Hydrogen Sulfide Alleviates Lipopolysaccharide-Induced Myocardial Injury Through TLR4-NLRP3 Pathway

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Summary

To investigate the effect of hydrogen sulfide (H_2S) on myocardial injury in sepsis-induced myocardial dysfunction (SIMD), male C57BL/6 mice were intraperitoneally injected with lipopolysaccharide (LPS) (10 mg/kg, i.p.) to induce cardiac dysfunction without or with the H_2S donor sodium hydrosulfide (NaHS) (50 μmol/kg, i.p.) administration 3 h after LPS injection. Six hours after the LPS injection, echocardiography, cardiac hematoxylin and eosin (HE) staining, myocardial damage and inflammatory biomarkers and Western blot results were analyzed. In mice, the administration of LPS decreased left ventricular ejection fraction (LVEF) by 30 % along with lowered H_2S levels (35 % reduction). It was observed that cardiac troponin I (cTnI), tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) levels were all increased (by 0.22-fold, 2000-fold and 0.66-fold respectively). HE staining revealed structural damage and inflammatory cell infiltration in the myocardial tissue after LPS administration. Moreover, after 6 h of LPS treatment, toll-like receptor 4 (TLR4) and nod-like receptor protein 3 (NLRP3) expressions were up-regulated 2.7-fold and 1.6-fold respectively. When compared to the septic mice, NaHS enhanced ventricular function (by 0.19-fold), decreased cTnI, TNF-α, and IL-1β levels (by 11 %, 33 %, and 16 % respectively) and downregulated TLR4 and NLRP3 expressions (by 64 % and 31 % respectively). Furthermore, NaHS did not further improve cardiac function and inflammation in $TLR4^{-/-}$ mice or mice in which NLRP3 activation was inhibited by MCC950, after LPS injection. In conclusion, these findings imply that decreased endogenous H_2S promotes the progression of SIMD, whereas exogenous H_2S alleviates SIMD by inhibiting inflammation *via* the TLR4-NLRP3 pathway suppression.

Key words

Sepsis • Myocardial dysfunction • Hydrogen sulfide • Toll-like receptor 4 • Nod-like receptor protein 3

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Introduction

Sepsis is a systemic inflammatory syndrome induced by the emission of high amounts of the endotoxin lipopolysaccharide (LPS) after body infection caused by blood microbes. As one of the most prevalent critical clinical disorders, sepsis can lead to multi-organ failure, infectious shock, and death if left untreated. Myocardial dysfunction is a vital component of sepsis-associated multi-organ failure, which can result in a high death rate in the intensive care unit. Multiple pathways have been linked to sepsis-induced myocardial dysfunction (SIMD), including an increased inflammatory response and pyroptosis [1,2]. As a non-invasive reliable model, LPS-induced myocardial dysfunction has been employed frequently. Intraperitoneal injection of LPS, for instance, significantly enhances the inflammatory factor expression while decreasing cardiac beat volume and ejection fraction. Endotoxin's heart-damaging effects have gotten

a lot of attention in recent years, with myocardial injury and cardiac dysfunction showing up early in sepsis and dramatically increasing patient mortality [3,4]. There are currently no specific cardioprotective medications available to help with sepsis-related cardiac problems. As a result, further research into the mechanisms of myocardial injury in sepsis along with the development of efficient preventive and therapeutic measures are critical clinical issues that must be addressed.

Based on cystathionine gamma-lyase (CSE) and cystathionine beta-synthase (CBS) a third essential system gas signaling molecule in addition to nitric oxide (NO) and carbon monoxide (CO), hydrogen sulfide (H_2S), is synthesized from cysteine [5]. Oxidative stress, angiogenesis, inflammation, vasodilation, and carcinogenesis are a few of the pathological and physiological processes that H_2S is involved in [6]. Through the suppression of inflammation and oxidative stress, H_2S has been shown to have potential therapeutic benefits in cardiovascular diseases [7] and some H_2S-releasing compounds has been used several basic studies and processed into clinical trials [8]. In our previous study, we found that H_2S ameliorated SIMD by suppressing inflammation and endoplasmic reticulum stress *via* inhibition of the toll-like receptor 4 (TLR4) pathway [9]. Furthermore, H_2S protects H9c2 cardiomyocytes against high glucose-induced inflammation and apoptosis by blocking TLR4/ nuclear factor-κB (NF-κB) pathway activation and NOD-like receptor protein 3 (NLRP3) expression in H9c2 cardiomyocytes [10]. Another study found that H_2S therapy alleviated sepsis-induced acute lung injury in mice by inhibiting the PDGFRβ/Akt/NF-κB/NLRP3 pathway [11].

The NLRP3 inflammasome is a macromolecular protein complex composed of NLRP3, an apoptosis-associated speck-like protein containing a CARD (ASC), and the precursors of the effector protein cysteine aspartate protease-1 (pro-caspase 1). It is a critical component of the innate immune system and its activation enhances the maturation and production of interleukin-1β (IL-1β) and interleukin-18 (IL-18) while cleaving gasdermin-D to cause pyroptosis [12].

