H$_2$S attenuates sepsis-induced cardiac dysfunction via a PI3K/Akt-dependent mechanism

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Abstract. The heart is the most vulnerable target organ in sepsis, and it has been previously reported that hydrogen sulfide (H$_2$S) has a protective role in heart dysfunction caused by sepsis. Additionally, studies have demonstrated that the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt) signaling pathway has a protective function during sepsis. However, the potential association between H$_2$S and PI3K/Akt in sepsis-induced cardiac dysfunction is unclear. Therefore, the PI3K inhibitor LY294002 was used to investigate the role of PI3K/Akt signaling in the protective effects of H$_2$S during sepsis-induced myocardial injury. A rat sepsis model was established using cecal ligation and puncture (CLP) surgery. Sodium hydrosulfide, a H$_2$S donor, was administered intraperitoneally (8.9 μmol/kg), and serum myocardial enzyme levels, inflammatory cytokine levels, cardiac histology and cardiomyocyte apoptosis were assessed to determine the extent of myocardial damage. The results demonstrated that exogenous H$_2$S reduced serum myocardial enzyme levels, decreased the levels of the inflammatory factors tumor necrosis factor (TNF)-α and interleukin (IL)-6, and increased the level of anti-inflammatory IL-10 following CLP. Staining of histological sections demonstrated that myocardial damage and cardiomyocyte apoptosis were alleviated by the administration of exogenous H$_2$S. Western blot analysis was used to detect phosphorylated and total PI3K and Akt levels, as well as NF-κB, B-cell lymphoma-2, Bcl-2-associated X protein (Bax) and caspase levels, and the results demonstrated that H$_2$S significantly increased PI3K and Akt phosphorylation. This indicated that the PI3K/Akt signaling pathway was activated by H$_2$S. Additionally, H$_2$S reduced Bax and caspase expression, indicating that apoptosis was inhibited, and decreased NF-κB levels, indicating that inflammation was reduced. Furthermore, the PI3K inhibitor LY294002 eliminated the protective effects of H$_2$S. In conclusion, the results of the current study suggest that exogenous H$_2$S activates PI3K/Akt signaling to attenuate myocardial damage in sepsis.

Introduction

Sepsis is a pathophysiological syndrome caused by infection and is a major public health problem (1). Its incidence rate has been reported to be 535 cases per 100,000 person-years and rising; in-hospital mortality remains high at 25-30% (2). Despite advances in care, sepsis is a major cause of mortality and critical illness in intensive care units worldwide (3). The heart is the most vulnerable organ during sepsis, and ~50% of patients with sepsis develop heart dysfunction (4,5). Hydrogen sulfide (H$_2$S) has been confirmed to serve an important role in the physiological and pathological processes of various systems, including the circulatory, nervous and respiratory systems (6). H$_2$S has a protective role in cells as a regulator of vascular tone, inflammatory responses and the clearance of reactive oxygen species, and it may even reduce the risk of myocardial ischemia (7,8). Multiple studies have confirmed that an appropriate dose of H$_2$S has cardiac protective effects (7,9,10), which may be associated with anti-apoptotic, anti-inflammatory and anti-oxidative mechanisms (11). A previous study by the present research team demonstrated that low doses of H$_2$S are able to improve the cardiac dysfunction caused by sepsis (12); however, the specific mechanisms involved are not clear.

Phosphoinositol-3-kinases (PI3Ks) are a conserved family of signaling transducers involved in the regulation of cell proliferation and survival. In studies of the mechanisms of cardiac dysfunction caused by sepsis, the PI3K/protein kinase B (Akt) signaling pathway has been proposed to have a key role in the development of dysfunction (13,14). Previous studies have revealed that PI3K and its downstream mediator, Akt, serve a role in sepsis via the regulation of cell activation, inflammation and apoptosis (15-17). Additionally, studies have

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demonstrated that H_2S is involved in the PI3K/Akt signaling pathway in pancreatitis and myocardial ischemia (18-20). However, it is unclear whether the protective effect of H_2S against myocardial damage during sepsis is associated with the PI3K/Akt pathway. Therefore, the current study used cecal ligation and puncture (CLP) to induce a rat model of sepsis, and the effect and mechanism of H_2S on myocardial injury in sepsis were evaluated by the administration of H_2S and inhibitors of PI3K in this model.

Materials and methods

Reagents. Sodium hydrosulfide (NaHS) was purchased from Sigma-Aldrich (Merck KGaA; Darmstadt, Germany; cat. no. 161527). LY294002 (a PI3K inhibitor) was purchased from ApexBio (Houston, TX, USA; cat. no. 8250-200 mg). Akt, phospho (p)-Akt and caspase-3 antibodies were obtained from Abcam (Cambridge, MA, USA; cat. nos. ab179463, ab192623 and ab13847, respectively). PI3K, p-PI3K and nuclear factor-κB (NF-κB) antibodies were obtained from Cell Signaling Technology, Inc. (Danvers, MA, USA; cat. nos. 4249, 4228 and 8242, respectively). B-cell lymphoma-2 (Bcl-2) and Bcl-2-associated X protein (Bax) antibodies were obtained from Boster Biological Technology, Ltd. (Wuhan, China; cat. nos. BA0412 and BA0315-2, respectively). Peroxidase-conjugated AffiniPure goat anti-rabbit immunoglobulin G (IgG), peroxidase-conjugated AffiniPure goat anti-mouse IgG and mouse anti-β-actin monoclonal antibodies were obtained from OriGene Technologies, Inc. (Beijing, China; cat. nos. ZB-2301, ZB-2305 and TA-09, respectively). *In situ* Cell Death Detection kit, POD was obtained from Roche Diagnostics GmbH (Mannheim, Germany; cat. no. 11684817910). Rat troponin I (TnI), interleukin (IL)-10, IL-6 and tumor necrosis factor-α (TNF-α) ELISA kits were provided by Elabscience Biotechnology Co., Ltd. (Wuhan, China; cat. nos. E-EL-R0055c, E-EL-R0016c, E-EL-R0015c and E-EL-R0019c, respectively).

