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Original Article

Hydrogen sulfide preconditioning protects against myocardial ischemia/reperfusion injury in rats through inhibition of endo/sarcoplasmic reticulum stress

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Abstract: Ischemia reperfusion (I/R) injury is a major cause of myocardial damage. Hydrogen sulfide (H_2S), a gaseous signal molecule, has drawn considerable attention for its role in various pathophysiological processes. Multiple lines of evidence reveal the protective effects of H_2S in various models of cardiac injury, however, the exact mechanism underlying this protective effect of H_2S against myocardial I/R injury is not fully understood. The present study was designed to investigate whether H_2S preconditioning attenuates myocardial I/R injury in rats and whether the observed protection is associated with reduced endo/sarcoplasmic reticulum (ER/SR) stress. We found that H_2S preconditioning significantly reduced myocardial infarct size, preserved left ventricular function, and inhibited I/R-induced cardiomyocyte apoptosis *in vivo*. Furthermore, H_2S preconditioning significantly attenuated I/R-induced ER/SR stress responses, including the increased expression of glucose-regulated protein 78, C/EBP homologous protein, and activate transcription factor in myocardium. Additionally, we demonstrate that H_2S preconditioning attenuates ER/SR stress and inhibits cardiomyocyte apoptosis in an *in vitro* model of hypoxia/reoxygenation in rat H9c2 cardiac myocytes. In conclusion, these results suggest that H_2S-attenuated ER/SR stress plays an important role in its protective effects against I/R-induced myocardial injury.

Keywords: Ischemia/reperfusion, hydrogen sulfide, endo/sarcoplasmic reticulum stress, myocardial protection

Introduction

Myocardial ischemia reperfusion (I/R) injury is the most important cause of cardiac damage. This process is mainly mediated by oxidative stress, calcium dysregulation, and inflammatory cell infiltration in infarcted myocardium [1]. The endo/sarcoplasmic reticulum (ER/SR) is responsible for synthesizing, modifying and folding of proteins, and senses oxidative stress. During ER/SR stress, unfolded proteins accumulate and aggregate during the pathological imbalance in ER/SR homeostasis, which is induced by perturbation of calcium homeostasis, glucose deprivation, and ischemia [2]. When ER/SR stress is intense or persistent, C/EBP homologous protein (CHOP), caspase-12, and JNK are activated, and ER/SR stress-induced apoptosis can be initiated [3]. ER/SR stress has been shown to play an important role in a broad spectrum of pathological conditions [3, 4]. Recently, an increasing body of evidence has demonstrated that ER/SR stress was involved in myocardial I/R injury [5-8].

In order to reduce myocardial I/R injury, therapeutic strategies such as pre- and postconditioning, as well as pharmacological interventions have been intensively investigated [9-13]. Hydrogen sulfide (H_2S) has become a molecule of great interest in recent years, and it is now recognized as the third endogenously produced gaseous messenger along with nitric oxide and carbon monoxide. Either endogenous or exogenous H_2S plays a prominent role in modulating many physiological processes [14]. Several studies have explored the beneficial effects of H_2S donors such as NaHS and Na_2S in myocardial I/R injury and other models of cardiac injury [14-19]. In addition, a more recent report has demonstrated that gaseous administration of H_2S also appears to be an effective way to attenuate the outcome of myocardial I/R injury [20]. However, the mechanisms underlying the

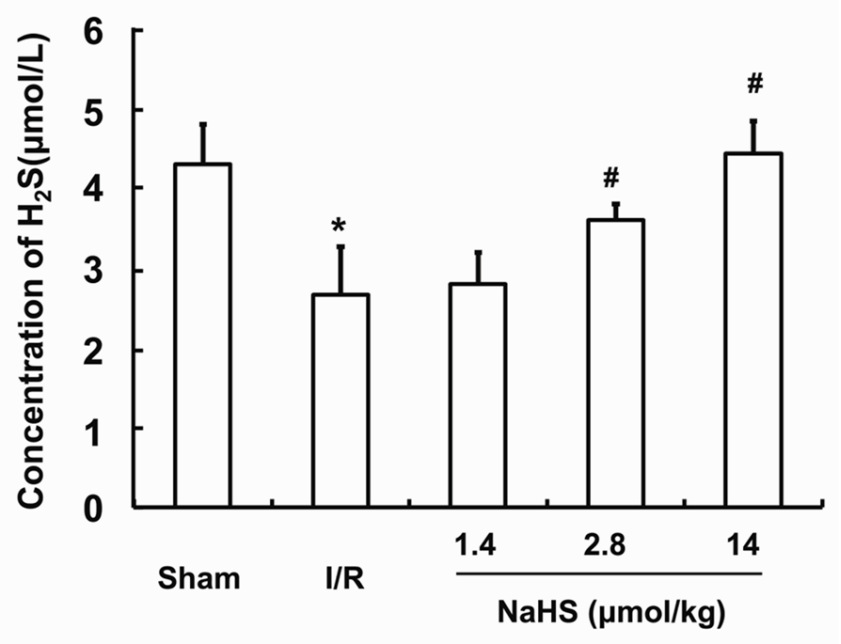

Figure 1. H_2S concentration in the plasma in rats. Rats were subjected to 30 min of left ventricle ischemia and reperfusion for 120 min, with or without different concentrations of NaHS preconditioning (1.4, 2.8 and 14 μmol/kg). The concentration of H_2S was measured from rat plasma as described in Materials and Methods. Data are presented as the mean ± SEM. $^*P < 0.05$, compared with sham-operated mice (n = 10). $^\#P < 0.05$, compared with I/R group (n = 8 per group).

protective effect of H_2S against myocardial I/R injury are incompletely understood.