The activation of the classical NLRP3 inflammasome pathway consists of two phases of priming and activation. The priming phase mainly includes the determination by TLR4, the damage-associated molecular patterns, or extracellular pathogen-associated molecular patterns. Its downstream signaling molecule, myeloid differentiation factor 88 (Myd88), activates NF-κB, ultimately inducing the expression of NLRP3 and IL-1β precursors. The activation phase is characterized by multiple stimuli that promote the activation and assembly of the NLRP3 inflammasome complex [13]. Thus, the TLR4/Myd88/NF-κB pathway is indispensable for stimulating the NLRP3 inflammasomal pathway. Accumulating evidence suggests that the NLRP3 inflammasome together with the TLR4/Myd88/ NF-κB signaling axis, mediate the onset of inflammatory responses in myocardial ischemia/reperfusion (MI/R) injury ultimately leading to myocardial injury. Therefore, inhibition of the NLRP3 inflammasome and the TLR4/Myd88/NF-κB axis can reduce the inflammatory responses and ameliorate MI/R injury [14-16]. The NLRP3 inhibitor, MCC950, significantly improves the LPS-induced myocardial inflammatory responses [17]. In addition, it was reported that cardiac connexin-hemichannels and pannexin-1 channels facilitated inflammatory NLRP3 assembly which leads to the secretion of pro-inflammatory factors [18], while H_2S exerted its anti-inflammatory effect in hypertensive inflammation *via* down-regulating pro-inflammatory connexins expression [19]. Thus, this study was aimed to evaluate whether the protective effects of H_2S on LPS-induced myocardial injury was mediated by inhibiting TLR4-NLRP3 pathway.

Methods

Animals and treatments

All animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH) of the United States and approved by the Ethics Committee for Laboratory Animals Care and Use of Hebei Medical University. Male C57BL/6J mice and $TLR4^{-/-}$ mice (C57BL/6 background), aged between 10-to-12-weeks were procured from Vital River Laboratories (Beijing, China). Mice were fed on a normal diet and reared under standard housing conditions. After acclimatization, the mice were treated as follows:

C57BL/6J mice were randomly divided into three groups, each with eight mice as follows: Control, LPS, and LPS+NaHS (50 μmol/kg) groups. The wild-type (WT) and $TLR4^{-/-}$ mice were classified into the following four groups consisting of eight mice each: WT Control, WT+LPS, $TLR4^{-/-}$+LPS, and $TLR4^{-/-}$+LPS+NaHS groups. In order to explore the

relationship between NLRP3 signaling pathway and H_2S, C57BL/6J mice were randomly divided into 3 groups: LPS, LPS+MCC950 and LPS+NaHS+MCC950 groups.

Ten mg/kg LPS (dissolved in normal saline) was intraperitoneally injected, following which saline or NaHS (50 μmol/kg, dissolved in normal saline) was intraperitoneally administered (3 h after LPS injection). MCC950 (50 mg/kg, dissolved in normal saline, MedChemExpress Co., Ltd., China), a NLRP3 inhibitor, was intraperitoneally injected 30 min before LPS administration. Taken together, every mice in each group were injected with the same volume of fluid at 0.2 ml.

Next, 6 h post-LPS treatment, two-dimensional echocardiography was used to evaluate left ventricular function. After echocardiographic measurements, the mice were euthanized by an overdose of pentobarbital (100 mg/kg; intraperitoneal injection). Subsequently, heart tissues and blood were collected. The plasma was aspirated and frozen at -80 °C till further use following centrifugation at 4000 rpm for 10 min at 4 °C. Left ventricular heart tissues were frozen at -80 °C and fixed with paraformaldehyde (4 % PFA).

Echocardiography

To evaluate left ventricular function, mouse two-dimensional echocardiography was performed using a VisualSonics Vevo 770 platform. Mice were anaesthetized with 1 % isoflurane and M-mode images of the left ventricle were recorded. Left ventricular ejection fraction (LVEF) was calculated using the Vevo analysis software.

Measurement of H_2S concentration in plasma

The H_2S concentration in plasma was determined by using a commercially assay kit (Solarbio Science & Technology Co., Ltd., China) according to the manufacturer's instructions.

Histological analysis

Cardiac specimens were fixed with 4 % PFA for 48 h and embedded in paraffin. Hematoxylin-eosin (HE) stained 5 μm-thick sections were utilized for histological analysis by light microscopy (Olympus BX40, Tokyo, Japan). Histological alterations were determined by a quantitative tissue damage assessment by blinded observers and scored (0 to 4) for myocardial necrosis and cellular infiltration. The scores were as follows: 0 (none), no myocardial lesion; 1, lesions involving <25 % of the myocardium; 2, lesions involving 25 to 50 % of the myocardium; 3, lesions involving 50 to 75 % of the myocardium; 4, lesions involving >75 % of the myocardium.