Experimental animals. Adult male Sprague Dawley rats (7-8 weeks old; body weight 211.58±11.42 g) were provided by the animal center of Xinjiang Medical University [Urumqi, China; animal use license no. SYXK (Sinkiang) 2011-010101]. The animals were housed in cages with pathogen-free conditions, and free access to food and water, under a 12-h light/dark cycle at a room temperature of 22°C with 45% humidity, and normal air conditions (21% O_2, 78% N_2 and 0.03% CO_2). Chloral hydrate (350 mg/kg; intraperitoneal injection) was used to anesthetize rats for surgery and blood collection. Subsequently, rapid cervical dislocation was used to sacrifice the animals painlessly. All experimental protocols in this study complied with the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (1996) and were approved by the Animal Protection and Use Committee of Shihezi University (Shihezi, China).

Animal model. Prior to surgery, all mice were fasted for 8 h, with water freely available. The CLP procedure was performed as previously described (21). Briefly, the rats were anesthetized with chloral hydrate and then secured to a sterile operating table. Under aseptic conditions, a 2-cm incision was made

in the abdomen, and the cecum was exposed layer by layer. Prior to ligation of the cecum, feces were gently squeezed to the distal end of the cecum, then the cecum was ligated with a thin wire, and the cecum was pierced with an 18-gauge needle. Any remaining contents were extruded through the puncture site. Subsequently, the cecum was pushed back into the abdominal cavity, the abdominal cavity was closed and the layers were sutured. The sham group underwent the same procedure without CLP. All rats were resuscitated using 0.9% sodium chloride brine (subcutaneous injection, ~40 ml/kg), and following surgery, rats were returned their cages with free access to food and water.

Experimental grouping. Rats (n=56) were randomly divided into 7 groups as follows: Sham group, underwent exposure of the cecum, but not ligation and perforation; sham + NaHS group, received the sham procedure and was administered a 2-ml/kg intraperitoneal administration of 8.9 μmol/kg NaHS 1 h following the surgery; sham + LY294002 group, underwent the sham procedure and was administered a 2-ml/kg intraperitoneal injection of 40 mg/kg LY294002 following the surgery; CLP group, treated with the CLP surgery; CLP + NaHS group, underwent CLP and was administered NaHS (dosage and method as in the sham + NaHS group); CLP + LY294002 group, underwent CLP and was administered LY294002 following the surgery (dosage and method as in the sham + LY294002 group); CLP + NaHS + LY294002 group, underwent CLP and was administered an intraperitoneal injection of NaHS and LY294002 at the aforementioned doses. The doses of LY294002 and NaHS were selected according to previous studies (5,12). Following the procedures, all the rats were free to drink and eat, and 12 h later they were anesthetized again. The rat abdominal cavity was opened and blood was obtained from the abdominal aorta. Following coagulation, blood samples were centrifuged (3,500 x g, 15 min) and clear supernatants were collected. A portion of each serum sample was sent to the First Affiliated Hospital of Shihezi University for the measurement of creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH). The remaining samples were frozen at -80°C for subsequent analysis. The rats were sacrificed by cervical dislocation immediately after blood collection. Heart tissue was obtained immediately after sacrifice. Half of each heart tissue sample was fixed in paraformaldehyde (4%) for histomorphological analysis, and the other half was stored at -80°C for western blot analysis and other experiments.

Measurements of CK-MB, LDH and cardiac TnI (cTnI) levels in serum. Serum CK-MB and LDH levels in the rats were measured using an automatic biochemical analyzer (Modular DPP H7600; Roche Diagnostics, Basel, Switzerland). An ELISA kit was used to measure serum cTnI levels according to the manufacturer's instructions.

Measurement of inflammatory cytokines in serum. ELISA kits were used to determine the TNF-α, IL-6 and IL-10 levels in serum according to the manufacturer's instructions.

Measurement of H_2S levels. H_2S levels in serum were detected using a H_2S testing kit purchased from Nanjing Institute of

Bioengineering (Nanjing, China). H_2S reacts with zinc acetate, N,N-dimethylphenylenediamine and ammonium ferric sulfate in the kit to form methylene blue, which has a maximum absorption peak at 665 nm. The H_2S content was calculated by determining the absorbance value of methylene blue, and the plasma H_2S level is expressed in μmol/l.

Histological analysis. The tissue fixed in paraformaldehyde was conventionally embedded, sliced (4-μm thick) and dewaxed with xylene, then washed with ethanol at various levels. Subsequently, sections were stained with hematoxylin and eosin (H&E) at room temperature for ~2 min, dehydrated, dewaxed and sealed. Finally, images of sections were captured using an optical microscope (Olympus Corporation, Tokyo, Japan).