Recently, H_2S was shown to protect rat H9c2 cardiac myocytes against doxorubicin-induced cardiotoxicity [21] and attenuates hyperhomocysteinemia-induced cardiomyocyte injury [22] through inhibition of ER/SR stress. In addition, Li et al reported that H_2S exerts its protection against the neurotoxicity of formaldehyde by overcoming ER/SR stress in PC12 cells [23]. These studies suggest an emerging picture of the importance of H_2S in regulating ER/SR stress. In this light, we hypothesized that H_2S may protect against myocardial I/R injury by attenuating excessive ER/SR stress. In the present study, we establish that H_2S preconditioning decreases infarct size, preserves left ventricular function, and reduces I/R-induced cardiomyocyte apoptosis in an *in vivo* model of myocardial I/R injury in rats. The observed protection is associated with reduced I/R-induced ER/SR stress responses, including the increased expression of glucose-regulated protein 78 (GRP78), C/EBP homologous protein (CHOP), and activate transcription factor (ATF-6). Additionally, we further demonstrate the cytoprotective effects of H_2S *in vitro* cell culture experiments in rat H9c2 cardiac myocytes exposed to hypoxia and reoxygenation (H/R). Our results suggest that H_2S plays an important role in myocardial cytoprotection during the evolution of myocardial infarction, and that H_2S administration may be of clinical importance in ischemic disorders.

Materials and methods

Materials

Sodium hydrosulfide (NaHS) was from Sigma-Aldrich (St Louis, MO, USA). GRP78, ATF6, CHOP and PDI antibodies were from Santa Cruz Biotechnology (CA, USA). Enhanced chemiluminescence (ECL) kit was from Amersham Biosciences (Arlington Heights, IL). DMEM and fetal bovine serum (FBS) were from Gibico BRL (Calsbad, CA, USA). TRIzol reagent was from Invitrogen (Carlsbad, CA).

Cell culture and H/R injury

Rat H9c2 cardiac myocytes (Wuhan University Center for Animal Experiment, Wuhan, China) were cultured in DMEM supplemented with 10% FBS. Cells on culture plates were placed into the hypoxia chamber for 3 h to induce hypoxia, and then re-oxygenated with maintenance medium for 24 h to induce reoxygenation.

Myocardial ischemia-reperfusion and H_2S treatment

Male adult Sprague-Dawley rats weighing 200-250 g were from Wuhan University Center for Animal Experiment. Surgical procedures used in the I/R were similar to methods described previously [20]. In brief, myocardial I/R injury was performed by temporary ligation of the left anterior descending coronary artery for 30 minutes through an incision in the fourth intercostal space under anesthesia. The heart was inspected for restoration of blood flow after

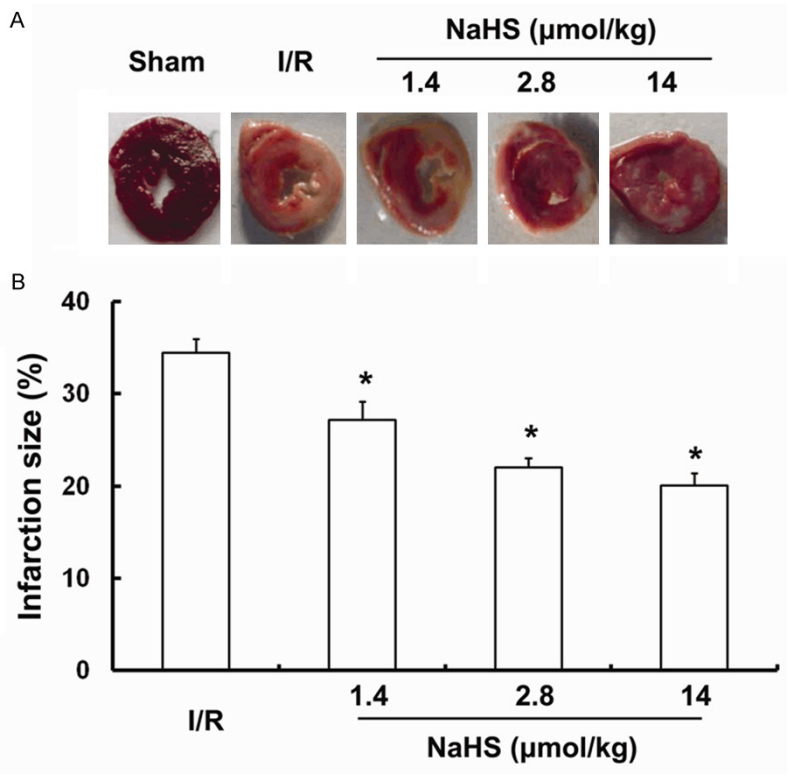

Figure 2. Effect of H_2S on myocardial infarct size in rats. A. The representative photographs of TTC stained sections. TTC-stained sections were obtained from rat hearts subjected to 30 min of ischemia followed by 120 min of reperfusion, with or without different concentrations of NaHS preconditioning (1.4, 2.8 and 14 μmol/kg). B. Myocardial infarct size was expressed as a percentage of total left ventricle volume. Data are presented as the mean ± SEM. $^*P < 0.05$, compared with I/R-operated rats (n = 8).

removing the ligature. Sham operated rats underwent the same procedure, except the placement of the ligature. The rats received one time of NaHS (exogenous H_2S donor, Sigma, St. Louis, MO) treatment within five seconds, at 1.4, 2.8, and 14 μmol/kg body weight (i.v.), respectively, starting 10 minutes prior to ischemia (n = 10 per group). All animal work were in agreement with institutional and legislator regulations and approved by the Committee on the Ethics of Animal Experiments of Wuhan University.