Measurement of inflammatory cytokines and cardiac troponin I (cTnI) in plasma

The IL-1β, tumor necrosis factor-α (TNF-α), and cTnI levels in the plasma were detected following the manufacturer's protocols for the corresponding enzyme-linked immunosorbent assay (Abclonal Biologicals Company, China) kits.

qPCR analysis

Frozen heart tissues were suspended in Trizol reagent (Invitrogen, United States), and the total RNA was extracted according to the manufacturer's instructions. Reverse transcription was performed using a Reverse Transcription Kit (Toyobo, Japan). A SYBRGreen RT-PCR Kit from Toyobo was used for quantitative real-time qPCR analysis with the StepOne PLUS Real-time PCR system (Applied Biosystems, United States), according to the manufacturer's instructions. Gene-specific primers were used to detect mice IL-1β (forward primer: 5′-GCAGTGGTTCGAGGCCTAAT-3′; reverse primer: 5′-GCTGCTTCAGACACTTGCAC-3′), TNF-α (forward primer: 5′-CCAGACCCTCACACTCACAAA-3′; reverse primer: 5′-TGTCTTTGAGATCCATGCCGT-3′), TLR4 (forward primer: 5′-ATCCCTGCATAGAGGTAGTTCC-3′; reverse primer: 5′-TTCAAGGGGTTG AAGCTCAGA-3′) and NLRP3 (forward primer: 5′-GGCTGCTATCTGGAGGAACT-3′; reverse primer: 5′-GCAACGGAC ACTCGTCATCT-3′). The samples were normalized against endogenous mice GAPDH (forward primer: 5′-GGTGAAGGTAGGTGTGAACGT-3′; reverse primer: 5′-CTCGCTCCTGGAAGATGGTG-3′), and fold changes were calculated using the formula $2^{-\Delta\Delta Ct}$.

Western blot analysis

Frozen left ventricle tissues were lysed with ice-cold RIPA buffer. After lysis, proteins were extracted and quantified by the bicinchoninic acid (BCA) method. Equal amount of protein samples were separated by electrophoresis, transferred onto membranes, and incubated with anti-TLR4 (1:500, Abcam, United States), anti-NLRP3 (1:500, Abcam, United States), anti-β-actin (1:500, Abcam, United States), overnight at 4 °C. Next,

the membrane was incubated in horse-radish peroxidase (HRP)-labeled secondary antibody (1:1000) at room temperature for 1 h. The intensity of the protein bands was assessed on the Bio-Rad imaging platform and quantified using the Image J software.

Statistical analysis

Results were expressed as mean ± SEM. Statistical analysis was performed using an SPSS software package, version 13.0 (SPSS, Inc., United States). The results for three or more groups were compared using one-way ANOVA followed by LSD *t*-test. $P<0.05$ was considered statistically significant.

Results

H_2S levels in plasma decrease and inflammation increases in SIMD mice

After 6 h post-LPS injection, the H_2S concentration in plasma was significantly lower in the LPS group ($P<0.01$) (Fig. 1A), and those of IL-1β, cTnI, and TNF-α were significantly higher ($P<0.01$), relative to the Control group (Fig. 1B-D); the relative mRNA levels of TNF-α, IL-1β, TLR4 and NLRP3 (Fig. 1E-H) and the protein expressions of TLR4 and NLRP3 in myocardial tissues were significantly higher after LPS injection (Fig. 1I-J). The above data proves that H_2S levels in plasma decrease and inflammation increases in SIMD mice and H_2S is involved in the development of SIMD.