Western blot analysis. The frozen myocardial tissue was lysed with lysis buffer (cat. no. R0030; Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) on ice, and following protein extraction, a NanoDrop spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to determine the concentration of tissue proteins. A total of 10 μl of protein samples (40 mg/ml) were separated by SDS-PAGE using 10% gels, and then transferred to a polyvinylidene fluoride membrane. The membrane was blocked with 5% bovine serum albumin (cat. no. 4240GR100; BioFroxx; NeoFROXX GmbH, Hesse, Germany) for 2 h at room temperature. Membranes were incubated with antibodies against Akt (1:800 dilution), p-Akt (1:800 dilution), NF-κB (1:800 dilution), PI3K (1:800 dilution), p-PI3K (1:800 dilution), Bcl-2 (1:200 dilution), Bax (1:200 dilution), caspase-3 (1:500 dilution) and β-actin (1:1,000 dilution) at 4˚C for 12-18 h. The membranes were then washed six times in TBS-Tween (5 min each time), and incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibody (1:5,000 dilution) for 2-3 h at room temperature (β-actin was detected using HRP-conjugated goat anti-mouse secondary antibody under the same conditions). Following incubation, the membranes were washed six times over a total of 30 min. The membranes were reacted with an enhanced chemiluminescence reagent (cat. no. WBKLS0100; EMD Millipore, Billerica, MA, USA) and developed using X-ray film. The band intensities were quantified using ImageJ software (Java 1.6.0.24; version 1.51k; National Institutes of Health, Bethesda, MD, USA).

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Apoptosis of myocardial cells was determined by a TUNEL assay using the *in situ* Cell Death Detection kit, POD. Slices (4-μm thick) of heart tissue were incubated in a 68˚C oven for 1 h, and then dewaxed in xylene and gradient alcohol. The sliced tissue was then washed three times with PBS (5 min each wash). After washing, the sections were placed in hydrogen peroxide and soaked for 10 min. Subsequently, further washes with PBS were performed, and then sections were steamed in a pressure cooker for 5 min with citrate buffer at pH 6.0, and washed again three times with PBS (5 min each wash). Samples were incubated with 50 μl TUNEL reaction mixture (1:12) at 37˚C for 1 h in the dark. The slides were then rinsed three times with PBS, 50 μl POD

substrate was added to each section, and the samples were incubated at 37˚C for 30 min in the dark. Following incubation, the sections were washed three times with PBS, and then 50 μl diaminobenzidine coloring solution was added dropwise, and the color development was stopped with distilled water when an appropriate degree of coloration was achieved. The sections were counterstained with hematoxylin for 2 min, then dehydrated in alcohol and sealed with neutral gum. Finally, a light microscope was used to calculate the apoptotic index. Dark brown staining indicated TUNEL-positive cells, and five high power fields of the slices were randomly selected to calculate the proportion of positive cells.

Statistical analysis. Each experiment was repeated three times. All data were analyzed using SPSS 22.0 software (IBM Corp., Armonk, NY, USA). All data are expressed as the mean ± standard error. The data were analyzed using one-way analysis of variance followed by least significant difference tests. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Confirmation of the rat model of sepsis. The rats in the CLP group exhibited the behavioral characteristics of sepsis, including malaise, fever, chills, piloerection, generalized weakness and reduced gross motor activity, as well as weight loss and increased proinflammatory cytokine levels in the serum. One mortality in the CLP group and two in the CLP+LY294002 group were observed.

Effects of NaHS on myocardial enzyme serum levels. As presented in Fig. 1, CK-MB, LDH and cTnI concentrations exhibited no significant differences among the sham, sham + NaHS and sham + LY groups. There was a significant increase in serum CK-MB, LDH and cTnI in the CLP group compared with the sham group, and rats in the NaHS treatment group exhibited reductions in CK-MB, LDH and cTnI levels compared with those in the CLP group. However, these reductions were attenuated by LY294002 (a PI3K/Akt pathway-specific inhibitor).

NaHS reduces inflammatory reactions in septic rats. Inflammatory factors were measured in the serum of model rats. Following CLP, the levels of the inflammatory factors TNF-α, IL-6 and IL-10 in the serum of rats were increased significantly compared with those in the sham group ($P<0.05$; Fig. 2). The administration of NaHS decreased the levels of TNF-α and IL-6 in the serum compared with those in the CLP group ($P<0.05$), whereas the level of IL-10 was significantly increased. However, the administration of LY294002 eliminated the effects of NaHS ($P<0.05$ vs. CLP + NaHS group; Fig. 2).

