those in the ALI group (Fig. 5). The results in the ALI + SB203580 group were similar to those in the ALI + taurine group, which suggested that SB203580 may protect against CLP-induced lung injury by inhibiting the p38/MAPK signaling pathway. The results demonstrated that
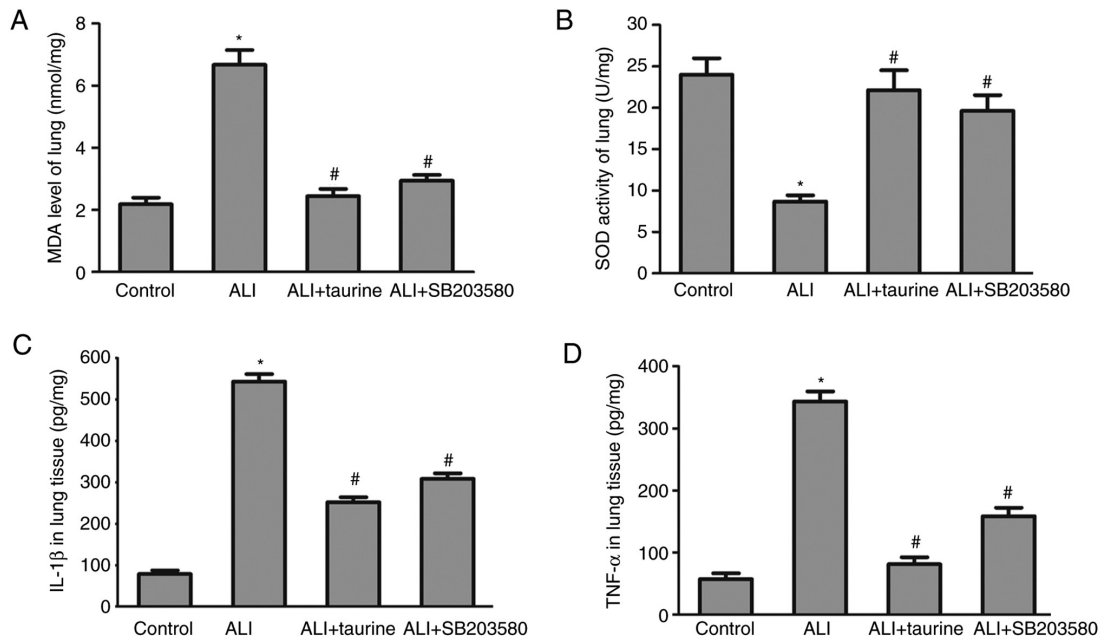
Figure 5. p38/MAPK signaling suppression inhibits sepsis-induced oxidative stress and inflammatory responses in cecal ligation and puncture-induced septic ALI model rats. SB203580 attenuated MDA levels, SOD activity, and TNF-α and IL-1β concentrations in the lung tissues of septic rats. (A) MDA levels and (B) SOD activity in the lung tissues of each group. (C) TNF-α and (D) IL-1β concentrations in the lung tissues of each group. Data are presented as mean ± standard deviation. $^*P<0.05$ vs. control; $^#P<0.05$ vs. ALI. ALI, acute lung injury; MDA, malondialdehyde; SOD, superoxide dismutase.
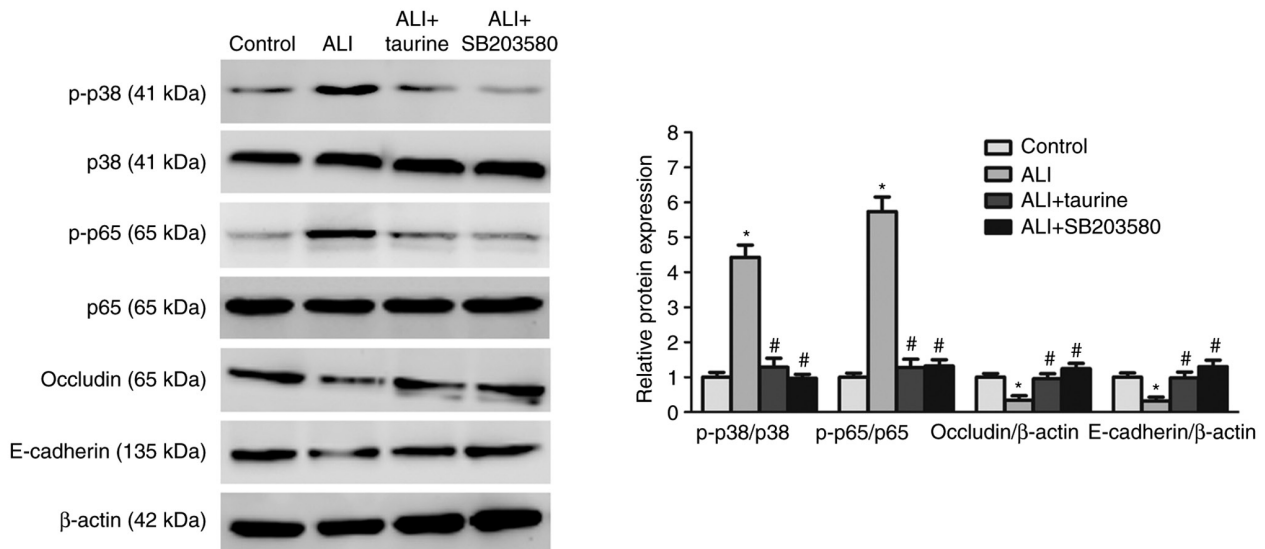