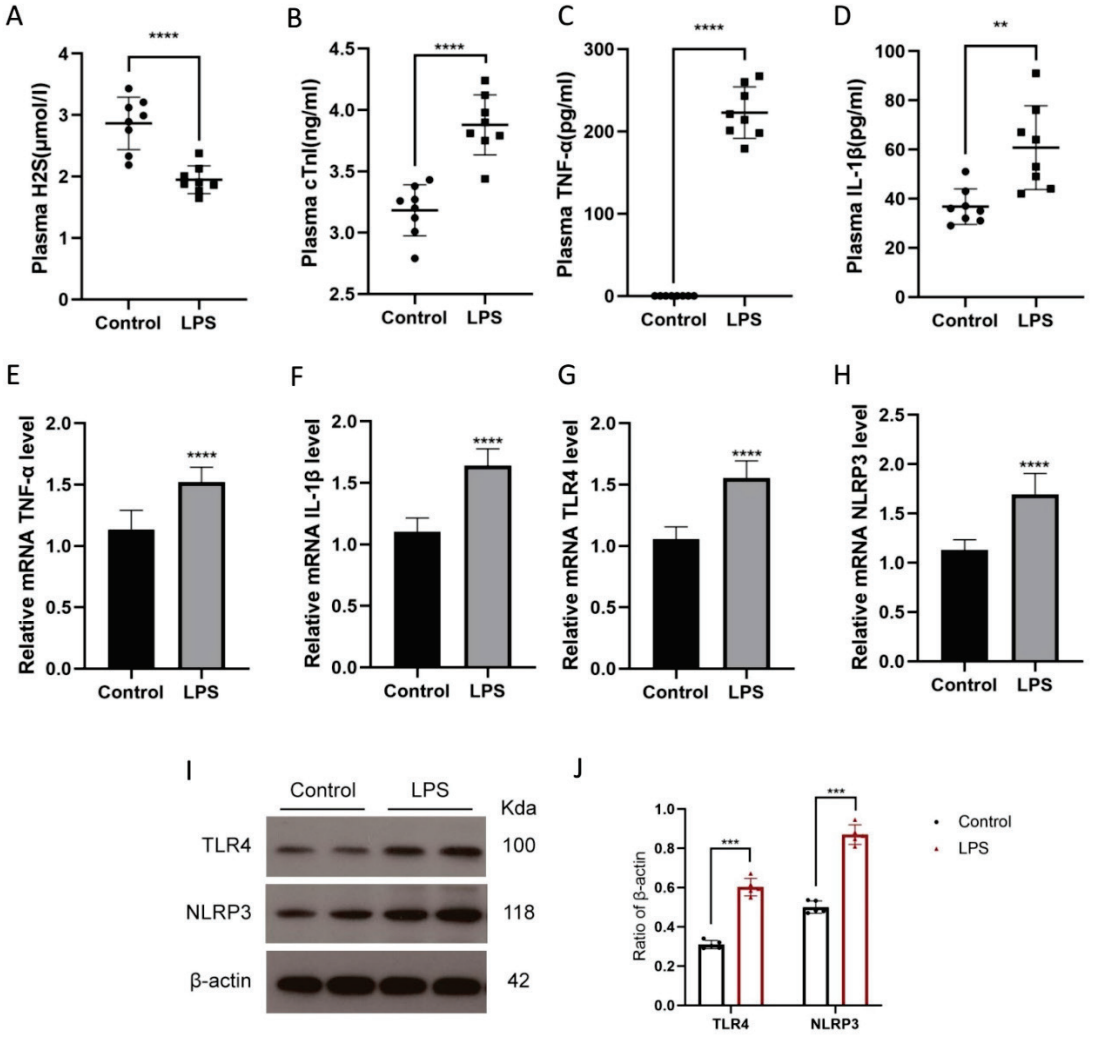

Fig. 1. H_2S levels in plasma decrease and inflammation increases in SIMD model mice. (**A**) H_2S levels in the SIMD mice model plasma. (**B**) cTnI levels in the SIMD mice model plasma. (**C**) TNF-α levels in the SIMD mice model plasma. (**D**) IL-1β levels in the SIMD mice model plasma. (**E-H**) Relative mRNA levels of TNF-α, IL-1β, TLR4 and NLRP3 in heart tissues. (**I-J**) Representative Western blots and quantification of TLR4 and NLRP3 protein expression in heart tissues. β-actin was used as the internal control. n=8 in every mice model group. n=5 in every Western blot group. Results are means ± SEM. ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$. $P<0.05$ was considered significant.

Exogenous H_2S ameliorates myocardial tissue injury

In order to explore the role of H_2S in septic myocardial tissue injury, we obtained myocardial tissue 6 h after LPS injection for detection. The results of mouse echocardiography showed that: NaHS increased cardiac LVEF and markedly improved myocardial dysfunction (Fig. 2A, C) relative to the Control group. These results suggested that NaHS played an important protective role in myocardial tissue injury caused by sepsis. Substantially less myocardial cell damage in the LPS+NaHS group relative to the LPS group was observed by HE staining (Fig. 2B, D). The results of HE staining showed that the damage degree of myocardial tissue in LPS+NaHS group was significantly reduced compared with that in LPS group, which also confirmed the protective effect of H_2S on damaged myocardial tissue. We then examined the secretion of inflammatory factors in mouse plasma. Moreover, NaHS significantly increased plasma H_2S levels and decreased the levels of cTnI, TNF-α, and IL-1β in the plasma (Fig. 2E-H). These results suggested that NaHS inhibited the secretion of inflammatory factors and reduce the injury of inflammatory factors to myocardium. Next, we detected whether NaHS played a role through TLR4 and NLRP3 at the protein level. The levels of protein expressions of TLR4 and NLRP3 in mouse myocardial tissue were also downregulated (Fig. 2I-J). Therefore, we concluded that exogenous H_2S ameliorated myocardial tissue injury through TLR4 and NLRP3 inflammasome.

