Significance of hydrogen sulfide in sepsis-induced myocardial injury in rats

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Abstract. Sepsis-induced myocardial injury is a detrimental disorder for intensive care medicine due to its high rates of morbidity and mortality. Data suggest that nuclear factor (NF)-κB serves a critical role in the pathogenesis of myocardial injury. Hydrogen sulfide (H_2S) serves an important role in the physiology and pathophysiology of regulatory mechanisms, particularly during an inflammatory reaction. However, the relationship between NF-κB and H_2S in sepsis-induced myocardial injury is not well understood, and the underlying mechanisms remain unclear. In the present study, 60 male Sprague Dawley rats were randomly divided into the following six groups: A sham group, cecal ligation and puncture (CLP) group, sham + propargylglycine (PAG) group, CLP + PAG group, sham + sodium hydrosulfide (NaHS) group and CLP + NaHS group, with 10 rats in each group. The rats in all groups were sacrificed 12 h after surgery for sample collection. Compared with the sham group, it was observed that the concentrations of Creatine Kinase-MB (CK-MB) and cardiac troponin I (cTnI) in the serum, and pathological scores of myocardial tissue were significantly increased in the CLP, CLP + NaHS and CLP + PAG groups (P<0.05). The pathological scores and concentrations of CK-MB and cTnI were significantly higher in the CLP + PAG group (P<0.05) and significantly lower in the CLP + NaHS group (P<0.05) when compared with the CLP group. The expression of cystathionine-γ-lyase (CSE) mRNA and content of interleukin (IL)-10 were significantly higher in the CLP group compared with the CLP + PAG group (P<0.05), while the expression of myocardial NF-κB and content of tumor necrosis factor (TNF)-α in the CLP group were significantly lowered compared with the CLP + PAG group (P<0.05). The expression of NF-κB and content of TNF-α were significantly increased in the CLP group when compared with the CLP + NaHS group (P<0.05), while the content of myocardial IL-10 in the CLP group was significantly lower than in the CLP + NaHS group (P<0.05). In conclusion, H_2S acted as an anti-inflammatory cytokine and biomarker in sepsis-induced myocardial injury. Furthermore, H_2S may downregulate the NF-κB subunit p65 to mediate inflammatory responses. The present data suggest that myocardial injury in sepsis may be relieved through the regulation of H_2S expression, and provide an experimental basis for the treatment of sepsis patients presenting with myocardial injury. In addition, myocardial injury in sepsis may be identified by monitoring changes in the expression of H_2S.

Introduction

Sepsis is a systemic inflammatory response syndrome caused by bacteria (or other microorganisms, including fungi), and is among the most serious complications of acute and critical illness (1). Sepsis may cause multiple organ damage and/or failure, and typically lead to acute lung injury (ALI), endothelial dysfunction, liver injury, blood coagulation dysfunction, bone marrow suppression, acid-base balance disorder and myocardial injury (2). Statistics show that 40-50% of patients with sepsis exhibit myocardial injury (3). The mechanism underlying the development of cardiac injury following sepsis is not well understood, though previous studies have indicated that inflammation serves a key role in sepsis (4,5). The incidence of sepsis is increasing with a mortality rate of ~30% each year in the USA (6). The terminal reason of death in severe sepsis patients is multiple organ dysfunction syndrome (MODS). Of patients with sepsis, 67% exhibited MODS at sepsis recognition, with 30% subsequently developing new or progressive multi-organ dysfunction (7). Currently, the treatment of sepsis remains focused on early fluid resuscitation (8,9). The effective prevention and management of myocardial injury during sepsis is an important part of treatment for sepsis (10).

Hydrogen sulfide (H_2S) is a poisonous gas (11). Endogenous H_2S is generated from cysteine; a reaction catalyzed by phosphotyrosine-5'-phosphate-dependent enzymes, including cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), and cysteine aminotransferase (12). CBS is highly expressed in the nervous system, while CSE is primarily expressed in vascular tissue and the myocardium (13). CSE is an important

catalytic enzyme in the production of H_2S in myocardium, and it may be irreversibly blocked by propargylglycine (PAG), which may inhibit the production of H_2S in the body (14). However, a number of studies have revealed that H_2S serves an important roles in the physiology and pathophysiology of regulatory mechanisms as an important neurotransmitter molecule in cardiovascular regulation, and as a novel gaseous signal molecule, hepatic circulation regulatory molecule, oxygen sensor, inflammatory mediator, endothelium-derived relaxation factor and external factor in vasodilation and energy metabolism, particularly during inflammatory reactions (15,16). Previous research has indicated that a low concentration of H_2S serves a protective effect in the regulation of tissues and inflammation during sepsis (17). Increasing the production of endogenous H_2S, may also inhibit airway inflammation (18,19). More recently, it has been observed that H_2S may suppress sepsis-induced ALI and reduce the inflammatory response during ALI (20). Furthermore, H_2S may inhibit the nuclear factor (NF)-κB signaling pathway (21,22), and H_2S has been demonstrated to serve an important role in the regulation of NF-κB expression and activity (23). In a rat model of sepsis, Chen *et al* (24) observed that H_2S reduced kidney injury caused by urinary-derived sepsis by inhibiting NF-κB expression, decreasing tumor necrosis factor (TNF)-α levels and increasing interleukin (IL)-10 levels, thus indicating that H_2S serves an important role in inflammation and immune regulation during sepsis. However, there are few studies into the role of H_2S in sepsis-induced myocardial injury, and the underlying mechanism is not well understood. The present study aimed to evaluate the effect of endogenous and exogenous hydrogen sulfide in sepsis-induced myocardial injury in rats, and to explain the important regulatory role of H_2S in sepsis-induced myocardial injury.

