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Hydrogen sulfide reduces regional myocardial ischemia injury through protection of mitochondrial function

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Abstract. Hydrogen sulfide (H_2S) is a signaling gasotransmitter, involved in various physiological and pathological processes. H_2S-donating drugs have been tested to conjugate the beneficial effects of H_2S with other pharmaceutical properties. It has been shown that the endogenous cystathionine-γ-lyase (CSE)/H_2S pathway participates in myocardial ischemia injury in isolated hearts in rats. The present study aimed to investigate the cytoprotective action of H_2S against acute myocardial ischemia injury in rats. Isolated rat hearts were perfused and subjected to ischemic conditions for 4 h. The hearts were assigned to five groups: Sham, model, infarct plus low-dose (5 µmol/l) NaHS, infarct plus middle-dose (10 µmol/l) NaHS and infarct plus high-dose (20 µmol/l) NaHS. The administration of NaHS enhanced the activity of CSE, increased the content of H_2S and reduced infarct volumes following myocardial ischemia injury. Furthermore, the administration of NaHS attenuated the injury to organelles (including the mitochondria, nucleus and myofilaments) by reducing lactate dehydrogenase activity, decreasing the level of mitochondrial malondialdehyde and increasing the activities of superoxide dismutase and glutathione peroxidase in the ischemic myocardial mitochondria. These protective effects of H_2S against myocardial ischemia injury appeared to be mediated by its antioxidant activities and the preservation of mitochondrial function.

Introduction

Cardiovascular diseases are the leading cause of mortality worldwide and their incidence is increasing concurrently with the development of society and changes in lifestyle (1). Ischemic heart disease, which can cause arrhythmia and myocardial infarction, is a current major focus of the cardiovascular pathologies (2) since it is projected that ischemic heart disease is likely to be the primary cause of mortality in the population by 2030 (3,4). Despite advances in understanding the pathogenesis of this disease, current treatments for ischemic heart disease are not sufficient (5-7) and, therefore, the effective relief of the injury caused by myocardial ischemia is a priority in medical research. Coronary artery ligation is the most common method of establishing an acute myocardial ischemia model and is widely used in studies investigating the mechanisms of myocardial ischemia (8). The method of Langendorff isolated retrograde heart perfusion is a prominent experimental technique used in the field of cardiovascular research and provides stability, reliability and convenience (9,10).

Hydrogen sulfide (H_2S) was the third endogenous signaling gasotransmitter to be identified, following nitric oxide (NO) and carbon monoxide (11-15). Endogenous H_2S is widely present in mammalian tissues and has numerous signaling functions, thus contributing to various physiological and pathological processes (16-21). It has been shown that H_2S can antagonize ischemic reperfusion injury and therefore exhibit cardioprotective effects (22-26). However, associations between H_2S and acute myocardial ischemia injury are less well established, and the mechanisms underlying the effects of H_2S remain unclear.

In this study, an acute myocardial ischemia injury rat model was established using the method of coronary artery ligation. The aim of this study was to observe whether H_2S could protect the hearts against ischemic injury and whether antioxidation was involved in the cardioprotection induced by H_2S.

Materials and methods

Animals. Male Sprague Dawley rats, weighing 250-290 g, were provided by the Center of Experimental Animals of Hebei Province (Shijiazhuang, China). Rats were acclimated for one week, with standard rat chow and water provided *ad libitum*. All experimental protocols were approved by the Animal Care and Use Committee of Hebei Medical University (Shijiazhuang, China) and were performed in accordance with the Guidelines of Animal Experiments from the Committee

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Myocardial ischemia injury. Coronary occlusion was performed as previously described (27). The rats were anesthetized with 10% chloral hydrate at 350 mg/kg via intraperitoneal injection. The heart was rapidly excised and washed with Krebs-Henseleit buffer (K-H buffer), then retrogradely perfused through the aorta at a constant pressure of 90 cm H_2O with K-H buffer containing 6.91 g/l NaC, 0.35 g/l KCl, 0.265 g/l $CaCl_2$•$2H_2O$, 2.1 g/l $NaHCO_3$, 2.2 g/l glucose, 0.296 g/l $MgSO_4$•$7H_2O$ and 0.1632 g/l KH_2PO_4 (pH 7.35-7.45 when aerated with 95% O_2/5% CO_2 at 37˚C). The left anterior descending (LAD) coronary artery was occluded with a 6.0-silk suture 2-3 mm from the tip of the left atrium, at the end of the stabilization period. Successful coronary occlusion was verified by the development of a pale color in the distal myocardium. Coronary flow rate (CF) was measured by timed collection of the coronary effluent for 1 min.