Myocardial infarct size determination

The infarct size was determined by 1% 2, 3, 5-triphenyltetrazolium chloride (TTC) staining as described previously [24]. In brief, at the end of reperfusion, the hearts were rapidly excised from the thorax and washed by 4°C physiological saline. The left ventricle (LV) was separated from the heart and was weighed, and then frozen for 3 h at -20°C. Then the LV was cut into 5 transverse slices (1-2 mm) and the slices were incubated in 1% TTC (pH 7.4) at 37°C for 10 min. The viable myocardium tissue was stained red while the infracted myocardium remained pale. The pale necrotic myocardial tissue was separated from the stained portions and weighed. The size of the myocardial infarction was determined by the following equation: $(Wt_{-Inf1} + Wt_{-Inf2} + Wt_{-Inf3} + Wt_{-Inf4} + Wt_{-Inf5})/(Wt_{-LV1} + Wt_{-LV2} + Wt_{-LV3} + Wt_{-LV4} + Wt_{-LV5}) \times 100\%$, where Wt_{-Inf} is the weight of the infracted myocardium from subscripted numbers 1-5 representing sections and Wt_{-LV} is the weight of LV from the same numbered sections.

Cardiac functional parameters

One catheter was inserted into the left cardiac ventricle via right carotid artery. Left ventricular systolic pressure (LVSP) and maximal rate of increase and decline in left ventricular pressure ($LV \pm dp/dt_{max}$) were monitored continuously during the protocol of IRI with BL-420 multichannels physiologic signal analysis system (Taimeng Technology Company, Chengdu, China).

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining for assessment of apoptosis

LV tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned. Apoptotic cells were identified by TUNEL using an apoptosis detection kit (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's protocol. TUNEL-positive myocytes and total myocytes were counted using Leica Qwin plus V3 software.

Measurement of H_2S concentration in plasma

H_2S concentration in the plasma was determined by the method described previously [25]. In brief, 0.5 mL of 1% zinc acetate and 2.5 mL

