Int J Clin Exp Med 2017;10(12):16352-16359
www.ijcem.com /ISSN:1940-5901/IJCEM0046586

Original Article

PTEN/PI3K/Akt pathway is involved is involved in the protection of hydrogen sulfide against myocardium ischemic/reperfusion injury

Tieduo Kang[1], Wenxian Liu[2]

[1]*Department of Cardiology, Beijing Anzhen Hospital, Capital Medical University, Beijing, China;* [2]*Intensive Care Unit of Department of Cardiology, Beijing Anzhen Hospital, Capital Medical University, Beijing, China*

Received December 14, 2016; Accepted April 10, 2017; Epub December 15, 2017; Published December 30, 2017

Abstract: Myocardial ischemia-reperfusion injury (MIRI) is one of the important reasons for the high mortality of ischemic heart disease in patients who have undergone a blood supply reconstruction. Hydrogen sulfide (H_2S) has been thought of to be just a toxic gas with a strong odor of rotten eggs for hundreds of years, but it has been demonstrated that H_2S plays a protective role in the pathogenesis and development of heart diseases, especially in MIRI. In the present study, we treated rats with H_2S after MIRI and then observed its effects on heart function parameters following in situ ischemia/reperfusion. Multiple oxidative products, myeloperoxidase and proinflammatory cytokines in the myocardium were measured to evaluate the anti-oxidative and anti-inflammatory effect of H_2S. The role of phosphatase and tensin homologue (PTEN)/PI3K/Akt pathway was examined to explore the underlying mechanism. The treatment of H_2S for seven consecutive days exhibited dramatic improvement in cardiac function, as manifested by increased LVSP and ± (dP/dt) max, and decreased LVDP. PTEN mRNA and expression were decreased by H_2S. PI3K/Akt pathway was activated by H_2S and PTEN inhibitor bisperoxovanadium (Phen) but de-activated by PI3K inhibitor LY294002. The heart function was enhanced by Phen but worsened by LY294002, suggesting the involvement of PTEN/PI3K/Akt pathway. These results revealed that H_2S may exert its cardioprotection by inactivation of PTEN, which in turn activates the PI3K/Akt signaling and increases survival in the MIRI model.

Keywords: Myocardium ischemic/reperfusion injury, hydrogen sulfide, phosphatase and tensin homologue, PI3K/Akt pathway

Introduction

Despite the rapid developments in medical care, acute myocardial infarction remains a major cause of morbidity and mortality throughout the modern world. Blood supply reconstruction, represented by PCI and thrombolysis, is still the most effective treatment of ischemic heart disease. However, data from large clinical trials show that mortality of ischemic heart disease remains high in patients who have undergone a blood supply reconstruction, and that 5-6% of patients died of complications within one month after blood supply reconstruction [1]. The proportion of patients who develop into long-term heart failure is nearly 25% [2]. Myocardial ischemia reperfusion injury (MIRI) is one of the important reasons. Reperfusion injury is initiated when blood flow returns to the ischemic tissue [3], and involves many cell injury pathways, such as membrane destabilization, intracellular calcium dysregulation, free radical production, mitochondrial injury and pro-apoptotic pathway activation [4]. Hydrogen sulfide (H_2S) has been thought of to be a toxic gas with a strong odor of rotten eggs for hundreds of years. Since in 1996 when Abe and Kimura discovered that H_2S facilitated the induction of hippocampal long-term potentiation [5], H_2S has been regarded as a novel gaseous signaling molecule, which may be involved in a multitude of pathophysiologic processes, such as oxidative stress, inflammation, apoptosis, and angiogenesis [6]. In recent years, it has been showed that H_2S plays a protective role in the pathogenesis and development of heart diseases [7].