Drug preparation and experimental protocol. NaHS, 2,3,5-triphenyl tetrazolium and Evans-Blue were purchased from Sigma-Aldrich (St. Louis, MO, USA). Lactate dehydrogenase (LDH), mitochondrial malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-P_x) detection kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). NaHS was dissolved in K-H buffer.

The animals were randomly assigned to five groups: i) Sham; ii) model; iii) infarct plus NaHS at a concentration of 5 μmol/l; iv) infarct plus NaHS at a concentration of 10 μmol/l; and v) infarct plus NaHS at a concentration of 20 μmol/l. The three treatment groups were treated with the H_2S-donor (NaHS) 2 h after the induction of ischemia. The LAD coronary artery was ligated for 4 h in the rats of the model and NaHS-treated groups, but the rats in the sham group were threaded without ligation. The sham and model groups were subjected to perfusion with normal perfusate, and the experimental groups were perfused with NaHS perfusate 2 h after ischemia (5, 10 or 20 μmol/l, accordingly).

Hemodynamics. A water-filled latex balloon was inserted via the left atrium into the left ventricle and was inflated to set a left ventricular end-diastolic pressure of between 6 and 8 mmHg during the initial equilibration. This allowed monitoring of the left ventricular systolic pressure, the maximum velocity of left ventricular systolic pressure (+dP/dt_{max}) and the maximum velocity of left ventricular diastolic pressure (-dP/dt_{max}). The coronary effluent was collected to measure coronary flow rate (ml/min).

Tissue H_2S concentration. The tissue H_2S content was measured as described previously with modifications (24). Briefly, cardiac tissue was homogenized in a 10-fold volume (w/v) of 50 mM ice-cold potassium phosphate buffer (pH 6.8). The cardiac tissue homogenate was then mixed with 0.5 ml 1% zinc acetate. A total of 0.5 ml 20 mM N,N-dimethyl-p-phenylenediamine sulfate in 7.2 M HCl was subsequently added, immediately followed by the addition of 0.4 ml 30 mM $FeCl_3$ in 1.2 M HCl. Following 20 min incubation, 0.5 ml 10% trichloroacetic acid was added to the reaction mixture prior to the addition of 2.5 ml distilled water. The absorbance of the resulting solution at 670 nm was measured using a BioTek microplate reader (BioTek Instruments Inc., Winooski, VT, USA). The H_2S content was calculated against a calibration curve of NaHS, and the H_2S concentration was expressed as micromoles/gram protein.

Tissue cystathionine-γ-lyase (CSE) activity. The H_2S production rate was measured as a reflection of CSE activity (28). Myocardial tissue was homogenized in 1:10 (wt/vol) 50 mM ice-cold potassium phosphate buffer (pH 6.8). The subsequent reactions were performed in a 25-ml Erlenmeyer flask. The reaction mixture (1 ml) consisted of 100 mM potassium phosphate buffer (pH 7.4), 10 mM L-cysteine, 2 mM pyridoxal 5'-phosphate and 10% (w/v) tissue homogenate. Trapping solution of 0.5 ml 1% zinc acetate was added to a Cryovial® test tube in the flask, which was used as the center well, and a small piece of filter paper (2.0x2.5 cm^2) was used to increase the air/liquid contact surface. The flask was flushed with N_2 prior to being sealed, and then was transferred from an ice bath to an agitated water bath at 37˚C to initiate the reaction. After 90 min, the reaction was terminated with the addition of 0.5 ml 50% trichloroacetic acid. The flask was incubated further for an hour at 37˚C in order to completely trap the H_2S released from the mixture. The content of the center well was transferred to a test tube containing 3.6 ml distilled water and 0.5 ml 20 mM N,N-dimethyl-p-phenylenediamine sulfate in 7.2 M HCl was added immediately, followed by the addition of 0.4 ml 30 mM $FeCl_3$ in 1.2 M HCl. After 20 min, the absorbance of the resulting solution at 670 nm was measured with a BioTek microplate reader. The H_2S content was calculated against the calibration curve of NaHS. The H_2S production rates were expressed as nanomoles per milligram protein per minute.