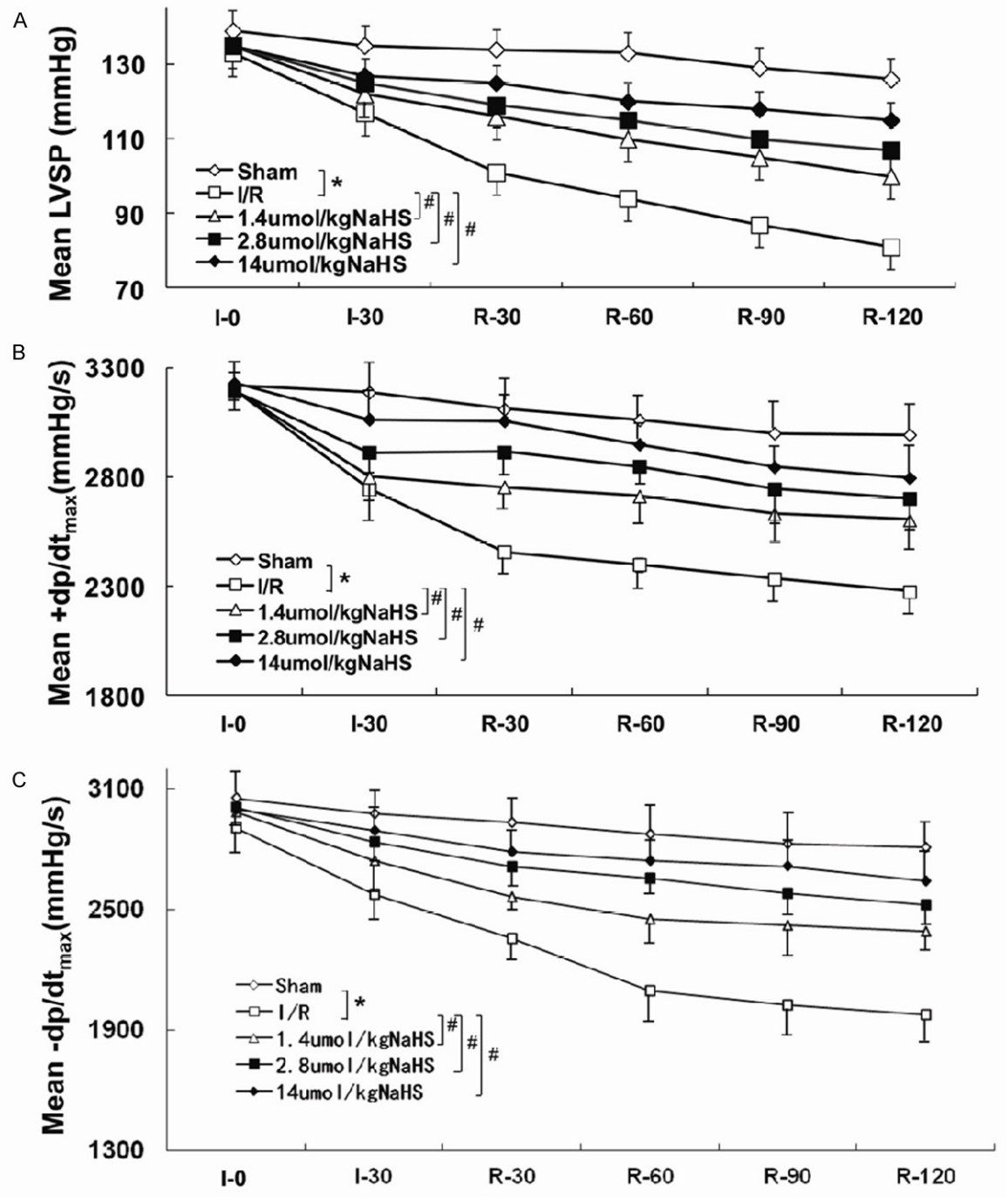

Figure 3. Effect of H_2S on left ventricle function after myocardial I/R injury in rats. Rats were subjected to 30 min of ischemia and reperfusion for 30, 60, 90 and 120 min, respectively, with or without different concentrations of NaHS preconditioning (1.4, 2.8 and 14 μmol/kg). A. LVSP. B. $+dp/dt_{max}$. C. $-dp/dt_{max}$. Data are presented as the mean ± SEM. $^{*}P < 0.05$, compared with sham-operated rats (n = 10). $^{\#}P < 0.05$, compared with I/R group (n = 8).

of distilled water were mixed with 0.1 mL of plasma. Then 0.5 mL of 20 mmol/L *N*, *N*-dimethyl-*p*phenylenediamine dihydrochloride in 7.2 mol/L HCl and 0.4 mL of 30 mmol/L $FeCl_3$ in 1.2 mol/L HCl were applied for 20 min at room temperature. After adding 1 mL of 10% trichloroacetic acid, the protein in the plasma was removed by centrifugation. The optical absorbance at 670 nm was measured with a spectrophotometer.

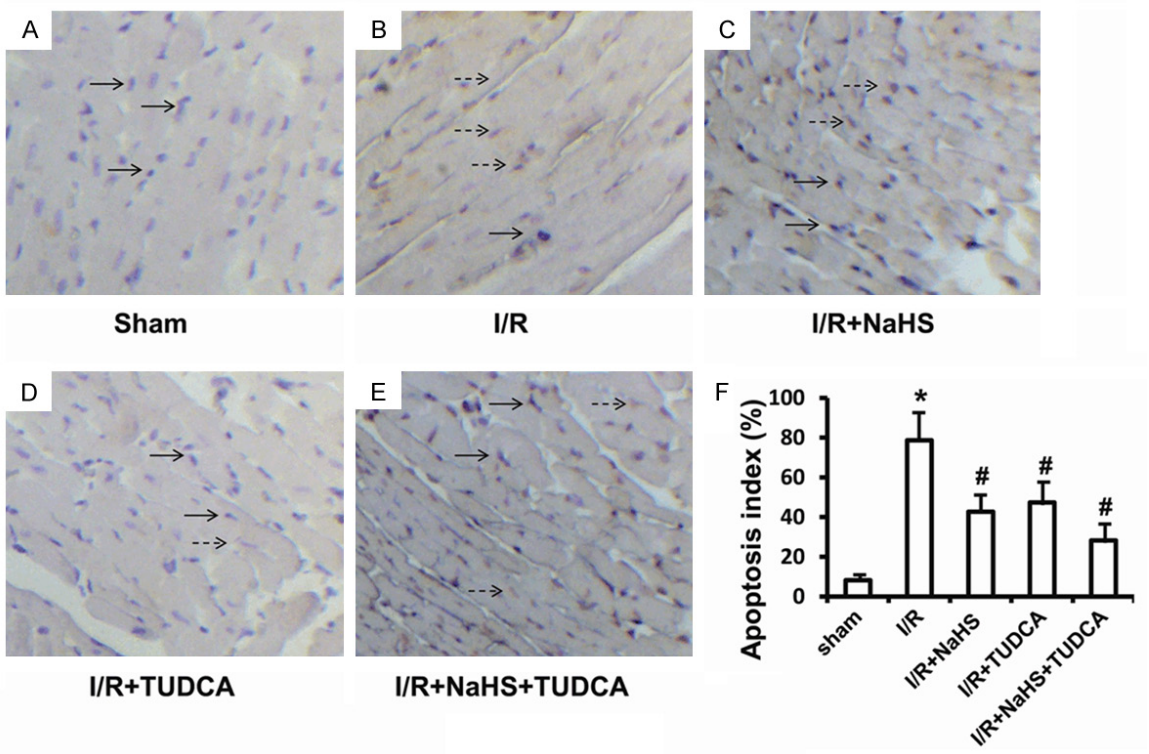

Figure 4. Effect of H_2S on I/R-induced myocardial apoptosis in rats. A-E. The representative images of TUNEL staining (original magnification, ×400). Tissue sections were obtained from rat hearts subjected to 30 min of ischemia followed by 120 min of reperfusion, with or without NaHS (14 μmol/kg) and/or TUDCA (an ER stress inhibitor, 25 mg/kg) pretreatment. The nuclei of TUNEL-positive cardiomyocytes were stained brown-yellow (dashed line arrow) and those of normal cells were stained blue (solid arrow). F. TUNEL-positive cells were counted in six random fields in the border of infarcted area of myocardium and expressed as percentage of the total number of nuclei. $^{*}P < 0.05$, compared with sham-operated rats (n = 10). $^{\#}P < 0.05$, compared with I/R group (n = 8).

Western blot analysis

Western blot analysis of GRP78, CHOP and ATF6 were performed with 10 μg of protein extract, obtained as described previously [26], using rat monoclonal antibodies (1:1000 dilution; Santa Cruz Biotechnology, CA) and peroxidase-conjugated rabbit-anti-rat IgG antibody (1:2000 dilution; Santa Cruz Biotechnology) as a secondary antibody. The signals were normalized to the glyceraldehyde-3-phosphate dehydrogenase signals (rabbit monoclonal antibodies, 1:1,000; Sigma, St. Louis, MO).