Materials and methods

Drugs and reagents. Sodium hydrosulfide (NaHS) was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany; cat. no. 1001915273). PAG was purchased from Sigma-Aldrich (Merck KGaA; cat. no. 101756193). Monoclonal antibody against the p65 subunit of NF-κB was purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA; cat. no. 8242). Horseradish peroxidase-conjugated AffiniPure goat anti-rabbit immunoglobulin G (IgG) and anti-β-actin monoclonal antibody were purchased from ZSGB-BIO (ZSGB-Biotechnology Company Beijing, China; cat. nos. 8242ZB-2301 and TA-09, respectively). A Takara MiniBEST Universal RNA Extraction kit was purchased from Takara Bio, Inc. (Otsu, Japan; approval no. AK801). A Thermo Scientific™ Revert Aid™ First Strand cDNA Synthesis kit was purchased from Thermo Fisher Scientific, Inc. (Waltham, MA, USA; approval no. K1622). Sequences of the polymerase chain reaction (PCR) primers, which were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) were as follows: For cystathionine-γ-lyase (CSE), forward, 5'-CCGATGACC TCAACGAACG-3' and reverse, 5'-GAGACGGTAGCC CAGGATAA-3' (national drugs approval nos. 8403261858 and 8403261859) and for β-actin, forward, 5'-CGTTGACAT CCGTAAAGACCTC-3' and reverse, 5'-TAGGAGCCAGGG CAGTAATCT-3' (national drug approval nos. 8403261860

and 8403261861). TNF-α, IL-10 and cardiac troponin I (cTnI) ELISA detection kits were purchased from Beijing Cheng Lin Biotechnology Co., Ltd. (Beijing, China; cat. nos. E-30633, E-30649 and E-30308). Ketamine hydrochloride injection (Provided by First Affiliated Hospital, School of Medicine, Shihezi University) was purchased from Fujian Gutian Yuanhang Medical Co., Ltd. (Ningde, China; national drug approval no. H35020148). 2x GoldStar Best MasterMix (Dye) was purchased from Beijing ComWin Biotech Co., Ltd. (Beijing, China; cat. no. CW0655S).

Animals. A total of 60 adult male Sprague Dawley (SD) rats (7-8 week old, body weight, 227.34±19.81 g) were obtained from the Animal Center of Xinjiang Medical University [Urumqi, China; animal use license: SYXK (Sinkiang) 2011-010101]. All experimental protocols in the present study conformed to 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 Care and Use Committee of Shihezi University (Shihezi, China). Animals were caged in groups of ten with free access to food and water and were maintained under a 12-h light/dark cycle at a room temperature of 22±1°C in a humidity of 45%, and the rats were housed in normal air with O_2 21%, N_2 78% and CO_2 0.03%. All experimental rats were anesthetized by intraperitoneally injecting (5 mg/100 g body weight) of anesthetics containing ketamine (40 mg/kg) and xylazine (10 mg/kg) for cecal ligation and puncture (CLP), sham operation, or cervical dislocation.

Establishment of sepsis model. An experimental model of sepsis was reconstructed by cecal ligation and puncture (CLP). The rats were subjected to CLP as previously described (25,26). The rats were anesthetized as described 5 mins prior to surgery. Briefly, under aseptic conditions, a 3-cm midline laparotomy was performed to expose the cecum and adjoining intestine. The cecum was tightly ligated with a 2.0-silk suture at its base, below the ileocecal valve, and was perforated twice with an 18-gauge needle. The cecum was then gently squeezed to extrude a small amount of feces from the puncture site. The cecum was returned to the peritoneal cavity and the laparotomy was closed with 3.0-silk sutures. Sham-operated animals underwent the same surgical procedure though the cecum was neither ligated nor punctured. Saline (3 ml/100 g) was administered to all rats intraperitoneally at the end of the procedure. All animals were returned to their cages with free access to food and water.

Experimental groups and protocol. The six groups of animals (n=10 per group) used in the present study were a sham-operated group (sham), which underwent a laparotomy; a sepsis group (CLP), which underwent CLP; a sepsis + PAG group (CLP + PAG), which underwent an intraperitoneal injection of 50 mg/kg PAG 1 h after CLP, administered at 2 ml/kg; a sepsis + NaHS group (CLP + NaHS), which underwent an intraperitoneal injection of 8.9 µmol/kg NaHS 1 h after CLP, administered at 2 ml/kg; a sham-operated + PAG group (sham + PAG), which underwent an intraperitoneal injection of 50 mg/kg PAG 1 h after laparotomy, administered at 2 ml/kg; and a sham-operated + NaHS group (sham + NaHS), which

underwent an intraperitoneal injection of 8.9 μmol/kg NaHS 1 h after laparotomy, administered at 2 ml/kg. The drug doses of PAG and NaHS and the administration method were derived from previous studies (20,27,28). The rats were kept at a constant environmental temperature of 37°C to maintain body heat following the procedures. All rats were observed during this 12 h period for only 5 mins. At 12 h after laparotomy, the rats were re-anesthetized with the same dose of ketamine, and their abdomens were opened and 5 ml blood was collected from the abdominal aorta. Following midline sternotomy, their hearts were removed, as previously described (29). After the specimens were collected, all rats (with the exception of 3 rats, which did not survive 12 h, all of which succumbed to septic shock) were sacrificed by cervical dislocation. Plasma samples and hearts were immediately transferred to a biochemistry laboratory and stored in a refrigerator (DW-86L626; Qingdao Haier Special Electric Refrigerator Co., Ltd., Qingdao, China) at -80°C for later determination of serum Creatine Kinase-MB (CK-MB) and cTnI levels. A section of the left myocardial tissue was fixed in 10% formalin solution for histomorphological analysis. A left myocardial tissue section was also used to determine the levels of TNF-α and IL-10, and to detect the expressions of NF-κB and CSE mRNA in the myocardial tissue.