Infarct size. Myocardial infarction was determined according to a previously described method (29). The heart was perfused with 1 ml 1% Evans Blue to stain the non-ischemic tissue 4 h after ischemia and frozen for between 3 and 24 h. Transverse sections (2-mm) were incubated in 1% triphenyltetrazolium chloride in phosphate buffer (pH 7.4) for 15 min at 37˚C. Non-infarcted tissue was stained red, whereas necrotic tissue remained unstained. The transverse sections were fixed with 10% formaldehyde and imaged. Images were captured using a digital camera and were transferred to a computer for subsequent management with Photoshop CS2 software (Adobe Systems, San Jose, CA, USA).

LDH. Myocardial tissue damage was assessed by determining the LDH activity in the coronary effluent collected at the end of stabilization, 3 and 4 h after the induction of ischemia. The activity of LDH was measured following the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute).

Ultrastructural changes to mitochondria in myocardial cells. Following 4 h of ischemia, the hearts were rapidly excised. Transmural tissue samples (1 mm^3) were obtained from the left anterior myocardium and immediately immersed in ice-cold 4%, 0.1 mol/l phosphate-buffered glutaraldehyde (pH 7.2). The

Table I. Effect of hydrogen sulfide on the cardiac function in rats following ischemia.

Group	LVDP (mmHg)	+dP/dt$_{max}$ (mmHg/sec)	-dP/dt$_{max}$ (mmHg/sec)	CF (ml/min)
Sham	63.12±1.46	3189±189	1762±122	3.65±0.12
Model	43.27±3.01[a]	1174±79[a]	861±54[a]	2.15±0.21[a]
I+L NaHS	49.77±2.07[b]	1724±126[b]	1066±38[b]	2.29±0.08[c]
I+M NaHS	55.50±1.60[b]	2298±167[b]	1278±45[b]	2.95±0.12[b]
I+H NaHS	61.75±1.67[b]	2844±139[b]	1587±43[b]	3.36±0.11[b]

Hearts were treated with normal perfusate or NaHS at concentrations of 5, 10 or 20 μmol/l 2 h after ischemia. The hemodynamic parameters were measured 4 h after ischemia. Data are presented as the mean ± standard error of the mean (n=8 rats in each group). [a]P<0.01 vs. the sham group; [b]P<0.01 and [c]P<0.01 vs. the model group. LVDP, left ventricular developed pressure; +dp/dt$_{max}$, maximum velocity of left ventricular systolic pressure; -dp/dt$_{max}$, maximum velocity of left ventricular diastolic pressure; CF, coronary flow rate; I+LNaHS, infarct plus low-dose NaHS; I+MNaHS, infarct plus middle-dose NaHS; I+HNaHS, infarct plus high-dose NaHS.

tissues were washed twice in dimethyl arsenate buffer and the tissue blocks were then postfixed for 2 h in 1%, 0.1 mol/l phosphate-buffered OsO_4 (pH 7.2). This was followed by a further two washes in dimethyl arsenate buffer and dehydration. The tissues were subsequently embedded in araldite. Ultra-thin sections were cut and double stained in uranyl acetate and lead citrate. The sections were observed under a transmission electron microscope to assess the ultrastructural features of the cardiomyocytes.

Isolated mitochondria. Mitochondria were isolated from the adult rat hearts by homogenization and differential centrifugation, as described previously (30). Briefly, the heart was rapidly excised and washed in buffer containing 70 mM sucrose, 210 mM mannitol, 1 mM EDTA and 50 mM Tris (pH 7.4) at 4˚C. Following changes of buffer, the cardiac samples were cut into small pieces and homogenized. The homogenate was centrifuged at 1,300 x g for 3 min at 2˚C. The supernatant was then collected and re-centrifuged at 10,000 x g for 8 min at 2˚C. The pellet was resuspended in EDTA-free homogenization buffer [70 mM sucrose, 210 mM mannitol (pH 7.4) with 50 mM Tris] and centrifuged at 10,000 x g for 10 min at 2˚C. The prepared mitochondria were diluted in isolation medium prior to use.

Mitochondrial MDA, SOD and GSH-P_x. The level of MDA and the activities of SOD and GSH-P_x were determined using reagent kits, in accordance with the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute).

Statistical analysis. Results are presented as the mean ± standard error of the mean. All tests were performed using SPSS 13.0 (SPSS, Inc., Armonk, NY, USA). Data were analyzed using one-way analysis of variance followed by Tukey's post hoc multiple comparison test. P<0.05 was considered to indicate a statistically significant difference.