For ischemia reperfusion (I/R) injury, a current research focus is reperfusion injury salvage kinase (RISK) [8], a group of signal pathways that can reduce cell death in reperfusion injury

[9]. Their activation can reduce the infarct size, increase myocardial contractility and improve local blood supply recovery. PI3K/Akt pathway is a representative one of RISK. It is associated with cell survival and inhibits the proapoptotic gene Bad and activates NF-kB [10, 11], then leads to transcription of downstream survival-promoting genes and reduces cell apoptosis. It also reduces beeline-1 (a regulator that activates autophagy) [12] and activates mammalian target of rapamycin (mTOR), thus inhibiting autophagy. In addition, activation of Akt can inhibit Caspase 3/9 [13] and GSK-3β [14], enhance the activity of endothelial nitric oxide synthase [14] and murine double minute 2 [15]. The gene of phosphatase and tensin homologue (PTEN), a natural inhibitor of PI3K, participates in growth, apoptosis, adhesion, invasion, and migration [16]. It has been reported that PTEN increases apoptosis in cardiomyocytes, whereas the inactivation of PTEN activates the Akt signaling, reduces apoptosis, and increases survival [17, 18]. The role of PTEN/PI3K/Akt pathway in the cadioprotection of H_2S needs clarification.

Hence, the present work was performed to explore the protective effect of H_2S against MIRI and its underlying mechanism. We treated rats with H_2S after MIRI and then observed its effects on heart function parameters following *in situ* I/R. Multiple oxidative products, myeloperoxidase (MPO) and proinflammatory cytokines in the myocardium were measured to evaluate the anti-oxidative and anti-inflammatory effect of H_2S. Next, the role of PTEN/PI3K/Akt pathway was further examined to explore the underlying mechanism of the protective effects of H_2S.

Materials and methods

Animals

Male Wistar rats,weighing from 200 g to 220 g, were purchased from the Beijing Laboratory Animal Center (Beijing, China) and used in this study. The study was performed in accordance with the Guide for the Care and Use of Laboratory Animals, 8th Edition. The animals were housed in the Beijing Anzhen hospital animal center, under conditions of 22°C-25°C temperature, 38%-45% relative humidity, and 12-/12-hour light/dark cycles, with free access to water and food. All the protocols were approved by the institutional animal care and use committee of the Capital Medical University.

Experimental protocol

In the first part of study, rats were randomly assigned to four groups: Sham, MIRI, MIRI+H_2S and H_2S. In the second part of study, rats were randomly assigned to five groups: Sham, MIRI, MIRI+H_2S, MIRI+H_2S+bisperoxovanadium (Phen), and MIRI+H_2S+LY294002. Rats in the Sham group received a sham myocardial I/R surgery. Rats in the MIRI group received a myocardial I/R surgery. Rats in the MIRI+H_2S group received a myocardial I/R surgery, followed by low concentration H_2S inhalation 8 hour/day for 7 days. Rats in the H_2S group received only low concentration H_2S inhalation 8 hour/day for 7 days. There were 18 rat in each group: 10 rats were used for functional studies; 8 rats were used for biomarker measurements. PTEN inhibitor Phen was purchased from EMD Chemicals (Gibbstown, NJ, United States) and intraperitoneal injected at a dose of 0.2 mg/kg/day during H_2S exposure. PI3K inhibitor LY294002 was purchased from Sigma-Aldrich (St. Louis, MO, USA) and intraperitoneal injected at a dose of 100 mg/kg/day during H_2S exposure.

Myocardial I/R procedure

The myocardial I/R procedure was similar to Yin et al [19]. Briefly, rats were anesthetized by intramuscular injection of ketamine hydrochloride (35 mg/kg) and xylazine (5 mg/kg). A middle cervical incision was made to expose the trachea, then a section of tubing was passed through the exposed trachea until the tip was 3 mm below the larynx. After the pericardium was opened, the left coronary artery was located and a 3-0 silk black braided suture was inserted around the artery near its origin. A snare was created by passing both ends of the suture through the tip of an angiocatheter that could then be tightened and released by sliding a Voss clip down the angiocatheter. After 60 minutes of ischemia, the occlusive snare was released for reperfusion. Sham rats underwent the same surgical procedures except that the suture was not snared.