Measurement of serum CK-MB and cTnI levels. In order to assess plasma samples, serum was collected from the blood samples using a 2-16K High Speed Refrigerated Centrifuge (Sigma-Aldrich; Merck KGaA) after centrifugation (7,000 x g) for 15 min at 4°C. Levels of CK-MB in the serum were measured using a Roche Modular DPP Automatic Biochemical Analyzer (Modular DPP H7600; Roche Diagnostics, Basel, Switzerland) by the inpatients department of the First Affiliated Hospital, Shihezi University. The CK-MB data were expressed in U/l. Levels of cTnI in the serum were quantified using a commercially available cTnI ELISA kit, according to the manufacturer's instructions. The cTnI results were expressed in ng/ml.

Histopathological examination. At 12 h post-surgery, the myocardial tissue of rats in different groups was collected and immediately washed twice with phosphate-buffered saline, then fixed in 10% neutral formalin at 25°C for 72 h. The samples were successively dehydrated (Followed by soaking in 75, 85, 90, 95 and 95% alcohol, anhydrous acetic acid, anhydrous ethanol and anhydrous ethanol, each for 30 min) and paraffin embedded. Tissue sections (4 μm) were then fixed in ethanol, as follows: Soaking in 70, 80 and 90% ethanol for 4-5 sec and then soaking in anhydrous ethanol for 5 min at room temperature. Sections were then stained with hematoxylin and eosin (H&E) and analyzed and photographed using a light microscope (Olympus Corporation, Tokyo, Japan). The method by Rezkalla *et al* (30) was used to calculate pathological scores for the myocardial tissue sections.

Analysis of CSE mRNA expression in myocardial tissue by semiquantitative reverse transcription (RT)-PCR. The Takara MiniBEST Universal RNA Extraction kit was used to extract total RNA from the myocardial tissues, following the manufacturers protocol. Total RNA then served as a template to obtain cDNA by RT with a Thermo Scientific™ Revert Aid™ First Strand cDNA Synthesis kit. To analyze gene expression,

the CSE cDNA was quantified and normalized using β-actin as a reference gene. For PCR, the 25 μl PCR reaction system comprised 12.5 μl Master Mix containing KCl, MgCl2, Tris-HCl, dNTP and Taq DNA polymerase, 0.5 μl forward primer, 0.5 μl reverse primer, 3 μl DNA template and 8.5 μl nuclease-free distilled water. The reaction conditions were as follows: Initial denaturation at 95°C for 2 min, 35 cycles at 94°C for 45 sec, 95°C for 45 sec, 58°C for 30 sec and 72°C for 7 min, followed by 72°C for a further 7 min. A total of 35 cycles was used to produce satisfactory results. The PCR product was analyzed by electrophoresis in a 2% agarose gel, then placed in a Universal Hood II Systems for Gel Doc™ and ChemiDoc™ Imaging Systems (Model no. EN61000-6-1; Bio-Rad Laboratories Ltd., Hercules, CA, USA) for an absorbance scan. β-actin served as a reference for calibration, and the absorbance ratio of the target gene to that of β-actin was used to determine the relative level of target gene expression. Three replicates were performed. Following gel imaging, Quantity One image analysis software (version 4.6.9; Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to process the results. CSE mRNA and β-actin band expression were calculated as follows: band area x band average pixel values. Then the expression level of CSE mRNA was calculated as follows: CSE expression/β-actin expression, and the relative content of target RNA was assessed. One-way ANOVA was used to analyze the statistical results.

Analysis of NF-κB expression in myocardial tissues by western blotting. Total protein was extracted from myocardial tissues using Radioimmunoprecipitation assay buffer (cat. no. R0010; Solarbio Science & Technology Co., Ltd., Beijing, China) at a ratio of 10 mg tissue to 100 μl buffer, and protein concentration was determined using the bicinchoninic acid method (cat. no. P0012; Beyotime Institute of Biotechnology, Haimen, China). Equal amounts of myocardial protein (25 mg/lane) were separated on a 10% SDS-PAGE gel and transferred onto 0.2 mm nitrocellulose membranes. The nitrocellulose blots were blocked by incubation in tris-buffered saline with Tween-20 (TBST; 10 mM Tris-HCl, pH 7.5; 150 mM NaCl and 0.1% Tween-20) containing 5% non-fat powdered milk for 1 h. The samples were subsequently mixed with anti-NF-κB p65 monoclonal antibody (1:200 dilution) or β-actin monoclonal antibody (1:1,000) and incubated at 4°C overnight. The blots were then washed five times with TBST for 15 min. Blots were incubated with horseradish peroxidase-linked anti-rabbit IgG for 1 h at room temperature, then washed five times in TBST for 15 min. A chemiluminescent peroxidase substrate (cat. no. 34094; Thermo Fisher Scientific, Inc.) was applied according to the manufacturer's instructions, and the membranes were exposed briefly to X-ray film. Protein expression was analyzed by Universal Hood II Systems for Gel Doc™ and ChemiDoc™ Imaging Systems (Model no. EN61000-6-1, Bio-Rad Laboratories Ltd). The optical density of each target protein band was assessed with Quantity One software (version 4.6.2; Bio-Rad Laboratories, Inc.) and normalized to the corresponding β-actin bands in the same sample. Three replicates were performed.