Results

Effect of H_2S on cardiac function during ischemia. The cardiodynamic variables are shown in Table I. Subsequent to myocardial ischemia injury, the values for left ventricular developed pressure (LVDP), ±dP/dt$_{max}$ and CF were significantly decreased in the model group relative to those in the sham group (P<0.01), whilst these parameters showed significant increases in the NaHS low-, middle- and high-dose groups as compared with those in the model group (P<0.05 or P<0.01).

Changes in H_2S content and CSE activity in myocardial tissue. The content of H_2S and the activity of CSE in cardiac tissue were significantly decreased in the model group as compared with those in the sham group (P<0.01, Fig. 1). However, these parameters showed significant increases in the NaHS low-, middle- and high-dose groups compared with those in the model group (P<0.05 or P<0.01, Fig. 1).

Effect of H_2S on infarct size. The myocardial infarct volume was significantly increased in the model group compared with that of the sham group (P<0.01, Fig. 2). No significant differences were observed in myocardial infarct volume between the NaHS low-dose group and the model group, whereas the infarct volumes were significantly decreased in the NaHS middle- and high-dose groups as compared with those in the model group (P<0.01, Fig. 2).

Effect of H_2S on LDH activity. No statistically significant differences were observed in the activity of LDH in the perfusate among the experimental groups prior to ischemia (Table II). The activity of LDH in the perfusate was significantly increased in the model group compared with that of the sham group (P<0.01, Table II). However, the activity of LDH in the perfusate was significantly decreased in NaHS low-, middle- and high-dose groups compared with that in the model group (P<0.01, Table II).

Effect of H_2S on the ultrastructure of mitochondria in myocardial cells. In the sham group, the ultrastructure of the myocardial cells exhibited regular mitochondria with uniform size, complete mitochondrial cristae and an intact nuclear membrane. In the model group, the myocardial cells were characterized by mitochondrial swelling, disappearance or deformation of mitochondrial cristae, disruption of the nuclear membrane and nuclear condensation. The NaHS low-,

Table II. Effect of hydrogen sulfide on the activity of LDH in the coronary effluent of each group.

	LDH activity (U/l)		
Group	BS 20 min	Ischemia 3 h	Ischemia 4 h
Sham	11.18±0.42	31.46±3.97	49.68±3.06
Model	11.24±0.38	82.35±4.43[a]	103.94±6.22[a]
I+L NaHS	11.20±0.38	66.02±3.69[b]	83.49±3.36[b]
I+M NaHS	11.22±0.35	51.32±4.16[b]	70.39±4.07[b]
I + H NaHS	11.17±0.42	44.28±2.52[b]	60.45±3.89[b]

Hearts were treated with normal perfusate or NaHS at concentrations of 5, 10 or 20 μmol/l 2 h after ischemia. The activity of LDH in cardiac tissue was measured prior to ischemia and 3 and 4 h after ischemia. Data are presented as the mean ± standard error of the mean (n=8 rats in each group). [a]P<0.01 vs. the sham group; [b]P<0.01 vs. the model group. I+LNaHS, infarct plus low-dose NaHS; I+MNaHS, infarct plus middle-dose NaHS; I+HNaHS, infarct plus high-dose NaHS; LDH, lactate dehydrogenase; BS, balance perfusion.