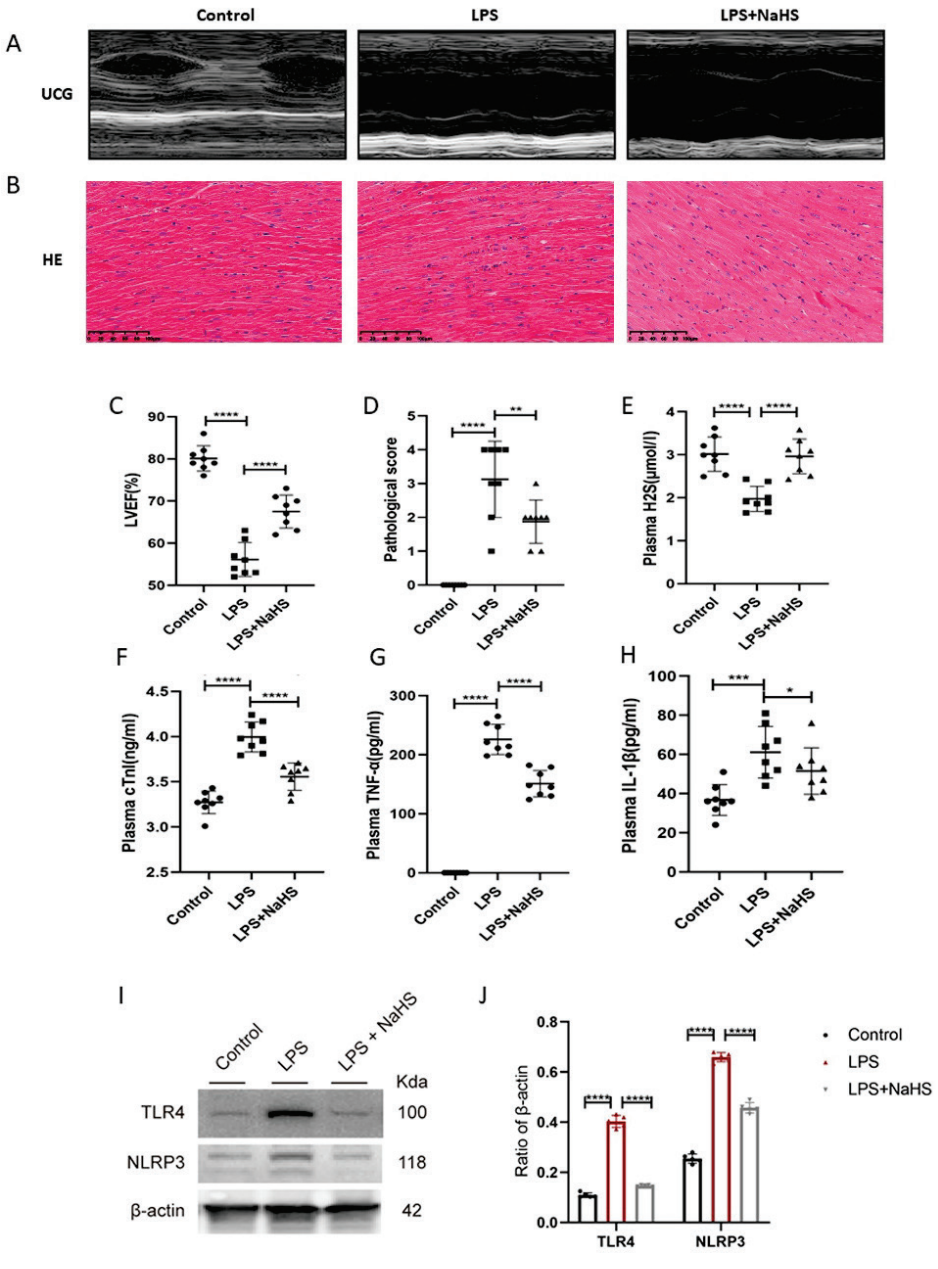

Fig. 2. Exogenous H_2S ameliorates myocardial tissue injury. (**A**) Representative M-mode images from Control, LPS, and LPS+NaHS groups. (**B**) Representative HE-stained left ventricular sections (scale bar = 50 μm). (**C**) The changes of left ventricular ejection fraction (LVEF). (**D**) Pathological score of the HE-stained left ventricular sections. (**E**) H_2S levels in the SIMD mice model plasma. (**F**) cTnI levels in the SIMD mice model plasma. (**G**) TNF-α levels in the SIMD mice model plasma. (**H**) IL-1β levels in the SIMD mice model plasma. (**I-J**) Representative Western blots and quantification of TLR4 and NLRP3 protein expression in heart tissues. β-actin was used as the internal control. n=8 in every mice model group. n=5 in every Western blot group. Results are means ± SEM. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001. P<0.05 was considered significant.

NLRP3 inhibitor, MCC950, attenuates LPS-induced myocardial injury

In order to explore the relationship between NLRP3 signaling pathway and H_2S, mice were divided into LPS LPS+MCC950 and LPS+NaHS+MCC950 groups. As compared to the LPS group, LPS+MCC950 and LPS+NaHS+MCC950, significantly improved myocardial dysfunction and increased LVEF (Fig. 3A, C). Less myocardial cell injury was observed in the LPS+MCC950 and LPS+NaHS+MCC950 groups relative to the LPS group by HE staining (Fig. 3B, D). These results suggested that NLRP3 inhibitor and NaHS had similar therapeutic effects in myocardial sepsis. Meanwhile, we found that the secretion of inflammatory cytokines related to myocardial inflammation, such as cTnI, TNF-α, and IL-1β, decreased upon the application of MCC950 alone and simultaneous application of MCC950+NaHS (Fig. 3E-G). These results indicated that NLRP3 inhibitor and NaHS had similar effects on the secretion of inflammatory factors related to myocardial inflammation and after inhibiting NLRP3 activation, NaHS does not further reduce inflammation.