Figure 6. SB203580 inhibits the p38/MAPK signaling pathway to promote lung repair. Protein expression levels of E-cadherin, occludin, p-p38/p38 and p-p65/p65 in the lung tissues of CLP-induced septic ALI model rats following treatment with SB20380 were determined by western blotting and semi-quantified. SB20380 treatment significantly increased the expression levels of E-cadherin and occludin, but significantly decreased the ratios of p-p38/p38 and p-p65/p65 in ALI rats. $^*P<0.05$ vs. control; $^#P<0.05$ vs. ALI. p, phosphorylated; ALI, acute lung injury.

the p38/MAPK signaling pathway may serve an essential role in the development of sepsis-induced ALI.

SB203580 inhibits sepsis-activated p38/MAPK signaling. The present study further indicated that treatment with SB203580 significantly reduced the ratio of p-p38/p38 in septic rats, suggesting that SB203580 inhibited activation of the p38/MAPK signaling pathway mediated by CLP-induced sepsis (Fig. 6). In addition, the western blotting results demonstrated that the protein expression levels of E-cadherin and

occludin were significantly increased in the ALI + taurine group compared with those in the ALI group. The results of the ALI + SB203580 group were also similar to those in the ALI + taurine group, indicating that SB203580 protected lung epithelium against sepsis-induced damage, which was similar to the protective effects of taurine. Furthermore, it was observed that the ratio of p-p65/p65 was also significantly decreased in the lung tissues of CLP-induced septic ALI model rats treated with SB203580 compared with those in the ALI group, which suggested that p38/MAPK may be an upstream of

the NF-κB signaling pathway. The results indicated that the suppressed p38 MAPK signaling pathway was associated with the expression level of NF-κB signaling-related proteins in CLP-induced septic ALI model rats treated with SB203580, which was similar to the taurine treatment group. Collectively, the results indicated that taurine may serve a protective role in lung epithelium damage in septic rats via modulating the p38 MAPK signaling pathway.