Hydrogen sulfide inhalation

Rats were kept in a 30-L plastic chamber and breathed air alone or air mixed with H_2S at 40 ppm for 8 h each day for 7 days. The inhalation procedure was previously described by Kida et al [20]. Rats breathed H_2S from 10 AM to 6 PM on each of 7 consecutive days after they received the myocardial I/R surgery. The H_2S

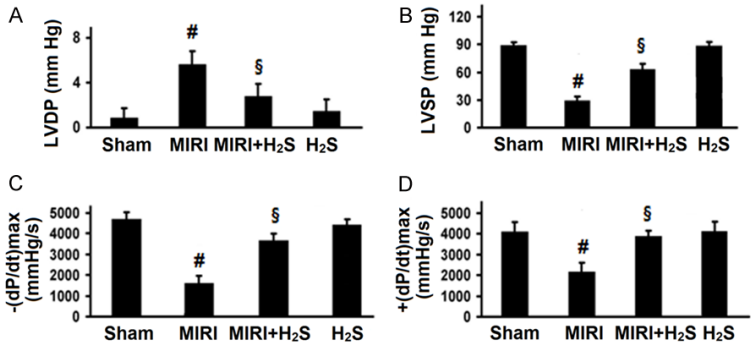

Figure 1. Changes in cardiac function by H_2S. Rats breathed air alone or air mixed with H_2S at 40 ppm for 8 h each day for 7 days after MIRI. MIRI decreased ± (dP/dt) max and LVSP but increased LVDP. H_2S which was reversed by H_2S. MIRI: myocardium ischemic/reperfusion injury; LVSP: left ventricular systolic pressure. LVDP: left ventricular developed pressure. Values are expressed as Mean ± SEM. #: P<0.05 compared to Sham; §: P<0.05 compared to MIRI.

gas/air mixture was flowing through the chamber to obtain a stable gas concentration (40 ppm).

Surgical procedures for evaluating hemodynamic parameters

The measurement of hemodynamic parameters was similar to Yin et al [19]. After the seven days of H_2S treatment was finished, rats were anesthetized, a small incision was made on the right side of the neck. The external right carotid artery was exposed and a microtip pressure transducer catheter (Millar Instruments, Houston, USA) was inserted into the artery. The proximal end of the catheter was connected to an electrostatic chart recorder (Gould, Cleveland, USA). The inserted tip of this catheter was then advanced until it reached the left ventricular lumen. The left ventricular systolic pressure (LVSP), left ventricular developed pressure (LVDP), and ± ventricular contractility (dP/dt max) measurements were obtained from the left ventricular pressure (LVP) signal.

Measurement of oxidative products, MPO and proinflammatory cytokines in myocardial tissues

Transmural tissue from the area at risk was homogenized in 2 mL of 10 mM phosphate buffer (pH 7.4). After centrifugation at 10,000 g for 30 min, MDA and protein carbonyl content determined using commercial kits (Beyotime Biotechnology, Jiangsu, China). All procedures were performed according to the manufacturer's instructions. For the 8-OHdG assay, DNA was extracted from the tissue using a DNA Extraction Kit (Wako Chemical, Osaka, Japan), then added to plate wells pre-coated with anti-8-OHdG antibody (Nikken SEIL Co., Fukuroi, Japan) and incubated for 45 min at 37°C. The wells were then sequentially treated with IgG, Streptavidin-Horseradish Peroxidase, and 3, 3',5,5'-tetramethylbenzidine. After incubation for 15 min, the reaction was terminated by sulfuric acid and the absorbance was read at 450 nm. MPO, proinflammatory cytokines TNF-α, and IL-1β levels were measured using ELISA kits, according to the manufacturer's instructions (Sigma, St. Louis, USA). Absorbance was measured with a microplate reader to obtain the MPO, TNF-α, and IL-1β levels. The protein concentration was determined using a standard BCA protein assay kit (Beyotime Institute of Biotechnology, Shanghai, China).

Real-time PCR of PTEN

RT-qPCR was performed with the CFX Connect Real-Time system (Bio-Rad, USA) using a SYBR Green PrimeScript RT kit (TaKaRa, Japan) based on the manufacturer's instructions. The PCR conditions included predenaturation at 95°C for 30 s followed by 40 cycles of denaturation at 95°C for 10 s and combined annealing/extension at 58°C for 30 s. The mRNA expression levels were calculated based on the comparative quantification method ($2^{-\Delta\Delta CT}$). β-actin were used as internal controls for PTEN mRNA quantitation.