H_2S levels. Although level of H_2S was increased in the CLP group, there was no significant difference in H_2S levels between the CLP and sham groups. Following the administration of NaHS, the serum H_2S content was increased significantly compared with that in the CLP group ($P<0.05$; Fig. 3). This finding indicated that the administration of NaHS increases serum H_2S content (Fig. 3).
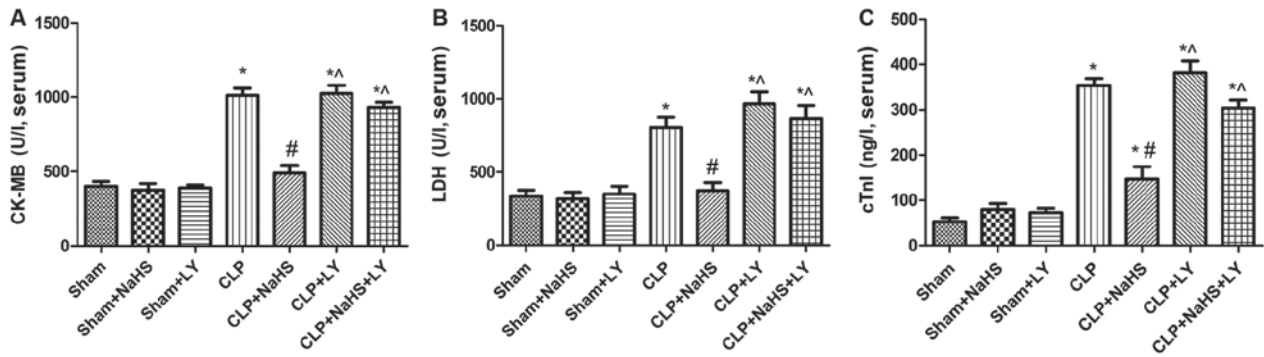
Figure 1. Changes in myocardial injury markers in rat serum. (A) CK-MB, (B) LDH and (C) cTnI. Data are expressed as the mean ± standard error. *P<0.05 vs. sham group; #P<0.05 vs. CLP group; ^P<0.05 vs. CLP + NaHS group. CK-MB, creatine kinase-MB; LDH, lactate dehydrogenase; cTnI, cardiac troponin I; CLP, cecal ligation and puncture; NaHS, sodium hydrosulfide; LY, LY294002.
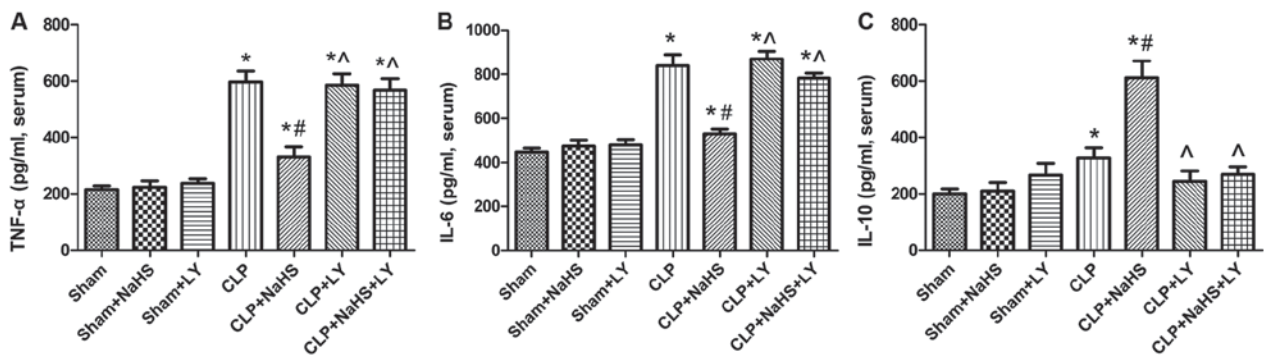

Figure 2. Changes in inflammatory factors in rat serum. (A) TNF-α, (B) IL-6 and (C) IL-10. Data are expressed as the mean ± standard error. *P<0.05 vs. sham group; #P<0.05 vs. CLP group; ^P<0.05 vs. CLP + NaHS group. TNF-α, tumor necrosis factor-α; IL, interleukin; CLP, cecal ligation and puncture; NaHS, sodium hydrosulfide; LY, LY294002.

NaHS alleviates myocardial damage caused by sepsis. H&E staining demonstrated that the sham group had normal myocardial fiber morphology and a regular arrangement of myocardial cells, with no abnormalities of the interstices and microvessels (Fig. 4). However, myocardial cells were disordered in the CLP group, with broken myocardial fibers, and parts of the myocardium exhibited vacuolization changes and inflammatory cell infiltration. The administration of NaHS reduced the degree of myocardial damage, and LY294002 eliminated the protective effect of NaHS (Fig. 4).

NaHS reduces myocardial apoptosis in septic rats. TUNEL staining was performed to investigate the effect of NaHS on myocardial apoptosis in rats with sepsis (Fig. 5). Myocardial apoptosis was significantly increased in the CLP group compared with the sham group (P<0.05). However, following the administration of NaHS, the apoptosis rate of cardiomyocytes was significantly reduced compared with that in the CLP group, and LY294002 treatment reversed these alterations (Fig. 5).

Effect of NaHS on PI3K, Akt, NF-κB, Bcl-2, Bax and caspase-3. The p-PI3K/PI3K and p-Akt/Akt ratios, and the levels of NF-κB, Bcl-2, Bax and caspase-3 were determined using western blot analysis to further understand the protective mechanism of NaHS against the myocardial injury caused by sepsis. The p-PI3K/PI3K and p-Akt/Akt ratios, and NF-κB,
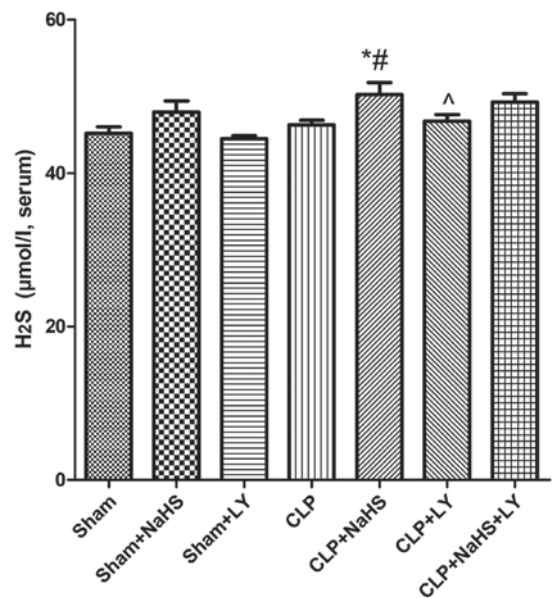

Figure 3. Changes in the serum levels of H₂S in rats. Data are expressed as the mean ± standard error. *P<0.05 vs. sham group; #P<0.05 vs. CLP group; ^P<0.05 vs. CLP + NaHS group. H₂S, hydrogen sulfide; CLP, cecal ligation and puncture; NaHS, sodium hydrosulfide; LY, LY294002.