Statistical analysis. Experimental data were presented as the mean ± standard error of the mean. SPSS 17.0 software (SPSS,

Inc., Chicago, IL, USA) was used for statistical analysis. Normal distribution variables among different groups were compared using one-way analysis of variance and non-normal distribution variables were compared using a Wilcoxon rank-sum test. Enumeration data were analyzed using a χ^2 test. P<0.05 was considered to indicate statistically significant results.

Results

Comparison of sepsis severity in model rats. The rats in the CLP group gradually appeared less active, were cold, had dull and upright fur, and stopped drinking water up to 6 h after the CLP procedure. Dyspnea occurred in 3 rats. In addition, 5 rats were in a passive supine position and responded slowly. Following the production of the model, the rats were placed in an animal room and observed by two researchers. There were 7 observation points: before modeling, 2, 4, 6, 8, 10 and 12 h after modeling, at which rats were observed for 5 mins. The within 12 h mortality rate of the CLP group and the CLP + PAG group were 10% (n=9) and 20% (n=8), respectively, and the mortality rates of the sham group and the CLP + NaHS group were 0%. The rats in the sham group were eating and exercising normally, in which had no anorectic and vertical hair. Rats in the CLP + NaHS group appeared anorectic, had vertical hair, and compared with the CLP group, rats in the CLP + NaHS group were less or completely inactive. Compared with the CLP + NaHS group, the symptoms (anorectic, vertical hair and reduced activity) were less severe than in the CLP group. Rats in the CLP + PAG group exhibited similar behavior to those in the CLP group. A comparison of the 12-h mortality rates of rats in each group is presented in Table I.

Pathological changes in the myocardial tissue of model rats. Pathological scores were calculated from the H&E stained myocardial tissues of rats in each group (Fig. 1 and Table II). In the CLP group, the pathological score was significantly higher than that in the sham group (P<0.05). No significant differences were observed in the pathological scores of the sham, sham + PAG and sham + NaHS groups (P>0.05). The pathological score of the CLP group was significantly higher than that in the CLP + NaHS group (P<0.05), and significantly lower than that in the CLP + PAG group (P<0.05).

Changes in the serum levels of CK-MB and cTnI. As depicted in Fig. 2, the CLP, CLP + NaHS and CLP + PAG groups exhibited significantly higher levels of CK-MB and cTnI in the serum when compared with the sham group (P<0.05). The serum levels of CK-MB and cTnI did not differ significantly between the sham, sham + PAG and sham + NaHS groups (P>0.05). The serum levels of CK-MB and cTnI in the CLP group were significantly higher than that in the CLP + NaHS group (P<0.05), and significantly lower than that in the CLP + PAG group (P<0.05; Fig. 2).

Changes in TNF-α and IL-10 levels in the myocardial tissue of model rats. As depicted in Fig. 3, the CLP, CLP + NaHS and CLP + PAG groups exhibited significantly higher levels of TNF-α and IL-10 in the myocardial tissue when compared with the sham group (P<0.05). The levels of myocardial

Table I. Comparison of 12-h mortality rates of rats in each group.

Groups	Mortality rate, %
Sham	0
Sham + NaHS	0
Sham + PAG	0
CLP	10
CLP + NaHS	0
CLP + PAG	20

CLP, cecal ligation and puncture; NaHS, sodium hydrosulfide; PAG, propargylglycine.

Table II. Pathological scoring of myocardial tissue determined by hematoxylin and eosin staining.

Group	Case, n	Pathological score
Sham	10	236.16±38.88
Sham + NaHS	10	248.92±83.35
Sham + PAG	10	217.28±37.01
CLP	9	796.91±89.57[a]
CLP + NaHS	10	516.14±79.77[a,b]
CLP + PAG	8	1,016.14±79.77[a-c]

Data are presented as the mean ± standard error. [a]P<0.05 vs. sham group, [b]P<0.05 vs. CLP group and [c]P<0.05 vs. CLP + NaHS group. CLP, cecal ligation and puncture; NaHS, sodium hydrosulfide; PAG, propargylglycine.

TNF-α and IL-10 did not differ significantly between the sham, sham + PAG and sham + NaHS groups (P>0.05). The levels of TNF-α in the CLP group were significantly higher than that in CLP + NaHS group (P<0.05), and significantly lower than that in the CLP + PAG group (P<0.05). The levels of IL-10 in the CLP group were significantly lower than that in the CLP + NaHS group (P<0.05), and significantly higher than that in the CLP + PAG group (P<0.05, Fig. 3). The current experimental design was to study the effect of endogenous and exogenous hydrogen sulfide on myocardial injury induced by sepsis, therefore the NaHS groups were not assayed for the expression of CSE mRNA.

Changes in CSE mRNA expression in the myocardial tissue of model rats. In the CLP group and the CLP + PAG group, the expression of CSE mRNA in the myocardial tissue was significantly increased compared with that in the sham group (P<0.05). The levels of CSE mRNA did not differ significantly between the sham and sham + PAG groups (P>0.05). Compared with the CLP group, the expression of CSE mRNA was significantly reduced in the CLP + PAG group (P<0.05; Fig. 4).