Flow cytometry analysis for identification and quantification of cell death

Identification and quantification of cell death in H9c2 cells were determined as described previously [26]. In brief, treated H9c2 cells were digested with trypsin, and washed twice with PBS, and then stained for 30 min with 0.5 mL staining solution consisting of PI (50 mg/mL, Molecular Probes, Eugene, OR), RNase A (10 mg/mL), and 0.1% Triton X-100 for a total count of cell death. The stained cells were subjected to flow cytometric analysis with a FACSCalibur (Becton Dickinson, San Jose, CA). Cell death was quantified as percentage of the sub-G1 peak, an indicator of cell death, in a total of 10^4 collected counts.

Statistical analysis

The results are expressed as mean ± SEM. Statistical significance was determined by two-way ANOVA. Dunns post-hoc analysis was applied where multiple comparisons were made. $P < 0.05$ was considered statistically significant.

Results

H_2S preconditioning decreased myocardial infarct size and preserves lv function

Rats were subjected to 30 min of LV ischemia and reperfusion for 30, 60, 90 and 120 min, respectively. We found that there were no elec-

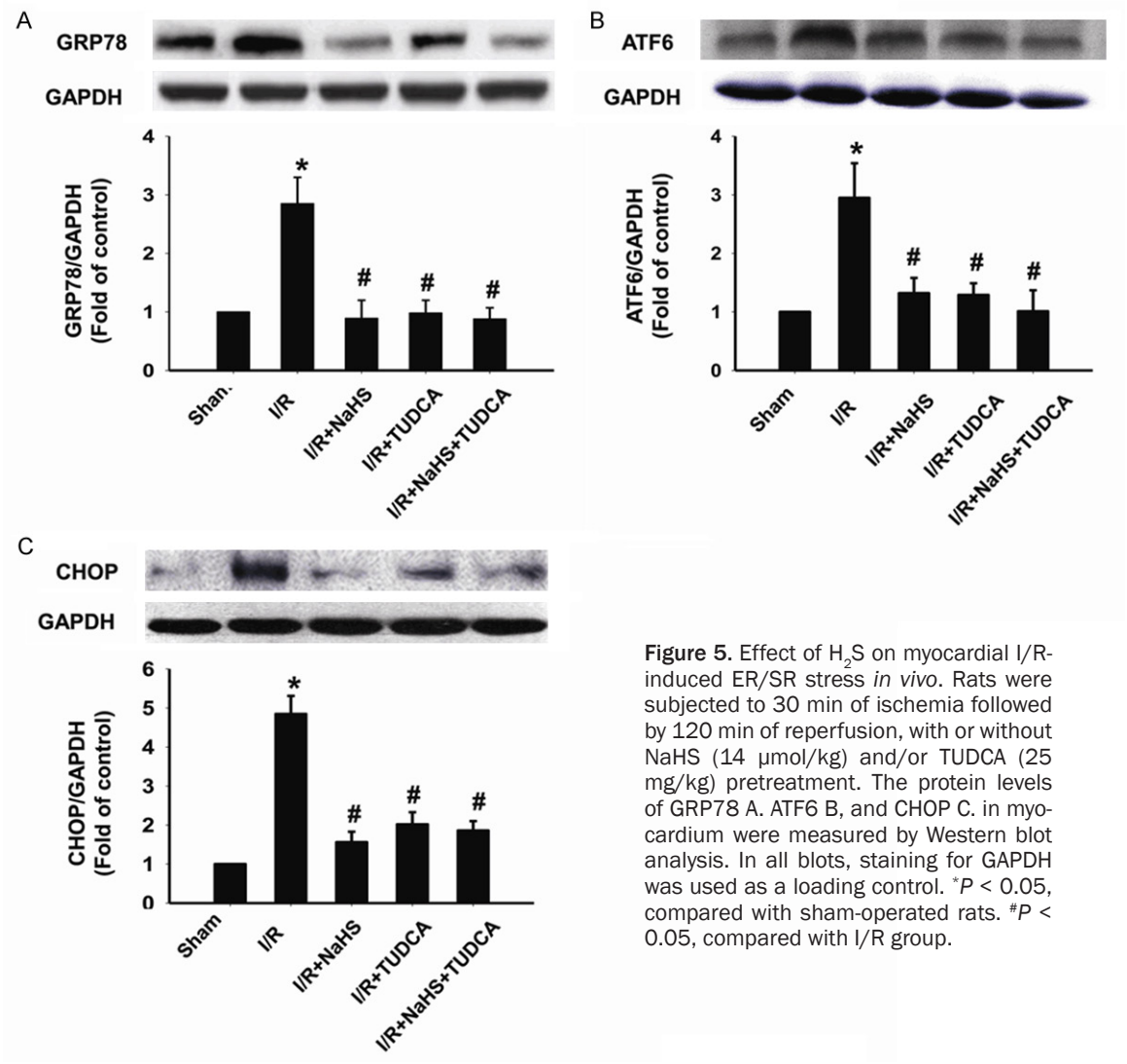

Figure 5. Effect of H_2S on myocardial I/R-induced ER/SR stress *in vivo*. Rats were subjected to 30 min of ischemia followed by 120 min of reperfusion, with or without NaHS (14 μmol/kg) and/or TUDCA (25 mg/kg) pretreatment. The protein levels of GRP78 A. ATF6 B, and CHOP C. in myocardium were measured by Western blot analysis. In all blots, staining for GAPDH was used as a loading control. $^{*}P < 0.05$, compared with sham-operated rats. $^{\#}P < 0.05$, compared with I/R group.

trocardiogram (ECG) changes in the sham group, while I/R injury caused marked elevation in ST-segment and T wave (data not shown). In addition, at the end of reperfusion for the indicated periods, the myocardial infarct size was 34%, 30%, 22% and 10%, respectively. These results showed that our *in vivo* model of myocardial I/R in rats were successful. To observe whether endogenous H_2S is involved in the myocardial I/R injury, H_2S concentration in the plasma was measured at the end of reperfusion. As shown in **Figure 1**, H_2S concentration was significantly decreased after myocardial I/R injury compared with that in the sham group.