Bax and caspase-3 levels were increased by CLP compared with those in the sham group, whereas the level of Bcl-2
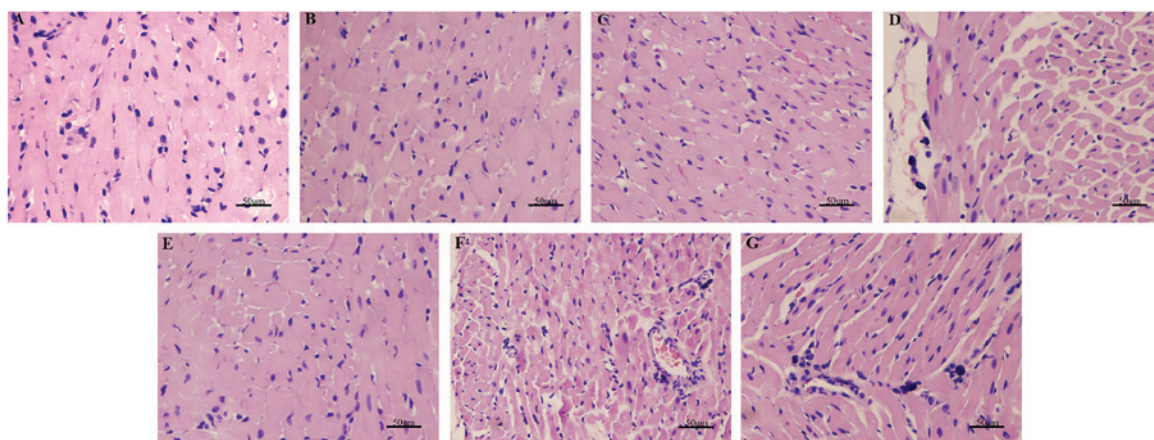
Figure 4. H&E staining demonstrating the morphology of myocardial cells in each group. Magnification, x200. (A) Sham, (B) sham + NaHS, (C) sham + LY, (D) CLP, (E) CLP + NaHS, (F) CLP + LY and (G) CLP + NaHS + LY group. H&E, hematoxylin and eosin; NaHS, sodium hydrosulfide; CLP, cecal ligation and puncture; LY, LY294002.
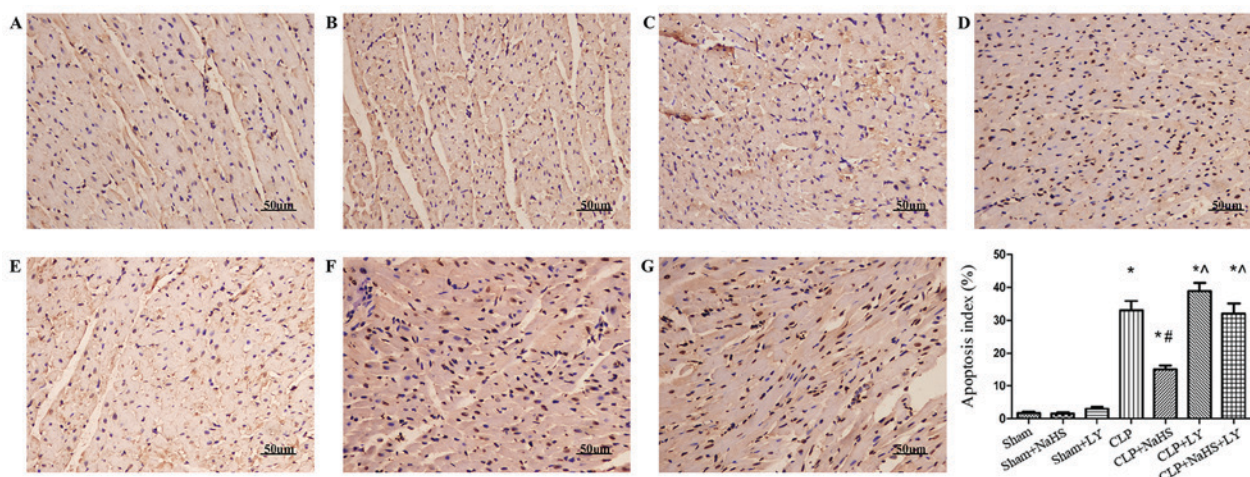

Figure 5. Apoptosis detected by TUNEL assay. Magnification, x200. (A) Sham, (B) sham + NaHS, (C) sham + LY, (D) CLP, (E) CLP + NaHS, (F) CLP + LY and (G) CLP + NaHS + LY group. The subgroup apoptosis index was calculated. *P<0.05 vs. sham group; $^{#}$P<0.05 vs. CLP group; $^{\wedge}$P<0.05 vs. CLP + NaHS group. TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; NaHS, sodium hydrosulfide; CLP, cecal ligation and puncture; LY, LY294002.

was decreased (Figs. 6 and 7). Following the administration of NaHS, the phosphorylation of PI3K and Akt was further increased and the expression of Bcl-2 was increased compared with that in the CLP group, whereas the expression levels of NF-κB, Bax and caspase-3 were decreased. The addition of LY294002 eliminated these NaHS-induced effects.