Changes in NF-κB expression in the myocardial tissue of model rats. Results of western blot analysis (Figs. 5 and 6) indicated
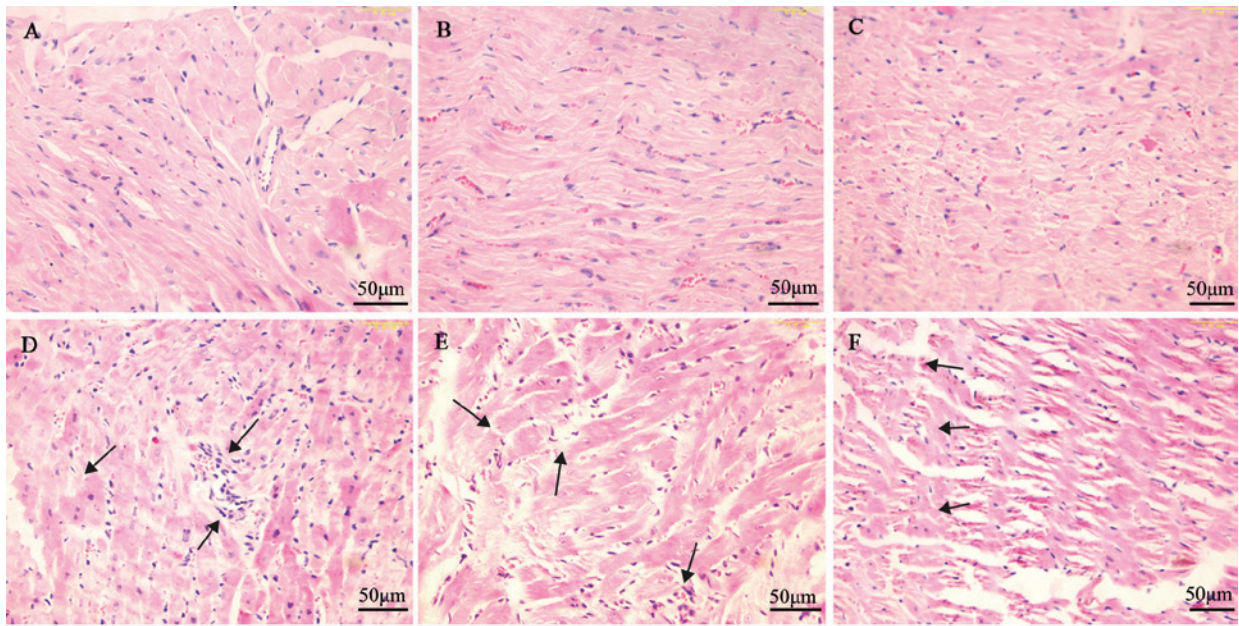
Figure 1. Examination of myocardial tissue pathology by hematoxylin and eosin staining. (A) Sham group, in which rats underwent a laparotomy; (B) sham + NaHS group, in which sham rats were treated with 8.9 μmol/kg NaHS 1 h after laparotomy; (C) sham + PAG group, in which sham rats were treated with 50 mg/kg PAG 1 h after laparotomy; (D) CLP group, in which rats underwent CLP; (E) CLP + NaHS group, in which CLP rats were treated with 8.9 μmol/kg NaHS 1 h after CLP; (F) CLP + PAG group, in which CLP rats were treated with 50 mg/kg PAG 1 h after CLP. Magnification, x200. CLP, cecal ligation and puncture; NaHS, sodium hydrosulfide; PAG, propargylglycine.
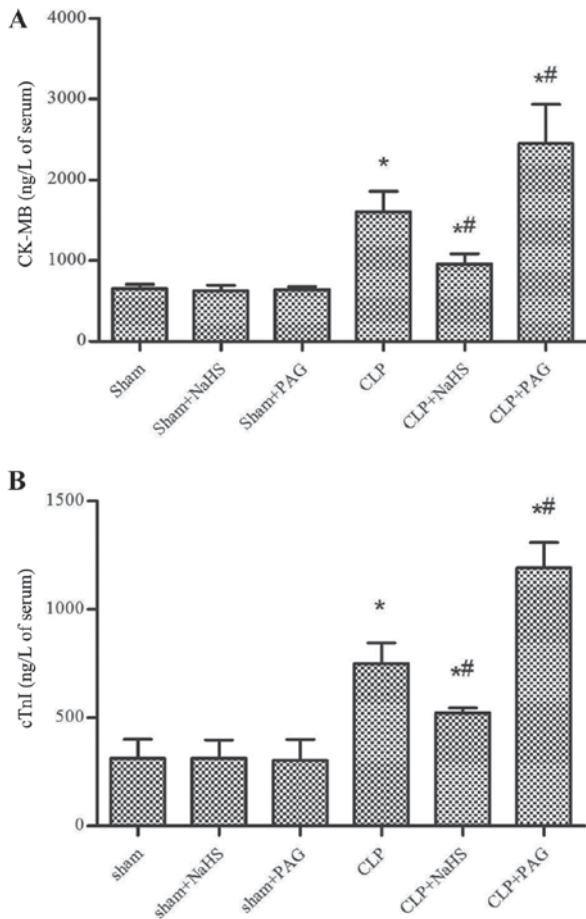