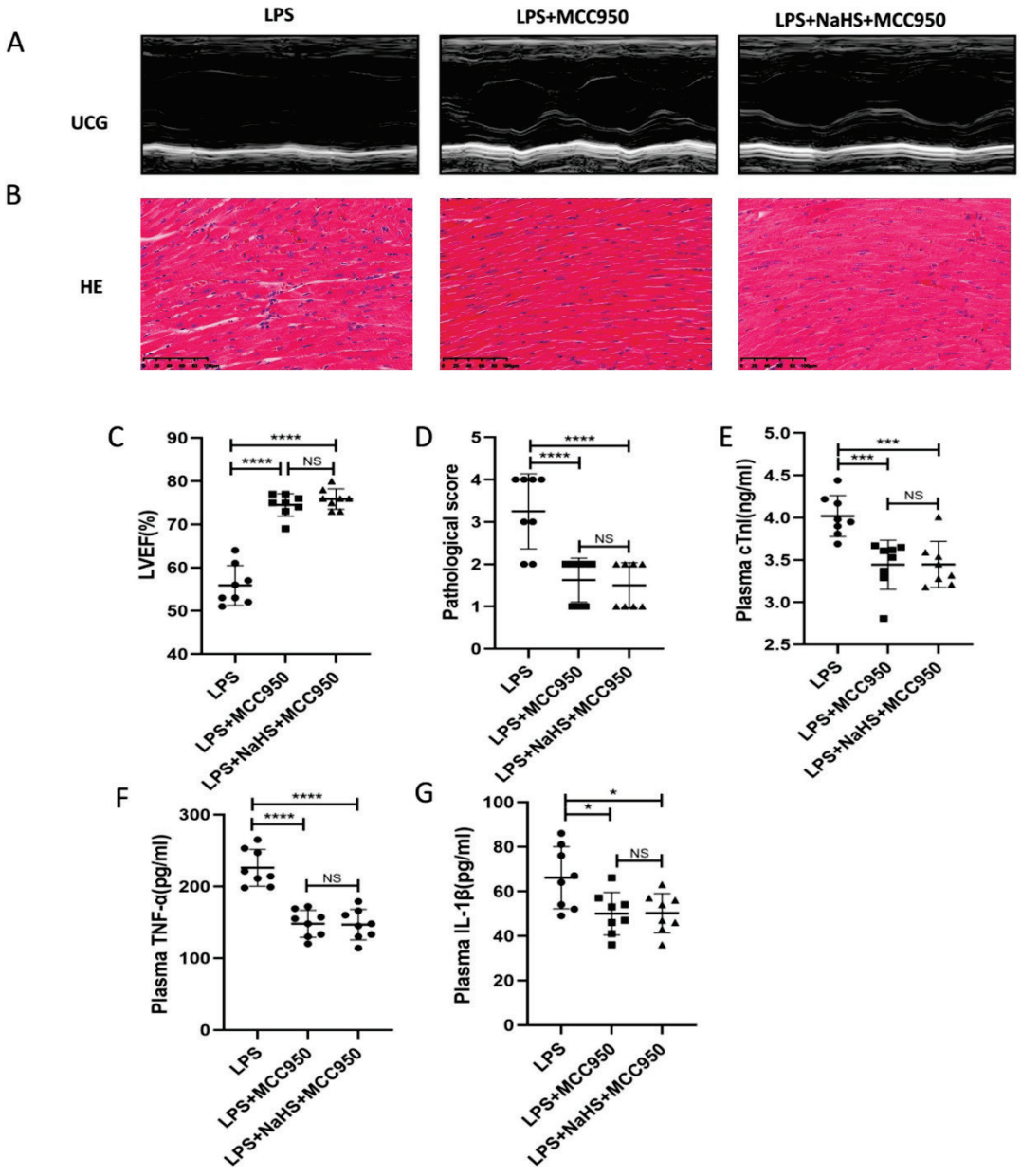

Fig. 3. NLRP3 inhibitor, MCC950, attenuates LPS-induced myocardial injury. (**A**) Representative M-mode images from LPS, LPS+MCC950 and LPS+NaHS+MCC950 groups (**B**) Representative HE-stained left ventricular sections (scale bar = 50 μm). (**C**) The changes of left ventricular ejection fraction (LVEF). (**D**) Pathological score of the HE-stained left ventricular sections. (**E**) cTnI levels in the SIMD mice model plasma. (**F**) TNF-α levels in the SIMD mice model plasma. (**G**) IL-1β levels in the SIMD mice model plasma. n=8 in each group. Results are means ± SEM. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001. P<0.05 was considered significant.

NaHS exerts protective effects mainly through the TLR4-NLRP3 signaling axis

To investigate whether NaHS plays a role in myocardial injury through TLR4 signaling pathway, WT and TLR4$^{-/-}$ mice were divided into four groups: WT Control, WT+LPS, TLR4$^{-/-}$+LPS, and TLR4$^{-/-}$+LPS+NaHS groups. The myocardial dysfunction and cardiac LVEF, both significantly improved upon TLR4$^{-/-}$+LPS and TLR4$^{-/-}$+LPS+NaHS interventions relative to the WT+LPS group (Fig. 4A, C). Meanwhile, we also obtained the corresponding tissues for histological staining, and found that less myocardial cell injury in the TLR4$^{-/-}$+LPS and TLR4$^{-/-}$+LPS+NaHS groups was observed as compared to the WT+LPS group by HE staining (Fig. 4B, D). This strongly suggested that NaHS played a role in myocardial injury through TLR4 signaling pathway. We further observed the effects of TLR4 and NaHS on the secretion of inflammatory factors related to myocardial inflammation. Moreover, both TLR4 knockdown alone and TLR4 knockdown along with NaHS treatment significantly decreased those of cTnI, TNF-α, and IL-1β in the plasma (Fig. 4E-G). These results demonstrated that NaHS exerted protective effects mainly through the TLR4-NLRP3 signaling axis.