Discussion

As reported in our previous study, exogenous H$_2$S can improve sepsis-induced heart dysfunction (12). In the current study, exogenous H$_2$S significantly reduced the expression of myocardial injury markers (CK-MB, LDH and cTnI), reduced the expression of pro-inflammatory factors (TNF-α and IL-6) and induced the expression of the anti-inflammatory factor IL-10 following CLP. Furthermore, the apoptosis of cardiomyocytes was reduced by H$_2$S in the CLP-induced sepsis model. However, the effects of exogenous H$_2$S on myocardial injury in sepsis were counteracted by the PI3K signaling pathway

inhibitor LY294002, indicating that the PI3K/Akt signaling pathway may partially mediate the effect of H$_2$S.

Sepsis is caused by a dysfunctional response to infection in the host, resulting in life-threatening organ dysfunction (22). Sepsis has high morbidity and mortality rates, and significant treatment costs in developed and developing countries (23). During sepsis, various factors, including cytokines, eicosanoids, reactive oxygen species and nitrogen species, accelerate the development of the symptoms observed in patients with sepsis and cause severe systemic inflammatory reactions, which can ultimately lead to multiple organ failure (24). The heart is one of the most vulnerable target organs in sepsis (4,5). Currently, there is no specific treatment for sepsis-induced cardiac insufficiency.

H$_2$S is one of a family of gaseous signaling molecules, which includes nitric oxide (NO) and carbon monoxide. H$_2$S is typically considered to be a toxic gas; however, in the past 30 years, the understanding of the effects H$_2$S has changed markedly (25). Numerous *in vitro* and *in vitro* experiments
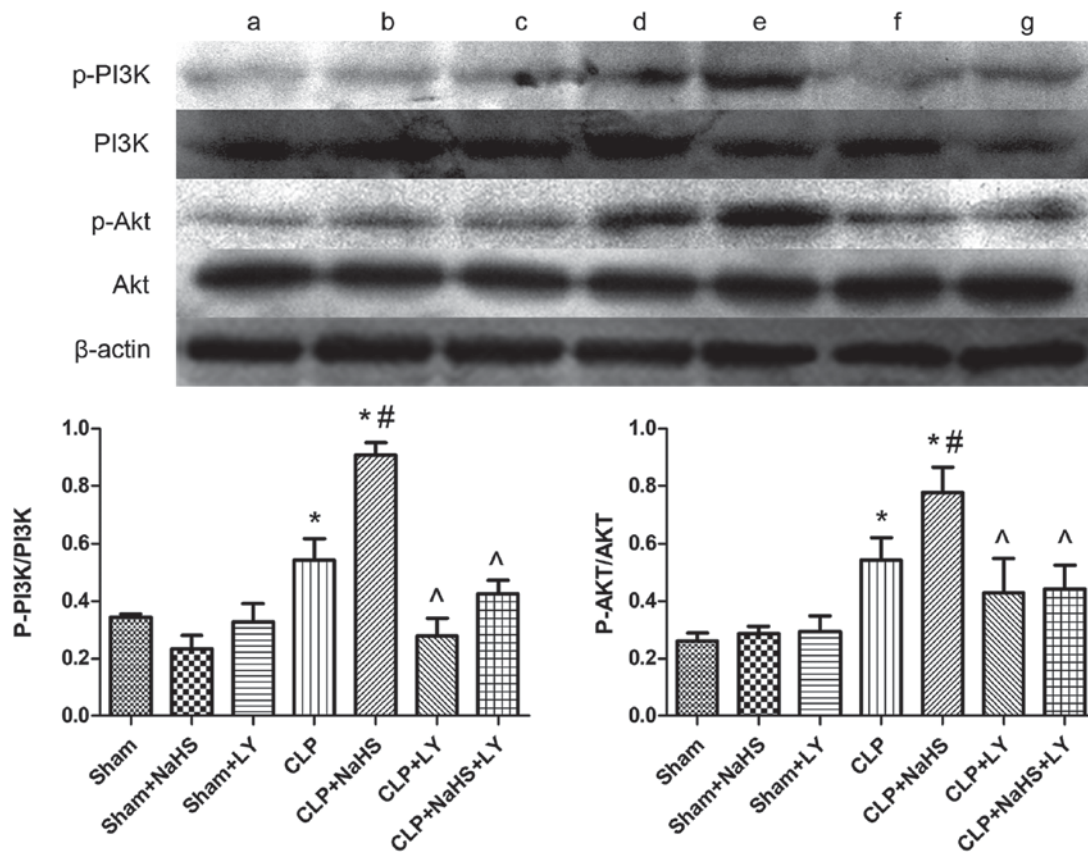
Figure 6. Changes in PI3K, p-PI3K, Akt and p-Akt in each group. Representative western blotting images are presented. Lanes: a, Sham; b, sham + NaHS; c, sham + LY; d, CLP; e, CLP + NaHS; f, CLP + LY; g, CLP + NaHS + LY. Quantified data are expressed as the mean ± standard error. *P<0.05 vs. sham group; #P<0.05 vs. CLP group; ^P<0.05 vs. CLP + NaHS group. PI3K, phosphatidylinositol-3-kinase; p-, phospho-; Akt, protein kinase B; NaHS, sodium hydrosulfide; CLP, cecal ligation and puncture; LY, LY294002.