Figure 2. Change in the serum levels of (A) CK-MB and (B) cTnI in sepsis rats. Data are presented as the mean ± standard error of the mean. *P<0.05 vs. sham group; #P<0.05 vs. CLP group. CK-MB, Creatine Kinase-MB; cTnI, cardiac troponin I; CLP, cecal ligation and puncture; NaHS, sodium hydrosulfide; PAG, propargylglycine.
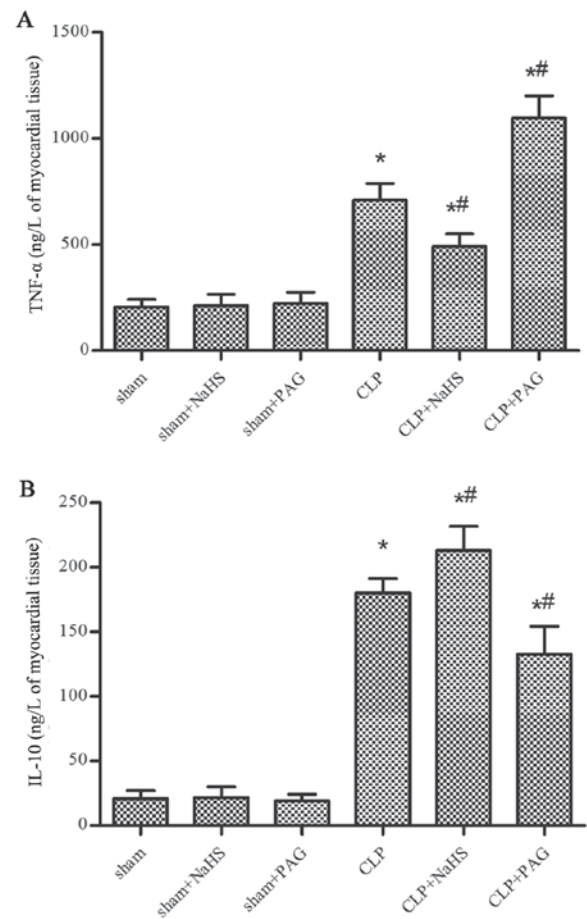
Figure. 3. Change in the myocardial tissue levels of (A) TNF-α and (B) IL-10 in sepsis rats. Data are presented as the mean ± standard error of the mean. *P<0.05 vs. sham group; #P<0.05 vs. CLP group. TNF, tumor necrosis factor; IL, interleukin; CLP, cecal ligation and puncture; NaHS, sodium hydrosulfide; PAG, propargylglycine.
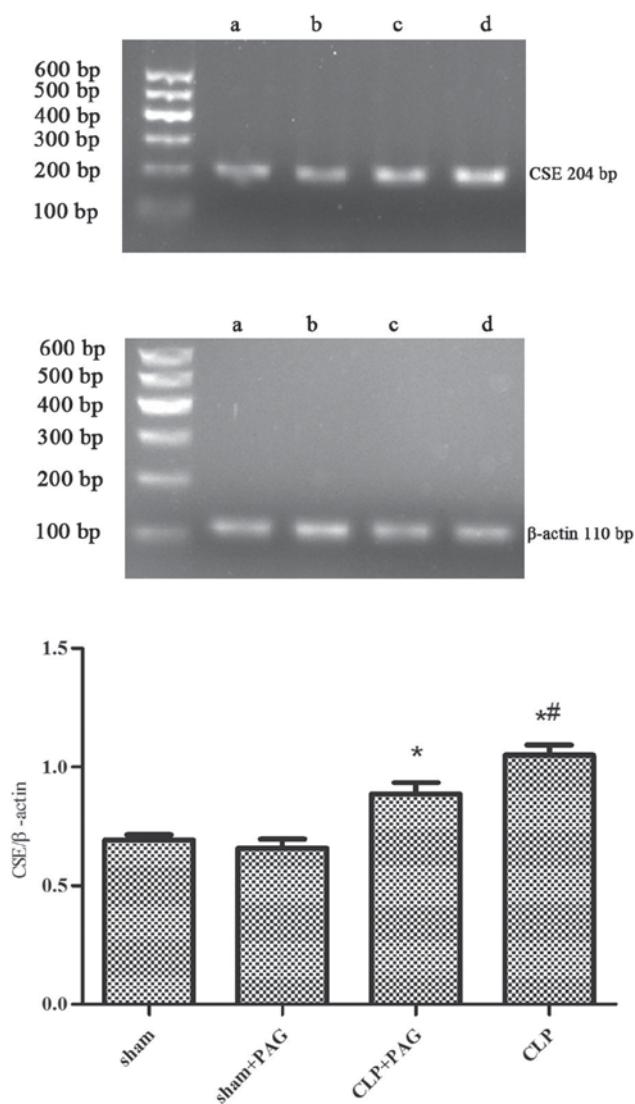
Figure 4. Analysis of CSE mRNA expression in the myocardial tissue of sepsis rats by reverse transcription-polymerase chain reaction. Lanes a, b, c and d show results of the sham, sham + PAG, CLP + PAG and CLP groups, respectively. *P<0.05 vs. sham group; #P<0.05 vs. CLP + PAG group. CSE, cystathionine-γ-lyase; CLP, cecal ligation and puncture; PAG, propargylglycine.