Western blot

Cardiac tissues were harvested and washed in ice-cold saline, and then homogenized in RIPA lysis buffer (25 mg/mL) with 1 mM phenylmethylsulfonyl fluoride on ice. All samples were centrifuged at 3000×g at 4°C for 15 min and the supernatants were collected. The protein concentration was determined using a standard BCA protein assay kit (Beyotime Institute of Biotechnology, Shanghai, China). Equal amounts (50 μg) of proteins were separated and trans-

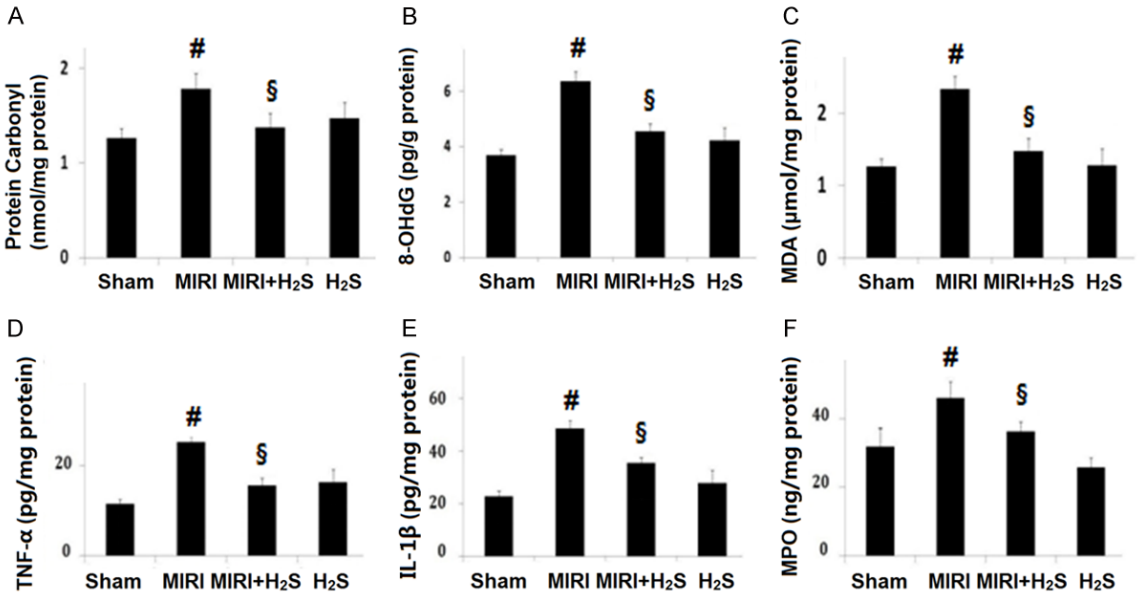

Figure 2. Changes in oxidative products, MPO and proinflammatory cytokines in myocardial tissues. It shows a significant increase in myocardial oxidative products (protein carbonyl, 8-OHdG and MDA), MPO and proinflammatory cytokines (TNF-a, and IL-1β) by MIRI and a decrease of them by H_2S. MIRI: myocardium ischemic/reperfusion injury; 8-OHdG: 8-hydroxy-2-deoxyguanosine; MDA: Malondialdehyde; MPO: myeloperoxidase; TNFα: Tumor necrosis factor; IL-1β: Interleukine-1 beta. Values are expressed as Mean ± SEM. #: P<0.05 compared to Sham; §: P<0.05 compared to MIRI.

ferred to a polyvinyl difluoride membrane. After blocking with 5% nonfat dried milk, the membranes were probed with antibodies PTEN, pAkt, Akt and β-actin and incubated with either horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse antibody (1:5000, ThermoFisher Scientific, Shanghai, China). Immunoreactive proteins were visualized and then scanned. The densitometry was performed to quantify the expression using Bio-Rad Quantity One 4.4.0 software (Bio-Rad, Hercules, CA, USA).

Statistical analysis

The statistical analysis was performed using SPSS 17.0 with one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls post hoc test. P<0.05 was considered significant.