have demonstrated that H_2S has protective effects in various tissues and cells (26-28). Abdelrahman et al (29) reported that H_2S has a protective effect on CLP-induced cardiac dysfunction, by reducing tachycardia, mortality, serum CK-MB, cTnI, C-reactive protein and LDH, and cardiac and aortic malondialdehyde levels. Chen et al (30) demonstrated that the exogenous administration of NaHS ameliorated septic-induced renal dysfunction by inhibiting inflammation and oxidative stress through the Toll-like receptor 4/NACHT, LRR and PYD domains-containing protein 3 signaling pathway. Furthermore, Ahmad et al (31) reported that the intraperitoneal injection of an appropriate dose of H_2S improved the survival rate of septic rats and reduced the inflammatory reaction. This finding is supported by the results of the present study, demonstrating that the exogenous administration of NaHS is able to attenuate inflammation during sepsis and reduce myocardial cell apoptosis. However, the findings of Zhang et al (32,33) were contrary to the results of the present study. These previous studies indicated that H_2S may exert pro-inflammatory effects during sepsis by regulating the inflammatory response via extracellular signal-regulated kinase-NF-κB pathway activation. We concur with the in-depth analysis by Ahmad et al (31) regarding the differences in findings from those of Zhang et al The differences between studies may be due to the following: i) The dose of NaHS used (0.89 μmol/kg was used in the current study, whereas Zhang et al administered a high dose (10 mg/kg) by intraperitoneal injection); and ii) the time of administration.

The serum H_2S level in each group was measured in the present study. The level of endogenous H_2S in the serum of rats appeared to increase following CLP, which was consistent with the findings of Bee et al (34). This phenomenon may be associated with the upregulation of inducible nitric oxide synthase (iNOS) expression during septic shock. Due to the increase in iNOS, the level of NO is also increased, which can induce cystathionine γ-lyase expression, resulting in higher H_2S levels (35). No significant difference in H_2S level between the sham and CLP groups was observed and, although the production of endogenous H_2S was increased following the occurrence of sepsis, the increase was not significant. Following the administration of exogenous NaHS, a statistically significant increase in the serum level of H_2S was detected, indicating that the administration of NaHS produced the desired effect.

CK, CK-MB and LDH are indicators used for the diagnosis and detection of myocardial injury (36,37). cTnI and cTnT have high specificity, and Tn levels are directly proportional to the degree of myocardial damage (38). In the current study, the analysis of serum myocardial injury markers in each group revealed that myocardial enzymes were significantly elevated in the sepsis group, indicating that sepsis had an adverse effect on the myocardium. The intraperitoneal injection of NaHS reduced the serum myocardial enzyme level. Similar changes in Tn levels were observed. H&E staining of the heart tissue revealed that the sham-operated group exhibited tightly arranged myocardial fibers with uniform coloring, and no edema, congestion or
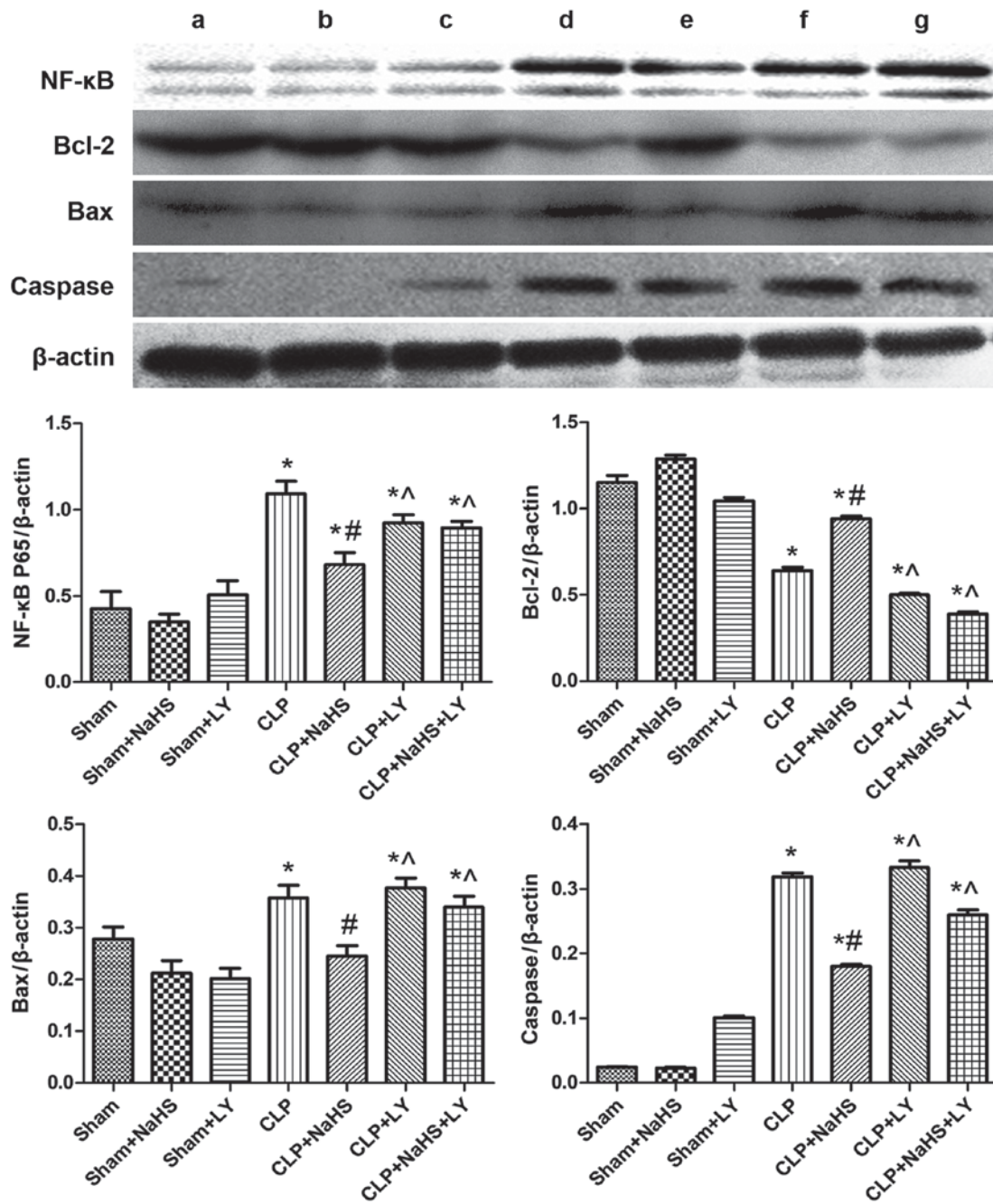
Figure 7. Expression of NF-κB, Bcl-2, Bax and caspase in each group. Representative western blotting images are presented. Lanes: a, Sham; b, sham + NaHS; c, sham + LY; d, CLP; e, CLP + NaHS; f, CLP + LY; g, CLP + NaHS + LY. Quantified data are expressed as the mean ± standard error. *P<0.05 vs. sham group; #P<0.05 vs. CLP group; ^P<0.05 vs. CLP + NaHS group. NF-κB, nuclear factor-κB; Bcl-2, B-cell lymphoma-2; Bax, Bcl-2-associated X protein; NaHS, sodium hydrosulfide; CLP, cecal ligation and puncture; LY, LY294002.