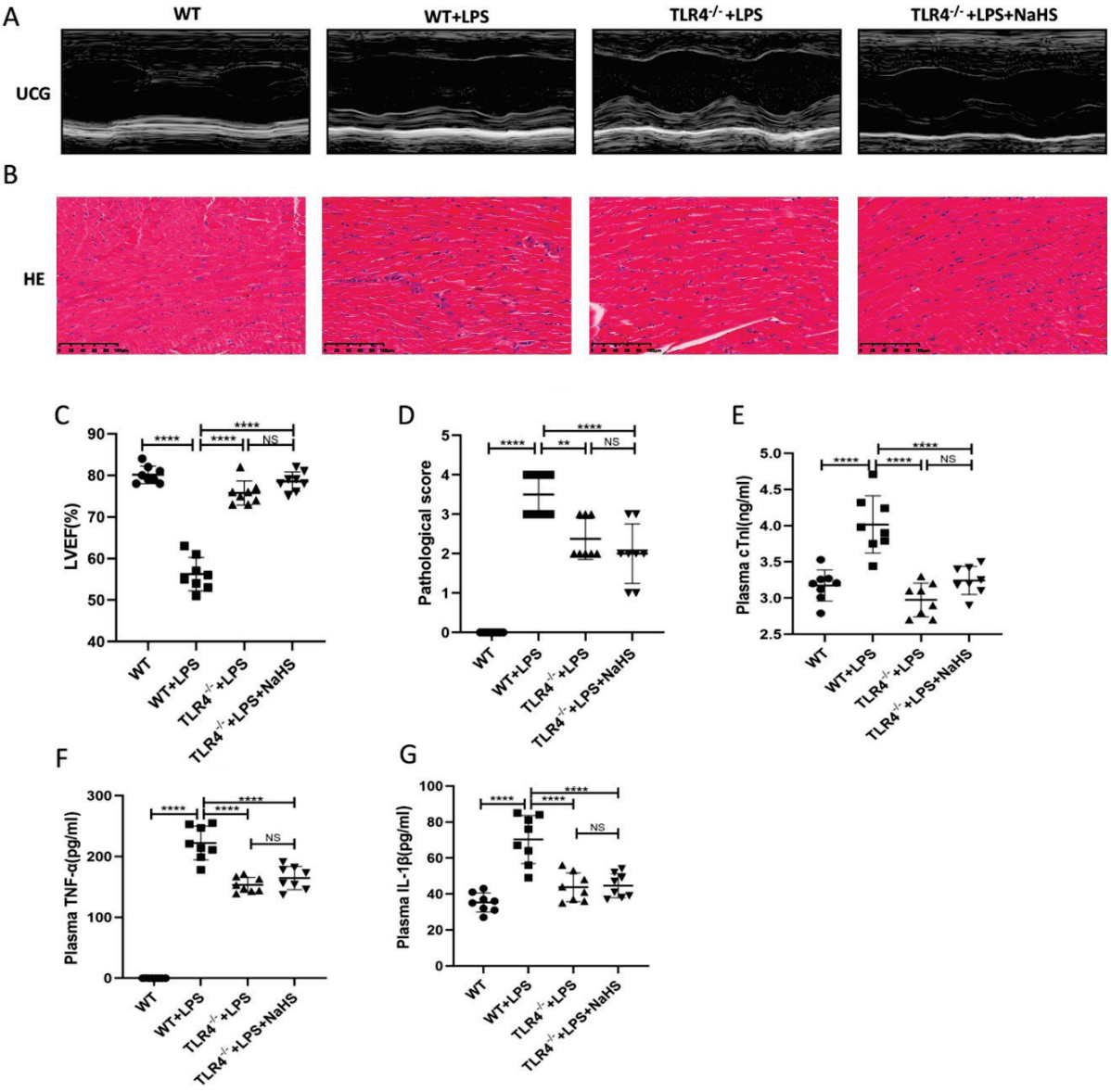

Fig. 4. NaHS exerts protective effects mainly through the TLR4-NLRP3 signaling axis (**A**) Representative M-mode images from WT, WT+LPS, TLR4$^{-/-}$+LPS, TLR4$^{-/-}$+LPS+NaHS groups (**B**) Representative HE-stained left ventricular sections (scale bar = 50 μm). (**C**) The changes of left ventricular ejection fraction (LVEF). (**D**) Pathological score of the HE-stained left ventricular sections. (**E**) cTnI levels in the SIMD mice model plasma. (**F**) TNF-α levels in the SIMD mice model plasma. (**G**) IL-1β levels in the SIMD mice model plasma. n=8 in each group. Results are means ± SEM. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001. P<0.05 was considered significant.

Discussion

Sepsis is a syndrome caused by a pathogenic microbial infection that ultimately leads to multi-organ failure in the body. Severe sepsis and infectious shock are important medical concerns in the treatment of critically ill patients [20]. The heart is the main organ affected by sepsis and the consequences are severe. The degree of myocardial damage is crucial for the prognoses of patients [21]. Myocardial damage in sepsis can manifest itself in different forms of cardiac dysfunction, resulting in decreased ejection fraction and insufficient cardiac output, thereby significantly increasing the risk of death in septic patients [22]. In the current study, we observed significant myocardial injury in the endotoxin, LPS-induced sepsis model, as evidenced by the significant increase in levels of IL-1β, TNF-α, and cTnI in plasma, along with a significant decrease in H_2S concentration in the plasma, as well as severely impaired cardiac function, evidenced by a significant decrease in the LVEF in the LPS injected mice groups. Based on this, the identification of protective drugs that can alleviate myocardial injury in sepsis is of substantial clinical value for improving the survival rate in septic patients.