Results

Changes in cardiac function by H_2S

Figure 1A-D shows the significant changes caused by MIRI and H_2S on hemodynamic parameters. MIRI treatment significantly increased LVDP, and decreased LVSP and ± (dP/dt) max (P<0.05 compared to the Sham group). The LVDP values in the MIRI+H_2S group was lower than that in the MIRI group (P<0.05), while the LVSP and ± (dP/dt) max values were higher than those in the MIRI group (P<0.05). The treatment of H_2S alone had no effect on cardiac function compared to Sham.

Changes in oxidative products and pro-inflammatory cytokines in myocardial tissues by H_2S

As shown in **Figure 2A-C**, myocardial oxidative products (protein carbonyl, 8-OHdG and MDA) assays demonstrated that MIRI caused significant oxidative stress, but H_2S could significantly mitigate the oxidative stress, indicated by the dramatic decreases in the protein carbonyl, 8-OHdG and MDA levels compared to the MIRI group (P<0.05). As shown in **Figure 2D-F**, MIRI caused significant increases in myocardial TNF-α, IL-1β and MPO levels (P<0.05 compared to the Sham group), which were inhibited by H_2S treatment, as shown by the dramatic decreases in the MPO, TNF-α, and IL-1β levels (P<0.05 compared to the MIRI group). The treatment of H_2S alone had no effect on oxidative products, MPO and proinflammatory cytokines compared to Sham.

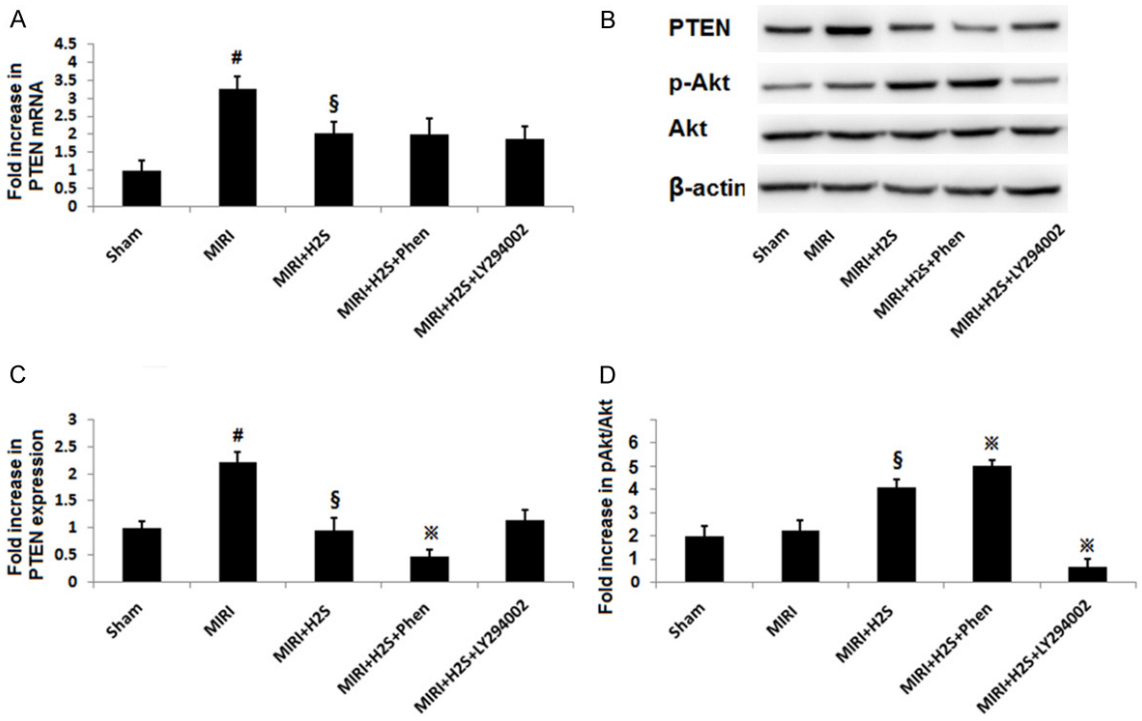

Figure 3. Changes in PTEN mRNA and PTEN/PI3K/Akt pathway activation. The myocardial PTEN mRNA, PTEN expression and Akt phosphorylation were measured after rats were treated with H_2S or PTEN inhibitor Phen or PI3K/Akt pathway inhibitor LY294002. PTEN: phosphatase and tensin homologue; Phen: bisperoxovanadium. Values are expressed as Mean ± SEM. #: P<0.05 compared to Sham; §: P<0.05 compared to MIRI; ※: P<0.05 compared to MIRI+H_2S.