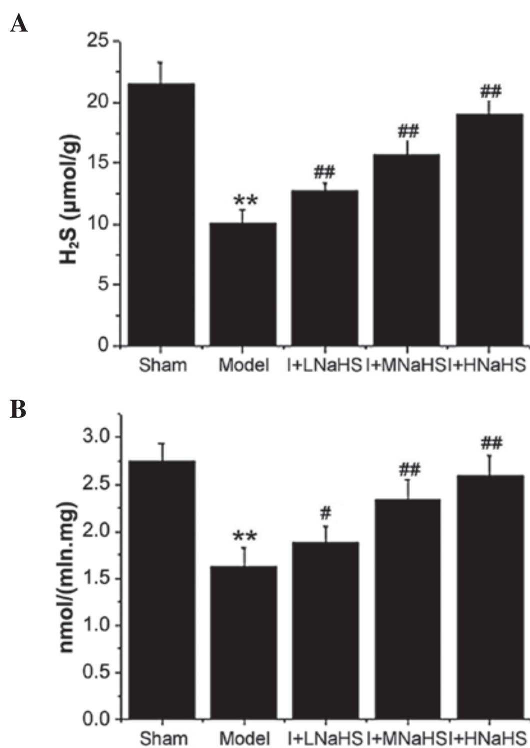

Figure 1. Changes in the content of H_2S and the activity of CSE in cardiac tissue following ischemia. Hearts were treated with normal perfusate or NaHS at concentrations of 5, 10 or 20 μM 2 h after ischemia. (A) The content of H_2S in the cardiac tissue and (B) the activity of CSE were measured 4 h after ischemia. Data are presented as the mean ± standard error of the mean (n=8 rats in each group).**P<0.01 vs. the sham group; #P<0.05 and ##P<0.01 vs. the model group. I+LNaHS, infarct plus low-dose NaHS; I+MNaHS, infarct plus middle-dose NaHS; I+HNaHS, infarct plus high-dose NaHS; H_2S, hydrogen sulfide; CSE, cystathione-γ-lyase.

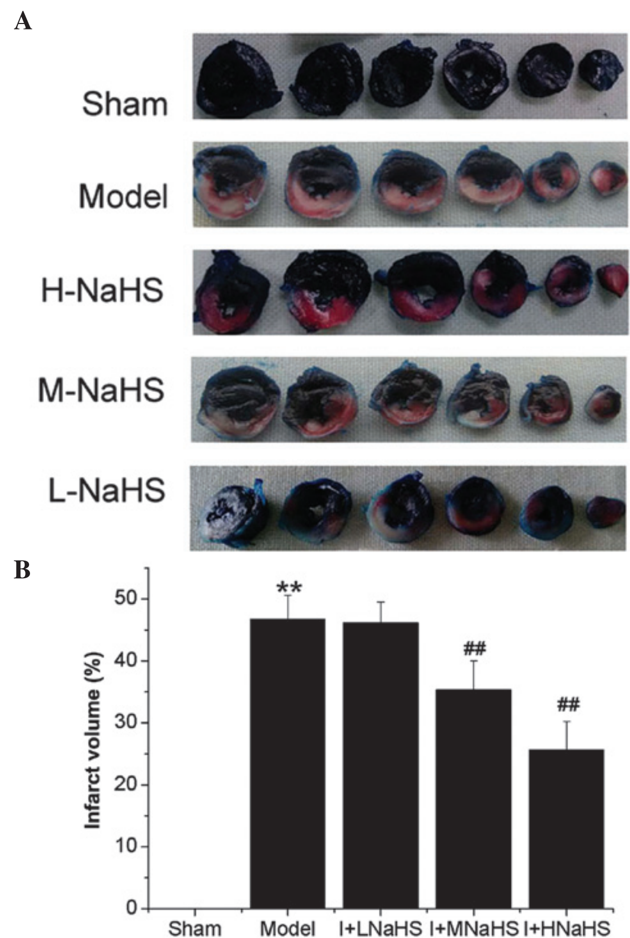

Figure 2. Effect of H_2S on the infarct volume following ischemia. Hearts were treated with normal perfusate or NaHS at concentrations of 5, 10 or 20 μM 2 h after ischemia. (A) Representative images of infarct volume in each group. (B) Quantification of the effect of H_2S on infarct volume (expressed as a percentage of total volume). Data are presented as the mean ± standard error of the mean (n=8 rats in each group).**P<0.01 vs. the sham group; ##P<0.01 vs. the model group. I+LNaHS, infarct plus low-dose NaHS; I+MNaHS, infarct plus middle-dose NaHS; I+HNaHS, infarct plus high-dose NaHS; H_2S, hydrogen sulfide.

middle- and high-dose groups showed less significant pathological changes in the myofilaments, mitochondria and nuclei as compared with the model group (Fig. 3).