Discussion

Sepsis, as one of the leading causes of death in patients who are critically ill, induces a complex systemic inflammatory response syndrome and subsequent multiple organ failure (31,32). Multiple organ dysfunction syndromes, particularly ALI/ARDS, are major causes of morbidity and mortality in patients with sepsis (33). ALI/ARDS, which is induced by sepsis, is characterized by the rapid onset of respiratory insufficiency, acute inflammatory response, alveolar epithelium damage, bleeding and lung interstitial edema (6,34,35). The pathogenesis of ALI/ARDS is not completely understood and there are no specific effective therapeutic strategies available (36,37). Therefore, it is important to investigate the molecular mechanisms underlying sepsis-induced ALI/ARDS to develop effective treatments.

Taurine, an endogenous free amino acid, is highly expressed in numerous mammalian tissues. Taurine has been reported to be an effective cell protector, which could protect against lung damage by mitigating oxidative stress and reducing the expression of inflammatory factors (20,21). Previously, Li *et al* (21) reported that taurine could reverse paraquat-induced disturbances in glutathione (GSH) content and GSH peroxidase activity. Moreover, taurine attenuated paraquat-induced A549 cell apoptosis via modulation of oxidative stress. Although it has been reported that taurine reverses paraquat-induced oxidative stress to protect against cell apoptosis, the mechanisms underlying taurine-mediated regulation of oxidative stress remain unclear. In contrast to the CLP-induced septic ALI rat model established in the present study, Men *et al* (26) demonstrated that taurine treatment attenuated lung injury following limb ischemia reperfusion (LIR). It has also been reported that taurine prevents oxidative stress-induced cell apoptosis, decreases MDA level, and increases SOD and catalase activities by inhibiting endoplasmic reticulum stress. However, whether taurine attenuates the expression levels of inflammatory factors to protect against LIR-induced lung injury is not completely understood. Our previous study indicated that taurine enhanced the protective effect of dexmedetomidine on sepsis-induced lung injury by suppressing activation of the NF-κB signaling pathway and decreased the expression levels of IL-6 and IL-1β (20). Taurine serves an essential role in regulating the T helper (Th)1/Th2 cytokine balance (38). In the present study, the protective effects of taurine on sepsis-induced lung injury were investigated. Taurine treatment preserved epithelium integrity by increasing the expression levels of epithelium markers E-cadherin and occludin to alleviate CLP-induced lung injury in rats. The proinflammatory cytokines TNF-α and IL-1β are implicated in the pathogenesis of sepsis-induced lung injury (39). The present study demonstrated that taurine treatment decreased

sepsis-induced increases in TNF-α and IL-1β expression levels in lung tissues, indicating that taurine treatment inhibited the inflammatory response in CLP-induced septic ALI model rats. Oxidative stress serves an essential role in the pathogenesis of ALI (40). Therefore, oxidative stress levels were assessed in the present study by detecting MDA levels and SOD activities. MDA levels are an index of membrane lipid peroxidation and can be used as a marker to indicate the degree of tissue peroxidation damage. SOD serves an important role in the balance of oxidation and antioxidation (25). The results of the present study indicated that taurine effectively attenuated oxidative stress damage in sepsis-induced lung injury, suggesting that taurine could be used as a protective factor to attenuate lung injury by decreasing sepsis-induced inflammatory responses and oxidative stress. Moreover, taurine may protect epithelium integrity by promoting the expression of epithelial markers. Therefore, the present study further suggested that taurine may serve an essential role in the lung repair process.