To analyze the effect of H_2S on protection against myocardial I/R injury, the rats were pretreated with NaHS (an H_2S donor) at 1.4, 2.8, and 14 μmol/kg body weight, respectively, starting 10 minutes prior to ischemia. As expected, circulation plasma levels of H_2S were significantly elevated following NaHS administration (**Figure 1**). Evaluation of myocardial infarct size revealed a cardioprotection with NaHS pretreatment, as assessed by TTC staining (**Figure 2**).

Additionally, the effect of H_2S on LV function after myocardial I/R injury was determined. Myocardial functional parameters, such as left ventricular systolic pressure (LVSP) and the rate of pressure development ($\pm dp/dt_{max}$) were measured at 30 min during ischemia, and 30, 60, 90 and 120 min during reperfusion, respectively. As shown in **Figure 3**, myocardial I/R

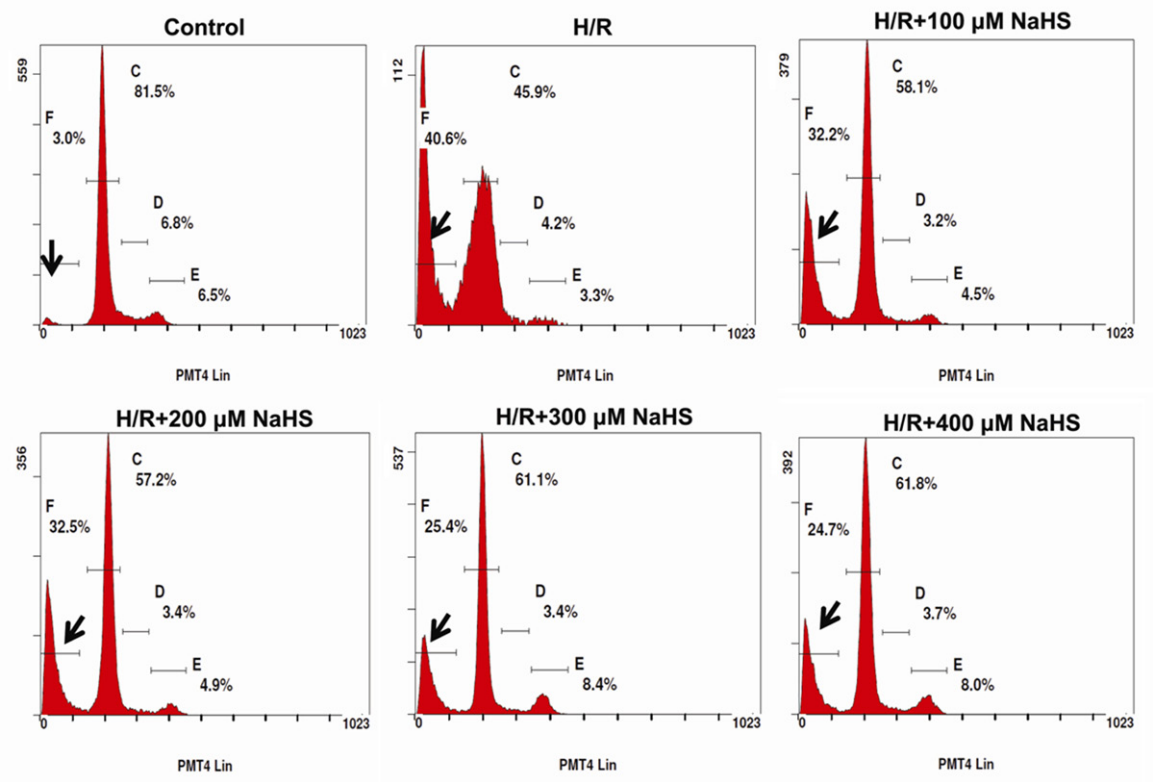

Figure 6. Effect of H_2S on H/R-induced cell death in rat H9c2 cardiac myocytes. H9c2 cells were exposed to hypoxia/reoxygenation (H/R) with or without different concentrations of NaHS pretreatment (100, 200, 300 and 400 μM). Representative histograms of flow cytometric analysis by propidium iodide (PI) staining were from 3 independent experiments. The peak of sub-G1 fraction (arrow) is an indicator of the total number of dead cells.

caused marked decreases in LVSP, $+dp/dt_{max}$ and $-dp/dt_{max}$, compared with those in the sham group. However, NaHS pretreatment significantly improved the cardiac contractile function. In addition, there were no significant differences in the above-mentioned parameters between NaHS-treated and vehicle-treated mice in the absence of I/R (data not shown).

Taken together, these results indicated that H_2S preconditioning exerts a protective effect against myocardial I/R injury in rats.