Effects of H_2S on mitochondrial MDA content and SOD and glutathione $GSH\text{-}P_x$ activity. The content of MDA in the cardiac mitochondria was significantly increased and the activities of SOD and $GSH\text{-}P_x$ were significantly decreased in the model group compared with those in the sham group (P<0.01, Fig. 4). By contrast, the content of MDA was signifi-

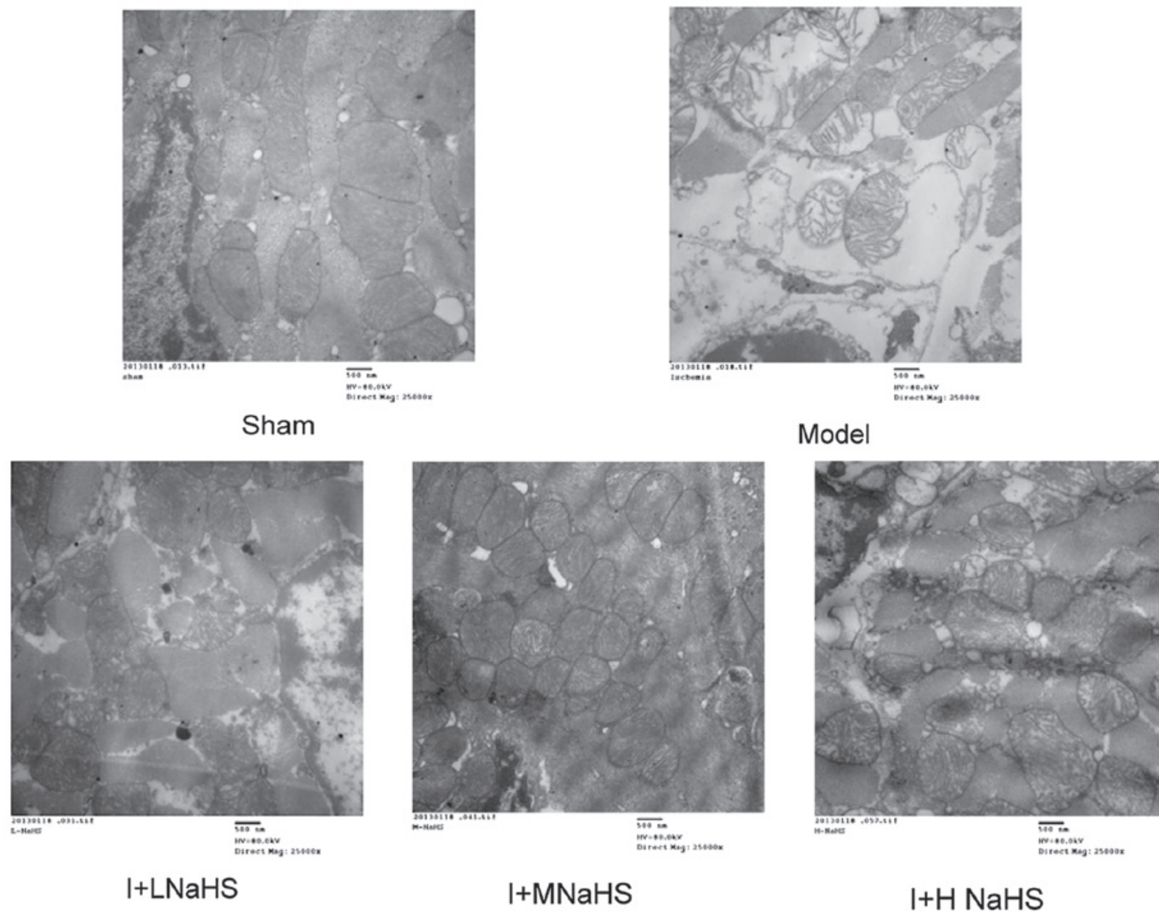

Figure 3. Pathological changes in the ultrastructure of myocardium, observed using electron microscopy. Hearts were treated with normal perfusate or NaHS at concentrations of 5, 10 or 20 μmol/l 2 h after ischemia (magnification, x25,000). I+LNaHS, infarct plus low-dose NaHS; I+MNaHS, infarct plus middle-dose NaHS; I+HNaHS, infarct plus high-dose NaHS.