Changes in PTEN mRNA and PTEN/PI3K/Akt pathway activation

We first measured the mRNA in the presence of H_2S, PTEN inhibitor Phen or PI3K/Akt pathway inhibitor LY294002. As shown in **Figure 3A**, PTEN mRNA was increased by MIRI but decreased by H_2S; it was not changed by Phen or LY294002. **Figure 3B-D** show the protein expression of PTEN and the activation of Akt. PTEN expression was enhanced by MIRI but decreased by H_2S and Phen, while LY294002 caused no change in PTEN expression. Akt was activated by H_2S and Phen, while de-activated by LY294002.

Changes in cardiac function by inhibitors of PTEN/PI3K/Akt pathway

To evaluate the roles of PTEN/PI3K/Akt pathway in the protection mediated by H_2S, we treated rats with inhibitors of PTEN/PI3K/Akt pathway and then examined the changes in cardiac function. As shown in **Figure 4A-D**, the LVDP was decreased by Phen but increased by LY294002 compared to the MIRI+H_2S groups (P<0.05). By contrast, LVSP and ± (dP/dt) max were increased by Phen but decreased by LY-294002 compared to MIRI+H_2S (P<0.05).

Discussion

With the advancement of scientific technology in recent years, researchers have discovered that H_2S can be produced by cystanthionine-γ-lysase (CSE) in the cardiovascular system and be an endogenous gaseous mediator exerting pronounced physiological effects as the third gasotransmitter in addition to nitric oxide (NO) and carbon monoxide (CO). Accumulating evidence indicated that exogenous H_2S could mediate the cardioprotective effects in myocardial ischemia model. However, the underlying mechanism still needs clarification.

Some investigators have explored the effects of H_2S on MIRI, but the mechanism remains controversial. The cardioprotection induced by H_2S have been studied by others, mainly on H_2S preconditioning. It was found that H_2S precon-