H_2S preconditioning reduces I/R-induced cardiomyocyte apoptosis in vivo

To further explore whether H_2S has any effect on myocardial I/R-induced apoptosis in rats, we performed TUNEL assay. The number of TUNEL-positive cells in six random fields on the border of the infarcted area per left ventricle was counted and the apoptotic index expressed as a percentage of total cells counted (**Figure 4**). TUNEL staining showed that I/R increased cardiomyocyte apoptosis; however, NaHS pretreatment significantly reduced I/R-induced apoptosis. In addition, we used an ER/SR stress inhibitor, tauroursodeoxycholic acid (TUDCA) to test whether ER/SR stress is involved in myocardial I/R-induced apoptosis. As shown in **Figure 4**, TUDCA pretreatment also reduced cardiomyocyte apoptosis induced by I/R. Importantly, the combination of NaHS with TUDCA enhanced the protective effect against myocardial I/R-induced apoptosis.

H_2S attenuates myocardial I/R-induced ER/SR stress in rats

Next, we investigated the mechanism underlying the protective effect of H_2S against myocardial I/R injury. We first sought to explore whether myocardial I/R induced ER/SR stress in our model by measuring the expression of protein markers of ER/SR stress such as GRP78, CHOP and ATF6 in myocardium using Western blot analysis. As illustrated in **Figure 5**, after myocardial I/R, the expression of GRP78, CHOP,

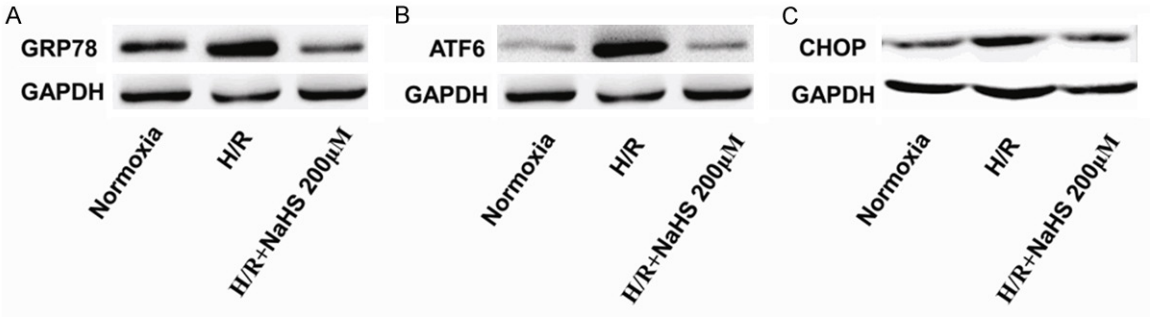

Figure 7. Effect of H_2S on H/R-induced ER/SR stress in H9c2 cells. H9c2 cells were exposed to H/R with or without NaHS pretreatment. The levels of GRP78 A. ATF6 B. and CHOP C. were measured by Western blot analysis. In all blots, staining for GAPDH was used as a loading control.

and ATF6 were significantly increased. In keeping with the previous studies [5, 6], our data indicate that ER/SR stress is involved in myocardial I/R injury in rats. Next, we tested the effect of H_2S on myocardial I/R-induced ER/SR stress. As shown in **Figure 5**, pretreatment with NaHS significantly attenuated the increases in the expression levels of GRP78, CHOP and ATF6 in rats induced by myocardial I/R.

Furthermore, we used TUDCA to confirm that the observed protection is associated with reduced I/R-induced ER/SR stress responses. As shown in **Figure 5**, pretreatment with TUDCA mimicked the above protective effect of H_2S through attenuating ER/SR stress. Taken together, these results suggest that H_2S preconditioning protects against myocardial I/R injury in rats by attenuating excessive ER/SR stress.

H_2S preconditioning inhibits H/R-induced apoptosis and attenuates ER/SR stress in h9c2 cells

Since ER/SR stress-induced myocardial apoptosis is known to play a significant role in the pathogenesis of myocardial I/R injury [7, 8], we analyzed the cytoprotective effect of H_2S on H/R-induced apoptosis in rat H9c2 cardiac myocytes *in vitro*. As shown in **Figure 6**, NaHS pretreatment markedly reduced H/R-induced H9c2 cell apoptosis, as determined by flow cytometric analysis.

To further demonstrate that ER/SR stress was involved in the cytoprotective effect of H_2S on H/R-induced apoptosis, we examined the expression of ER/SR stress-associated protein markers, including GRP78, ATF6 and CHOP *in vitro*. As expected, H/R induced a significant increase of GRP78, ATF6 and CHOP expression in H9c2 cells, and NaHS pretreatment inhibited their expression (**Figure 7**). CHOP is a critical pro-apoptotic factor in ER/SR stress-associated apoptosis [27, 28]. Therefore, these results suggest that suppression of ER/SR stress may contribute to the cytoprotection of H_2S against H/R-induced apoptosis in H9c2 Cells.

Discussion

Growing evidence suggests that endogenous H_2S, as a gas signal molecule, might be an important cardiovascular modulator and thus plays an important role in pathophysiological regulation of cardiovascular diseases [14-19]. However, the exact mechanism underlying this protective effect of H_2S is not fully understood. The present study was designed to investigate whether H_2S preconditioning attenuates myocardial I/R injury in rats and whether the observed protection is associated with reduced ER/SR stress. The main findings of the present work are the following: i) H_2S preconditioning significantly reduces myocardial infarct size, preserves LV function, and reduces I/R-induced cardiomyocyte apoptosis *in vivo*; ii) H_2S attenuates myocardial I/R-induced ER/SR stress; iii) H_2S attenuates ER/SR stress and inhibits H/R-induced apoptosis in rat H9c2 cardiac myocytes. These results implicate excessive ER/SR stress inhibition in the protective effect of H_2S against myocardial I/R injury.