The mechanism of action underlying myocardial injury in sepsis remains unclear, however, recent studies suggest that inflammatory responses and oxidative stress injury play key functions in its pathogenesis. Pro-inflammatory cytokines, such as IL-1β and TNF-α, trigger the responses by the inflammatory cascades in an autocrine and paracrine manner, which exert a negative effect on the myocardium, leading to myocardial injury in sepsis and eventually cardiac dysfunction [23]. Furthermore, dysregulation of the toll-like receptors, essential for the recognition of microbial products and host defense against infection, also enhance the inflammatory responses of the body by increasing the levels of inflammatory factors in myocardial tissue and serum, thereby enhancing neutrophil migration, which in turn promotes cardiac dysfunction in sepsis [24,25]. Simultaneously, in the state of sepsis, the balance between oxidant and antioxidant statuses of the body is severely hampered and a large number of reactive oxygen species of mitochondrial and non-mitochondrial origins can cause damage to the heart by directly disrupting the membrane structure and organelles of cardiomyocytes, eventually leading to dysfunction or even cellular autolysis [26,27]. Moreover, oxidative stress injury and inflammatory responses positively regulate one another, on the one hand, and trigger apoptosis on the other, thereby impairing the functions of cardiomyocytes. Therefore, evaluating effective anti-oxidative stress and anti-inflammatory therapeutic measures may facilitate the mitigation of myocardial injury in septicemic mice.

As the third gaseous signaling molecule after NO and CO, H_2S exerts a wide range of biological effects, including counteracting myocardial ischemic injury, attenuating oxidative stress damage, inhibiting apoptosis, and attenuating inflammatory responses. Thus, it has huge market potential in therapeutics for several diseases [28]. In our previous study, we found that TLR4 protein and endoplasmic reticulum stress proteins were over expressed in the SIMD mice, while H_2S ameliorated SIMD by suppressing inflammation and endoplasmic reticulum stress *via* inhibition of the TLR pathway [9]. In the present study, exogenous H_2S was shown to protect cardiomyocytes against LPS-induced inflammatory injury, evidenced by the reduced levels of pro-inflammatory cytokines and activation of the NLRP3 pathway. It was reported that H_2S attenuated sepsis-induced cardiac dysfunction *via* PI3K/Akt pathway [29]. In an *in vivo* study, S-Propargyl-cysteine, a sulfur-containing amino acid, attenuated LPS-induced inflammatory response in H9c2 cells involved in a H_2S-dependent mechanism [30]. The above-mentioned results indicated that H_2S was a putative treatment agent for the therapy of myocardial injury due to sepsis. According to recent findings, treatment with NaHS significantly attenuated the stimulation of TLR4 and NF-κB transduction pathways in high-glucose environment-induced myocardial injury, suggesting that the TLR4 and NF-κB signaling pathways may exert a major protective effect on LPS-induced myocardial injury through H_2S [10].

Our findings suggested that NaHS treatment could significantly suppress neutrophil infiltration and production of inflammatory factors, TNF-α and IL-1β, in myocardial tissues from the LPS+NaHS group of mice as compared to the LPS group of mice. This, in turn, attenuated myocardial tissue inflammatory responses, thereby protecting the cardiac functions of mice. Liu et al. found that endogenous H_2S attenuated the LPS-triggered inflammatory response by regulating histone demethylase JMJD3 expression [31]. H_2S also could inhibited neutrophil transmigration and reduced IL-1β release into the bronchoalveolar lavage fluid in LPS-induced acute lung injury [32]. In our study, NaHS treatment

significantly reduced the protein level expressions of myocardial TLR4 and NLRP3 after LPS injection. The stimulation of IL-1β and IL-18, along with cell necrosis, was significantly attenuated due to the inhibition of the NLRP3 protein expression [33]. LPS-induced over-activation of the NLRP3 inflammasomal pathway, along with cardiac dysfunction were observed *in vivo* [34]. NLRP3 inflammasome has been identified in several cells, including cardiomyocytes. NLRP3 reportedly exists in an inactivated state in the cytoplasm. Once released, its subsequent oligomerization leads to the recruitment of pro-caspase-1, which then promotes self-cleavage and activation. The eventual expressions of IL-1β and IL-18 are through activated caspase-1, an enhancer of multiple inflammatory responses. Moreover, in TLR4 KO mice the myocardial injury caused by LPS was ameliorated significantly. Based on the above observations, we reasonably hypothesized that the protective effects of NaHS on LPS-induced myocardial injury were mainly through the attenuation of the TLR4-NLRP3 signaling pathway, however, the specific mechanism needs further investigation. Actually, as a potent vasoactive agent, H_2S has both vasorelaxing and vasoconstricting effects on the cardiovascular system [35] and its regulatory effect on blood pressure during sepsis cannot be ignored. However, the vasoactive effects of H_2S in sepsis remain controversial. It was reported that during sepsis higher plasma H_2S levels inversely correlated with blood pressure [36], while, administration of GYY4137, a slow-releasing H_2S donor to anesthetized rats after LPS treatment decreased the slowly developing hypotension [37]. Up to now, the knowledge about vasoactive role of H_2S in sepsis is still fragmental, which needs to be further elucidated in subsequent studies.

In conclusion, exogenous H_2S exerts protective effects on LPS-induced inflammation and injury in cardiomyocytes by attenuating the activation of the TLR4-NLRP3 signaling axis. Therefore, H_2S is a potential therapeutic agent for the prevention of myocardial injury due to sepsis.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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