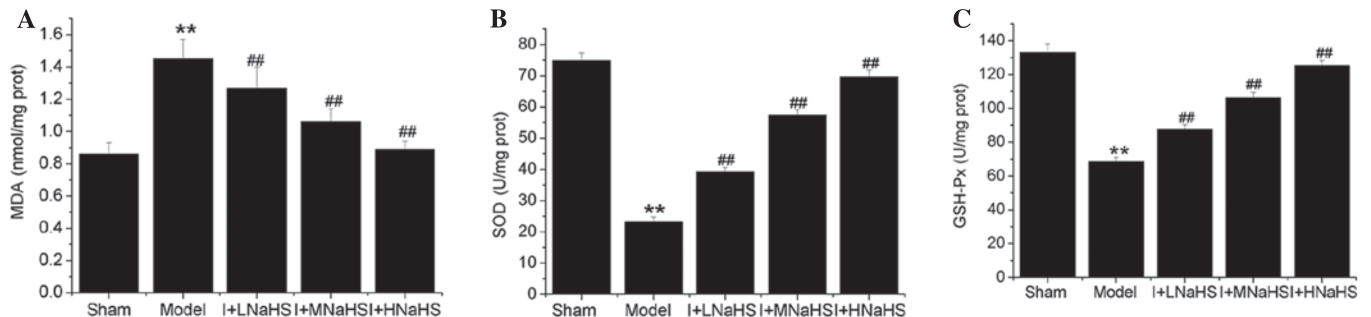

Figure 4. Effect of hydrogen sulfide on the antioxidative activities in the mitochondria of cardiomyocytes in isolated ischemic rat hearts. Hearts were treated with normal perfusate or NaHS at concentrations of 5, 10 or 20 μmol/l 2 h after ischemia. (A) MDA content, (B) SOD activity and (C) GSH-P_x activity in the different groups. Data are presented as the mean ± standard error of the mean (n=8 rats in each group). **P<0.01 vs. the sham group; ##P<0.01 vs. the model group. I+LNaHS, infarct plus low-dose NaHS; I+MNaHS, infarct plus middle-dose NaHS; I+HNaHS, infarct plus high-dose NaHS; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-P_x, glutathione peroxidase.

cantly decreased and the activities of SOD and GSH-P_x were significantly increased in the cardiac mitochondria in the NaHS low-, middle- and high-dose groups compared with those in the model group (P<0.01, Fig. 4).

Discussion

It has been shown H_2S is a novel gasotransmitter produced in numerous mammalian cells and tissues (31-33). H_2S is generated primarily from L-cysteine by CSE in the cardiovascular system (11,34,35); CSE activity has been detected in the heart (32) and vascular smooth muscle (36). There is a growing evidence to suggest that there are correlations among CSE activity, H_2S concentration and cardiovascular disease (37-40).

It has been suggested that H_2S exerts antioxidant, anti-inflammatory, and anti-apoptotic effects (41,42). H_2S has been shown to inhibit lipid peroxidation during heart ischemia-reperfusion and to decrease the ischemia-induced

death of myocardial cells via an oxygen free radical-reducing mechanism (42). This suggests that H_2S may act as an important modulator in cardiovascular physiology and pathophysiology. Since the role of H_2S in the pathogenesis of ligature-induced regional myocardial ischemia has not been investigated *in vitro,* to the best of our knowledge, the present study demonstrated for the first time the role of H_2S in a ligature-induced myocardial ischemia injury model in rats *in vitro*. An isolated rat heart model was used such that extrinsic humoral and autonomic nervous system influences, in addition to the effect of H_2S on peripheral vascular tone, could be excluded (43).

In our previous study, comparisons with the sham group revealed that the LVDP, $\pm dP/dt_{max}$ and CF were significantly decreased in the ischemia group at 30 min and 1, 2, 3 and 4 h after ischemia and that the infarct volumes in the ischemia group were markedly increased between 1 and 4 h after ischemia. Associated with these injuries, the content of H_2S and the activity of CSE in the cardiac tissue were significantly decreased compared with those of the sham control group during the 1-4 h after ischemia. These data revealed that the CSE/H_2S pathway may participate in the pathophysiological regulation of myocardial ischemia injury in isolated rat hearts. The CSE activity is dependent on pyridoxal 5'-phosphate (PLP), which is reduced in ischemic disease, such as stroke (44). Low PLP levels may inhibit the CSE activity and lead to decreased myocardial and plasma H_2S generation. Furthermore, CSE inhibition may decrease glutathione levels (45), and this reduction may explain the increase in infarct size (46).