Figure 5. Change in the expression of NF-κB p65 in the myocardial tissue of sepsis rats following treatment with PAG. Lanes a, b, c and d show results of the sham, sham + PAG, CLP + PAG and CLP groups, respectively. *P<0.05 vs. sham group; #P<0.05 vs. CLP + PAG group. NF-κB, nuclear factor-κB; CLP, cecal ligation and puncture; PAG, propargylglycine.

that the expression of NF-κB was significantly increased in the CLP group, the CLP + PAG group and the CLP + NaHS group when compared with the sham group (P<0.05). NF-κB expression in the myocardial tissue did not differ significantly between the sham and sham + PAG groups (P>0.05; Fig. 5), or between the sham and sham + NaHS groups (P>0.05; Fig. 6). Compared with the CLP group, the expression of NF-κB was significantly increased in the CLP + PAG group (P<0.05; Fig. 5) and significantly decreased in the CLP + NaHS group (P<0.05; Fig. 6).

Discussion

H₂S was initially considered to exert toxic effects on the human body (6). However, more recently, studies have indicated that H₂S is an important anti-inflammatory mediator *in vivo* (31,32), and physiological concentrations of H₂S have been demonstrated to serve key roles in the regulation of
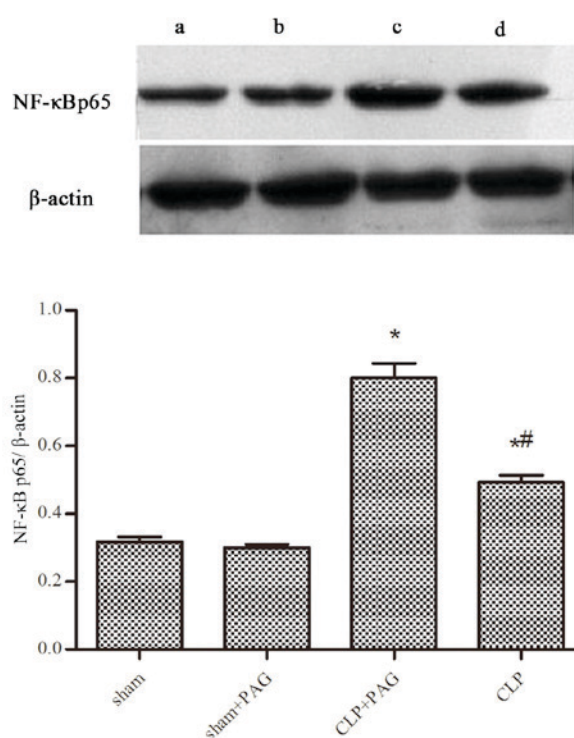
inflammation during sepsis (33). Endogenous H₂S is generated from cysteine; a reaction catalyzed by phosphotyrosine-5'-phosphate-dependent enzymes, including cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE), and cysteine aminotransferase (12). CBS is highly expressed in the nervous system, while CSE is mainly expressed in vascular tissue and the myocardium (13). CSE may be irreversibly blocked by PAG, which can inhibit the production of H₂S in the body (14). A third of the total H₂S in the body is present in gaseous form, while two-thirds exist as HS⁻. A balance between NaHS and HS⁻ exists to ensure the presence of H₂S and maintain a stable pH environment (34). Under physiological conditions, the plasma level of H₂S has been recorded at ~46 μmol/l in SD rats (35). The properties of NaHS are stable and its concentration in solution may be controlled, and thus it is often used in experiments. Therefore, the drug was used in the present study as a donor of H₂S.

Sepsis, when combined with myocardial injury, may aggravate its own development and increase the risk of multiple organ failure and mortality (36). Despite extensive study into sepsis-induced myocardial damage, the underlying mechanism is not well understood, though potentially involves myocardial ischemia-reperfusion injury, circulating myocardium inhibitory factor, mitochondrial dysfunction and oxidative stress injury (37). Elucidating the underlying mechanisms of myocardial injury in sepsis, and methods to prevent damage to heart function during early-stage sepsis, have been the focus of medical research. In the current study, a rat model of sepsis was established by CLP, which successfully induced the symptoms and signs of sepsis. Indeed, CLP is considered to
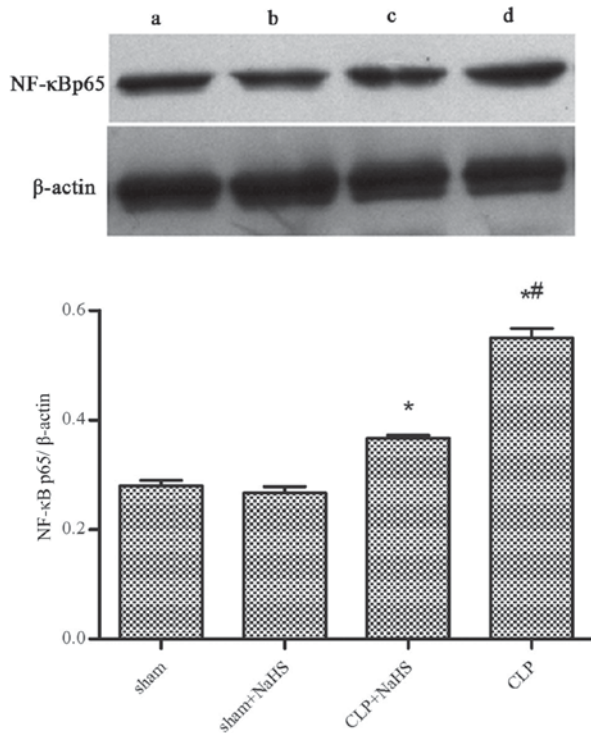
Figure 6. Change in the expression of NF-κB p65 in the myocardial tissue of sepsis rats following treatment with NaHS. Lanes a, b, c and d show results of the sham, sham + NaHS, CLP + NaHS and CLP groups, respectively. *P<0.05 vs. sham group; #P<0.05 vs. CLP + NaHS group. NF-κB, nuclear factor-κB; CLP, cecal ligation and puncture; NaHS, sodium hydrosulfide.

be a gold standard sepsis model (28). Male SD rats of similar age were also used in the current study to avoid the potential effects of gender, age, hormone levels and other factors on the results.