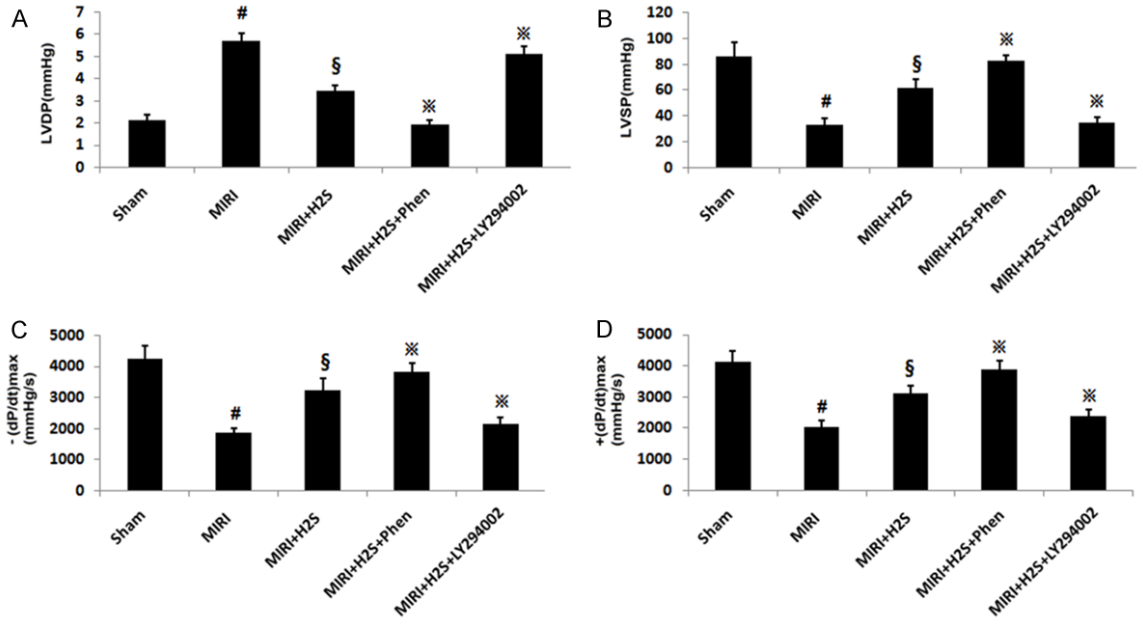

Figure 4. Changes of cardiac function by inhibitors of PTEN/PI3K/Akt pathway. The cardiac function was measured after rats were treated with H_2S or PTEN inhibitor Phen or PI3K/Akt pathway. MIRI: myocardium ischemic/reperfusion injury; MIRI: myocardium ischemic/reperfusion injury; LVSP: left ventricular systolic pressure. LVDP: left ventricular developed pressure; Phen: bisperoxovanadium. Values are expressed as Mean ± SEM. #: P<0.05 compared to Sham; §: P<0.05 compared to MIRI; ※: P<0.05 compared to MIRI+H_2S.

ditions the db/db diabetic mouse heart against I/R injury by activating Nrf2 signaling in an Erk-dependent manner [21]. Kang et al reported the involvement of miR-1 in the protective effect of H_2S against cardiomyocyte apoptosis induced by I/R [22]. A study of Pan found that H_2S preconditioning protected the heart against I/R insults by PKC activation [23]. H_2S post-conditioning protects isolated rat hearts against I/R injury via activation of the JAK2/STAT3 signaling pathway [24]. It is also found that H_2S infusion reduced myocardial infarct size and improved regional left ventricular function in an I/R model by suppressing cardiomyocyte apoptosis and autophagy [25]. Consistent with previous studies, in the present study, we confirmed that MIRI impaired cardiac function and induced oxidative products including protein carbonyl, 8-OHdG, and MDA in the myocardium. It also activated MPO and pro-inflammatory factors, such as TNF-α and IL-1β. The treatment of H_2S for seven consecutive days exhibited dramatic improvement in cardiac function, as manifested by increased LVSP and ± (dP/dt) max, and decreased LVDP, as well as ameliorated oxidative stress and inflammation.

PTEN, a tumor suppressor gene, has been found to participate in growth, apoptosis, adhesion, invasion, and migration [16]. It was reported that the increased PTEN expression by recombinant adenovirus in cultured neonatal rat primary cardiomyocytes caused cardiomyocyte apoptosis [26]. On the other hand, the inhibition of PTEN limits myocardial infarct size and improves left ventricular function after MI [27]. The inactivation of PTEN activates the Akt signaling, reduces apoptosis, and increases survival [17, 18]. To explore the underlying mechanism, we first examined the PTEN mRNA expression and the PI3K/Akt pathway activation. PTEN mRNA was increased by MIRI but decreased by H_2S. Similarly, PTEN protein expression was enhanced by MIRI but decreased by H_2S and Phen. PI3K/Akt pathway was activated by H_2S and Phen but de-activated by LY294002. These results indicate that PTEN were regulated by MIRI and H_2S and could mediate the PI3K/Akt pathway in the MIRI model. Next, we re-measured the cardiac function in the presence of Phen or LY294002. The results revealed that the LVDP was decreased by Phen but increased by LY294002 compared to MIRI+H_2S. By contrast, LVSP, ± (dP/dt) max were increased by Phen but decreased by LY294002 compared to MIRI+H_2S, suggesting the involvement of PTEN/PI3K/Akt pathway.

These results revealed that H_2S may exert its cardioprotection by inactivation of PTEN, which in turn activates the PI3K/Akt signaling and increases survival in the MIRI model.

To conclude, the present study showed that H_2S could effectively protect heart against I/R injury. It may act as a strong antioxidant and protect the cardiac system through its antioxidant role. It may also restrain the extent of inflammation and limit the extent of MIRI by preventing cytokine release. These findings reveal that the PTEN/PI3K/Akt pathway plays an important role in the cardioprotection exerted by H_2S and suggest that exogenous elevated H_2S serve to protect heart against I/R injury and may serve as an important therapeutic target.

Disclosure of conflict of interest

None.