To clarify the mechanisms underlying taurine-mediated protection of lung tissues after CLP induction, p38/MAPK and NF-κB signaling pathways involved in sepsis-induced inflammatory responses and oxidative stress were investigated. Recent studies have demonstrated that the p38/MAPK signaling pathway is one of the most important signaling pathways, serving a critical role in the phosphorylation of substrates involved in the sepsis-induced inflammatory response (12,39). Certain studies have indicated that the p38/MAPK signaling pathway also serves an essential role in regulating oxidative stress-related signaling and participates in the pathogenesis of ALI (12,41,42). It is important to identify the changes in p38/MAPK activity associated with sepsis-induced ALI; therefore, the protein expression levels of p-p38 in the p38/MAPK signaling pathway were detected in the lung tissues of CLP-induced septic ALI model rats. The results demonstrated that taurine suppressed sepsis-induced activation of the p38/MAPK signaling pathway. Furthermore, the NF-κB signaling pathway, which was previously reported to be involved in the regulation of inflammatory mediator generation and immune responses (43,44), was also investigated in CLP-induced septic ALI model rats. The results demonstrated that taurine treatment decreased the ratio of p-p65/p65 to block NF-κB signaling in CLP-induced septic ALI model rats. To investigate the role of the p38/MAPK signaling pathway in the pathogenesis of sepsis-induced lung injury, SB203580, an inhibitor of the p38/MAPK signaling pathway, was used to treat the CLP-induced septic ALI model rats. In the present study, the H&E staining and respiratory function test results indicated that SB203580 attenuated lung injury, reduced inflammatory cell infiltration and promoted lung restoration in CLP-induced septic ALI model rats. Furthermore, MDA levels and SOD activity, which are important indexes of oxidative stress, were significantly decreased after SB203580 treatment in CLP-induced septic ALI model rats, which indicated that inhibition of the p38/MAPK signaling pathway could ameliorate oxidative stress. The concentrations of proinflammatory cytokines, TNF-α and IL-1β, were significantly decreased in the SB203580 treatment group compared with those in the ALI group, suggesting that SB203580 administration reduced local inflammation in CLP-induced septic ALI model rats. Moreover, sepsis-induced p38/MAPK

phosphorylation was attenuated by treatment with SB203580. In addition, SB203580 administration also decreased the ratio of p-p65/p65, a NF-κB signaling pathway-related protein, in CLP-induced septic ALI model rats. Similarly, the results indicated that inhibition of p38/MAPK signaling could restore the expression levels of lung epithelium markers, suggesting that SB203580 protected against sepsis-induced lung epithelium damage. The results observed following SB203580 treatment of septic rats were similar to those observed in the taurine treatment group, which suggested that taurine attenuated lung injury and promoted lung repair by blocking the p38/MAPK signaling pathway. The present study also revealed that inhibition of the p38/MAPK signaling pathway could alter the activity of the NF-κB signaling pathway, which suggested that the p38/MAPK signaling pathway may crosstalk with the NF-κB signaling pathway for the generation of inflammatory factors and the immune response in sepsis-induced ALI. However, the present study only used the CLP-induced septic ALI rat model to investigate the protective effects and molecular mechanisms underlying taurine in sepsis-induced lung injury. Due to this limitation, a lung epithelial cell line should be treated with LPS to establish an *in vitro* experiment model to verify the results of the present study and investigate the role of p38/MAPK signaling or downstream signaling of p38/MAPK in regulating the inflammatory response and oxidative stress during the pathogenesis of LPS-induced lung epithelium damage.

In conclusion, to the best of our knowledge, the present study was the first to investigate the protective effects of taurine on sepsis-induced lung injury via suppressing the p38/MAPK signaling pathway, inhibiting the inflammatory response and oxidative stress levels, and preserving lung epithelium integrity. The present study demonstrated that taurine displayed potential therapeutic effects on sepsis-induced lung injury, and the p38/MAPK signaling pathway may serve as a promising therapeutic target for sepsis-induced lung injury.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JCh and XX performed the majority of the experiments. JCa and LJ performed the statistical analyses and performed some of the experiments. BS and WZ designed the study and analyzed the data. All authors contributed to preparing the manuscript. All authors have read and approved the final manuscript. BS and WZ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

All animal studies were conducted in accordance with the Guide for the Care and Use of Experimental Animals established by National Society for Medical Research and approved by the Ethics Committee of Nanjing Medical University (approval no. SYXK 2019-0042).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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