exudation. Following CLP, the presence of edema, degeneration and myocardial fiber breakage was observed in parts of the myocardial tissue. Interstitial blood vessels were congested and inflammatory cells had infiltrated the tissue. The administration of NaHS reduced the degree of myocardial damage compared with that in the sepsis group. The results of Abdelrahman *et al* (29) support the findings of the present study.

Following the onset of sepsis, a large amount of endotoxin is released, causing a series of pro-inflammatory factor cascades to be activated, including TNF-α and IL-1 (39,40). Among the pro-inflammatory cytokines, TNF-α induces cardiomyocyte apoptosis (41). A previous study reported that the infusion

of TNF-α monoclonal antibodies to septic mice transiently improves ventricular function, suggesting that TNF-α may reduce damage to the myocardium during sepsis (42). Another study demonstrated that the inflammation and apoptosis induced during sepsis is associated with the production and release of reactive oxygen species and inflammatory mediators. These substances may be involved in the activation of inducible pathways, such as NF-κB (43). Additionally, sepsis induces the expression of iNOS, which in turn produces high levels of NO. Excessive NO may cause cytotoxicity due to an increase in peroxynitrite, which can lead to cardiac dysfunction (44). In the current study, the CLP-induced inflammatory responses and

cytotoxicity were characterized by elevated levels of TNF-α, IL-6, CK-MB and LDH in serum. Bcl-2 was decreased, and Bax and caspase levels were increased by CLP, and the TUNEL assay confirmed that the apoptosis rate was increased following CLP. These results indicated that CLP induced inflammation and apoptosis. Furthermore, the administration of NaHS to rats that received CLP reduced the levels of the inflammatory response markers in the serum and reduced cardiomyocyte apoptosis. These results indicated that H_2S ameliorated the degree of sepsis-induced myocardial damage, potentially via anti-inflammatory and anti-apoptotic effects.

In numerous studies of sepsis, PI3K and downstream Akt have been demonstrated to be involved in the regulation of cell activation, inflammation and apoptosis (5,45,46). Studies have reported that heat shock protein A12B and heat shock protein 27 can attenuate cardiac dysfunction caused by endotoxins. The mechanism of this effect may be associated with the activation of PI3K/Akt (47,48). Additionally, other studies have demonstrated that H_2S activates the PI3K/Akt pathway (18,49). The results of the current study suggest that the exogenous administration of H_2S significantly increases PI3K and Akt phosphorylation in rats with CLP-induced sepsis. In order to confirm this hypothesis, a PI3K/Akt pathway specific inhibitor, LY294002, was used. The results revealed that the anti-inflammatory and anti-apoptotic effects of H_2S were eliminated by the intraperitoneal injection of LY294002 in the model rats.

H_2S has been widely used in animal studies; however, it is not clear whether H_2S has beneficial or damaging effects *in vivo*. Currently, clinical experiments have confirmed that H_2S is anti-inflammatory, reduces myocardial fibrosis and protects the myocardiu (50). However, different H_2S donors may have different mechanisms of action (51). Future studies are required to investigate the specific mechanisms of H_2S donors in complex cardiovascular signaling pathways.

In conclusion, the present study demonstrated that exogenous H_2S therapy provides an important protective effect against sepsis-induced myocardial damage via activation of the PI3K/Akt pathway. H_2S inhibits inflammation and apoptosis, and reduces myocardial dysfunction during sepsis.

Acknowledgements

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

JPL, JHL and QC jointly conceived and designed this study. JPL, GW and LL conducted the animal experiment. JPL analyzed the data and completed the first draft. JHL, PT, BG and QC reviewed and improved the paper. PT and BG revised the manuscript critically for important intellectual content. All authors read and approved the manuscript.

Ethics approval and consent to participate

The present study was approved by the Animal Protection and Use Committee of Shihezi University (Shihezi, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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