Analysis of myocardial markers demonstrated that the serum concentrations of CK-MB and cTnI were increased in rats subjected to CLP, with the highest levels observed in CLP + NaHS group, followed by the CLP and CLP + PAG groups (in successive order). The greatest injury to the myocardial tissue was observed in the CLP + PAG group, followed by the CLP, CLP + NaHS and sham groups (in successive order). These results suggested that inhibition of endogenous H_2S expression may aggravate myocardial injury during sepsis, while exogenous H_2S may alleviate myocardial injury induced by sepsis in rats. Therefore, H_2S may alleviate myocardial injury and exert protective effects on myocardial tissue during sepsis.

NF-κB, as a key nuclear transcription factor, is widely expressed in eukaryotic cells and is a member of the Rel protein family (38,39). At present, 5 members of this family have been identified in mammals; p65 (RelA), RelB, C-Rel, p50/p105 (NF-κB1) and p52/p100 (NF-κB2) (40). These proteins are typically present as homo- or heterodimers. p65 is the main subunit of the NF-κBp50/p65 dimer, which serves major roles in physiological processes (40). In particular, studies have indicated that NF-κB serves an important role in the generation and release of inflammatory mediators and cytokines (41,42). TNF-α also acts as a dynamic factor within intracellular cascades. TNF-α may induce the degradation of NF-κB inhibitor (IκB) and thus enhance NF-κB activity

to promote the synthesis and release of TNF-α, IL-1 and IL-6, which forms a cascade reaction (43). A previous study documented that the expression of proinflammatory cytokines was increased in patients with sepsis, while the expression of anti-inflammatory cytokines was decreased, which was associated with poor prognosis (44). Therefore, the condition of patients with sepsis may be improved by reducing the levels of IL-6, IL-8 and IL-1 and increasing the levels of IL-10.

In the present study, increased expressions of NF-κB and TNF-α in the myocardial tissue of the CLP group were accompanied by an increased pathological score and concentration of cTnI, while the concentration of IL-10 appeared to be inversely associated with myocardial pathological score and cTnI concentration. This may have been due to the inhibition of NF-κB expression by H_2S, as active NF-κB downregulates the expression of proinflammatory cytokines, including TNF-α, and upregulates the expression of the anti-inflammatory factor IL-10 (24).

The expression of CSE mRNA was significantly higher in the CLP group when compared with the CLP + PAG group. Furthermore, the expression of CSE mRNA was significantly higher in the CLP + PAG group when compared with the sham and sham + PAG groups, indicating that myocardial CSE expression was significantly inhibited in the sepsis model, which may have reduced the production of H_2S in the myocardial tissue. The expressions of NF-κB and TNF-α in the CLP group were significantly lower than that in the CLP + PAG group, and the expression of IL-10 in the CLP group was significantly higher than that in the CLP + PAG group. In addition, the level of IL-10 in the CLP group was significantly lower than that in the CLP + NaHS group. Li et al (45) observed that H_2S may reduce the level of serum TNF-α, increase the level of IL-10, and attenuate the inflammatory response and tissue damage in an early model of acute abdominal infection in rats; while reduced levels of H_2S may increase the inflammatory response. Ma et al (44) reported that TNF-α may induce IκB degradation, enhance NF-κB activity and promote the synthesis and release of TNF-α, IL-1, IL-6 in neutrophils to form a cascade reaction. H_2S has been documented to exert an inhibitory effect on cytokine production and inflammatory cell chemotaxis in the early stage of sepsis, which inhibits the inflammatory reaction and inflammatory cascades, thus protecting the tissue (45). In a myocardial ischemia model, Liu et al (46) observed that the protective effect of NaHS on myocardial tissues was associated with its modulation of inflammatory factors and anti-inflammatory effects. Chen et al (24) also documented that exogenous H_2S may inhibit NF-κB expression in a rat model of sepsis, which may subsequently downregulate IL-10 and upregulate TNF-α, thereby reducing sepsis-induced renal injury. These results indicate that reduced production of endogenous H_2S in a rat model of sepsis may increase the expressions of NF-κB and TNF-α and decrease the expression of IL-10 in myocardial tissue, which in turn may aggravate sepsis-induced myocardial injury. Therefore, administration of exogenous H_2S may attenuate sepsis-induced myocardial injury by downregulating NF-κB and the proinflammatory cytokine TNF-α and upregulating the anti-inflammatory factor IL-10.

In conclusion, H_2S may be involved in the pathogenesis of sepsis and may serve a protective role in sepsis-induced

myocardial injury. Its underlying mechanism of action may involve the inhibition of NF-κB expression, downregulation of the proinflammatory factor TNF-α and upregulation of the anti-inflammatory factor IL-10. The present data indicate that myocardial injury during sepsis may be relieved through the regulation of H_2S expression, and thus provide an experimental basis for the treatment of patients with sepsis-induced myocardial injury.

Acknowledgements

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