The cardiodynamic parameters and myocardial infarction volume were selected as the parameters to assess the functional performance of the isolated rat heart following ischemia. To explore the effects of H_2S on regional myocardial ischemia injury, the isolated rat heart was treated with NaHS following the induction of ischemia. NaHS has a fast releasing rate in aqueous solution, producing one-third H_2S as compared with the concentration of the salt (47,48), without changing the pH of the medium (49). In the present study, the content of H_2S and the activity of CSE in the cardiac tissue were significantly decreased in the model group as compared with those in the sham group. By contrast, the content of H_2S and the activity of CSE in the cardiac tissue were significantly increased in the NaHS low-, middle- and high-dose groups as compared with those in the model group. The LVDP, $\pm dP/dt_{max}$ and CF were significantly increased in the NaHS low-, middle- and high-dose groups as compared with those in the model group. The infarct volume is strongly associated with the prognosis of acute myocardial infarction and negatively correlated with improvements in the cardiodynamic parameters. In particular, impairments in the cardiac contractility become increasingly severe with increasing infarct volume; therefore, a decrease in infarct volume may be a useful parameter for the evaluation of the effectiveness of anti-myocardial ischemia drugs (50). In the present study, the infarct volumes were significantly decreased in NaHS middle- and high-dose groups compared with those in the model group. Taken together, these data demonstrate that H_2S exerts protective effects against myocardial ischemia injury in isolated rat hearts. Similar to NO, H_2S has been found to be a vasodilatory agent that acts through alterations in K^+ channel activity and elevated cyclic guanosine monophosphate levels in vascular smooth muscle cells (51,52). Consistent with these observations, our findings revealed that reduced levels of H_2S are associated with the constriction of blood vessels. Furthermore, the vasodilatory effects of NaHS can dilate coronary arteries and increase CF in ischemic diseases, thereby reducing ischemia-induced cellular damage.

Mitochondrial ultrastructural (53) and functional (54) injury occurs early and progresses through the course of ischemia (54,55). Mitochondrial damage results in a loss of mitochondrial function, impairing energy production and cell physiology, and an enhancement of pathological function, producing oxidative-, calcium- and apoptosis-mediated myocyte injury (56). Mitochondrial oxidative damage participates in a variety of pathologies, including cardiovascular disorders and neurodegenerative diseases. As such, the protection of mitochondria from oxidative damage may be an effective therapeutic strategy. The effect of H_2S on myocardial oxidative stress following ischemia was therefore assessed in the present study. The production of reactive oxygen species by mitochondria leads to the formation of lipid peroxidation products and, in turn, can induce oxidative stress, thereby causing cellular and mitochondrial damage (57,58). The release of free radicals, coupled with the ischemia-induced decrease in antioxidant activity, leaves the myocardium susceptible to injury. It has been observed that phospholipid peroxidation and subsequent damage to complex I jointly increase membrane leakage, mitochondrial swelling, cytochrome *c* release and caspase activation, resulting in cell death (59). The leakage of the cytosolic enzyme LDH is correlated with a loss of cell membrane integrity, and measuring the change in MDA content indicates the degree of damage caused by membrane lipid peroxidation. In this study, lipid peroxidation was observed to increase in the heart upon ligation of the LAD coronary artery. This was apparent through increases in the MDA content and LDH level relative to the sham group. However, the addition of NaHS significantly decreased the MDA content and LDH level. This result is consistent with the findings on the effects of H_2S on a rat model of myocardial infarction *in vivo* (60). In the majority of mammalian species, SOD and $GSH\text{-}P_x$ appear to be the most active antioxidant enzymes in the myocardium to provide defense for cellular organelles against oxidative damage caused by reactive oxygen species (61). In the present study, NaHS administration significantly increased the activities of SOD and $GSH\text{-}P_x$ in the cardiac mitochondria following ischemia. These data demonstrate that treatment with NaHS enhances the capacity of antioxidant enzymes, and the underlying mechanism of action is attributed to the protective effects exerted against the oxidative stress-mediated injury in the mitochondria. Transmission electron microscopy revealed a marked relief in mitochondrial swelling and increased matrix density in isolated ischemic rat hearts receiving NaHS, further suggesting a role for the preservation of mitochondrial function in the observed cytoprotection.

In conclusion, the pathophysiological process of ligature-induced regional myocardial ischemia injury is associated with the impaired endogenous CSE/H_2S pathway. Exogenously administered H_2S via NaHS can effectively protect myocytes and contractile activity and limit the extent of myocardial infarction. These protective effects of H_2S against myocardial ischemia injury appear to be mediated by its antioxidant activities and preservation of mitochondrial

function. Taken together, these data strengthen evidence that H_2S may be a cardiovascular protective regulator for preventing or treating cardiovascular diseases. However, the exact mechanisms underlying the action of H_2S require further investigation.

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