It has been shown that excessive ER/SR stress is involved in myocardial I/R injury [5-8]. Therefore, regulation of ER/SR stress plays a

crucial role in the protective effects of H_2S against myocardial I/R injury. H_2S is increasingly being recognized as an important gaseous messenger in the cardiovascular and nervous systems. Evidence is accumulating that therapeutic H_2S donor compounds exert protective effects in various animal models of inflammation, I/R injury and circulatory shock [29]. Indeed, in our *in vivo* model of myocardial I/R injury in rats, we found that H_2S concentration in plasma was significantly decreased after I/R injury, suggesting that endogenous H_2S is associated with the myocardial I/R injury. In agreement with the previous studies [17], our findings further support the notion that H_2S is able to exert an effective protection against the injury of hearts subjected to I/R. Elrod and his colleagues reported that H_2S limited the extent of myocardial infarction in mice and that the protection was associated with a preservation of mitochondrial function [17]. However, in the present study, we demonstrated that pretreatment of NaHS attenuates the increased expressions of GRP78, CHOP and ATF6 in rats induced by myocardial I/R. It is well documented that ER/SR chaperon GRP78, a critical regulator of ER/SR homeostasis, is generally used as a biomarker of the presence of ER/SR stress [5, 7, 23]. CHOP, ubiquitously expressed at very low levels in normal cells, is an important mediator for ER/SR stress-induced apoptosis. Induction of CHOP is responsive to ER/SR stress [27]. ATF6, a 670 amino acid ER/SR-transmenbrane protein, is shown to activated in cardiac myocytes by ER/SR stress and is recognized as an important mediator of ER/SR stress [30]. Therefore, our results demonstrate that H_2S is able to downregulate the elevated ER/SR stress induced by myocardial I/R. This is in agreement with recent studies that have demonstrated that H_2S-suppressed excessive ER/SR stress contributes to its protective effect on doxorubicin-induced cardiotoxicity [21], hyperhomocysteinemia-induced cardiomyocytic injury [22], 6-hydroxydopamine-induced neurotoxicity [31], and formaldehyde-induced neurotoxicity [23]. These previous reports and our findings have shown that either exogenous or endogenous increases in H_2S exert protective effect in various models of cardiac injury or neurotoxicity through inhibition of ER/SR stress.

It should be noted that, in the heart, ER/SR is an important organelle specializing in the regulation of Ca^{2+} fluxes in addition to protein synthesis functions. Multiple lines of evidence have demonstrated that disruption of the Ca^{2+} homeostasis in the ER/SR is a potent trigger of ER/SR stress, and that ER/SR stress sensor, BiP/GRP78, plays an important role as a Ca^{2+} buffer in the lumen of ER/SR [32]. In addition, recent studies have indicated that H_2S and nitric oxide (NO), these two gaseous molecules may have overlapping pathophysiological functions [33]. Moreover, numerous reports suggest that H_2S-NO cross-talk mediates their effects on several organ systems, including cardiovascular, immune and neurological tissues [33-36]. These findings led us to put forward the possibility that NO was also involved in H_2S-mediated cardioprotection in myocardial I/R injury in rats. Thus, further studies will be needed to investigate the association between ER/SR stress and intracellular Ca^{2+} after H_2S treatment, and to explore H_2S-NO chemical interactions in I/R models.

Apoptosis of cardiomyocytes has been shown to play an important role in the development of myocardial I/R injury [37]. Furthermore, several reports have demonstrated that ER/SR stress-induced myocardial apoptosis plays a significant role in the pathogenesis of myocardial I/R injury [7, 38]. One of the components of the ER/SR stress-mediated apoptosis pathway is CHOP, also known as growth arrest-and DNA damage-inducible gene 153 (GADD153). CHOP-mediated apoptosis is implicated in the pathophysiology of cardiovascular diseases [27, 28]. In the present study, our results suggest that administration of H_2S inhibits ER/SR stress-induced cardiomyocyte apoptosis both *in vivo* and *in vitro*. Additionally, we found that H_2S attenuates H/R-induced ER/SR stress in rat H9c2 cardiac myocytes, as demonstrated by down-regulation of GRP78, ATF6 and CHOP expression. Several lines of evidence indicate that there are a number of potential mechanisms through which H_2S may exert cardioprotective effects, including K_{ATP} channels [39], regulation of mitochondrial function [17], cytoprotective gene Nrf-2 signaling [40], reduction of myocardial ROS production and inhibition of inflammation, necrosis and fibrogenesis [20, 41], mTORC2 phosphorylation of Akt1 [42], and upregulation of endothelial nitric oxide synthase [43, 44]. Here, our results suggest that the cardioprotective effects of H_2S against myocardial I/R injury in rats can be attributed,

at least in part, to inhibition of ER/SR stress-induced apoptosis. Thus, studies conducted by our group and by other investigators have indicated that the pathways implicated in the cardioprotective action of H_2S are multiple. Further insights into the mechanistic details of the protective effects of H_2S in various models of cardiac injury are necessary to understand the pathophysiological actions of this intriguing gaseous mediator.

In conclusion, our findings indicate that H_2S preconditioning could ameliorate myocardial I/R injury by attenuating excessive ER/SR stress and inhibiting myocardial apoptosis. Our study suggests a promising future of H_2S based preventions and therapies for myocardial I/R injury.

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Disclosure of conflict of interest

None.

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