Address correspondence to: Wenxian Liu, Intensive Care Unit of Department of Cardiology, Beijing Anzhen Hospital, Capital Medical University, No. 2 Anzhen Road, Chaoyang District, Beijing 100029, China. Tel: +86010-6446552; E-mail: lwxicu@163.com

References

[1] Brodie BR. What anti-thrombotic therapy is best with primary PCI for acute ST elevation myocardial infarction: how should the HORIZONS trial change current practice? Catheter Cardiovasc Interv 2008; 71: 816-821.

[2] Keeley EC, Boura JA, Grines CL. Primary angioplasty versus intravenous thrombolytic therapy for acute myocardial infarction: a quantitative review of 23 randomised trials. Lancet 2003; 361: 13-20.

[3] Ibanez B, Heusch G, Ovize M, Van de Werf F. Evolving therapies for myocardial ischemia/reperfusion injury. J Am Coll Cardiol 2015; 65: 1454-1471.

[4] Bartos JA, Debaty G, Matsuura T, Yannopoulos D. Post-conditioning to improve cardiopulmonary resuscitation. Curr Opin Crit Care 2014; 20: 242-249.

[5] Abe K, Kimura H. The possible role of hydrogen sulfide as an endogenous neuromodulator. J Neurosci 1996; 16: 1066-1071.

[6] Wang R. Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. Physiol Rev 2012; 92: 791-896.

[7] Shen Y, Shen Z, Luo S, Guo W, Zhu YZ. The Cardioprotective effects of hydrogen sulfide in heart diseases: from molecular mechanisms to therapeutic potential. Oxid Med Cell Longev 2015; 2015: 925167.

[8] Hausenloy DJ, Yellon DM. New directions for protecting the heart against ischaemia-reperfusion injury: targeting the feperfusion injury salvage kinase (RISK)-pathway. Cardiovasc Res 2004; 61: 448-460.

[9] Zhao ZQ, Morris CD, Budde JM, Wang NP, Muraki S, Sun HY, Guyton RA. Inhibition of myocardial apoptosis reduces infarct size and improves regional contractile dysfunction during reperfusion. Cardiovasc Res 2003; 59: 132-142.

[10] Mehrhof FB, Muller FU, Bergmann MW, Li P, Wang Y, Schmitz W, Dietz R, von Harsdorf R. In cardiomyocyte hypoxia, insulin-like growth factor-I-induced antiapoptotic signaling requires phosphatidylinositol-3-OH-kinase-dependent and mitogen-activated protein kinase-dependent activation of the transcription factor cAMP response element-binding protein. Circulation 2001; 104: 2088-2094.

[11] Kupatt C, Hinkel R, Lamparter M, von Bruhl ML, Pohl T, Horstkotte J, Beck H, Müller S, Delker S, Gildehaus FJ, Büning H, Hatzopoulos AK, Boekstegers P. Retroinfusion of embryonic endothelial progenitor cells attenuates ischemia-reperfusion injury in pigs: role of phosphatidylinositol 3-kinase/AKT kinase. Circulation 2005; 112 Suppl: I117-122.

[12] Takagi H, Matsui Y, Sadoshima J. The role of autophagy in mediating cell survival and death during ischemia and reperfusion in the heart. Antioxid Redox Signal 2007; 9: 1373-1381.

[13] Uchiyama T, Engelman RM, Maulik N, Das DK. Role of Akt signaling in mitochondrial survival pathway triggered by hypoxic preconditioning. Circulation 2004; 109: 3042-3049.

[14] Tong H, Chen W, Steenbergen C, Murphy E. Ischemic preconditioning activates phosphatidylinositol-3-kinase upstream of protein kinase C. Circ Res 2000; 87: 309-315.

[15] Toth A, Nickson P, Qin LL, Erhardt P. Differential regulation of cardiomyocyte survival and hypertrophy by MDM2, an E3 ubiquitin ligase. J Biol Chem 2006; 281: 3679-3689.

[16] Ciuffreda L, Falcone I, Incani UC, Del Curatolo A, Conciatori F, Matteoni S, Vaccaro V, Cognetti F, Milella M. PTEN expression and function in adult cancer stem cells and prospects for therapeutic targeting. Adv Biol Regul 2014; 56: 66-80.

[17] Mocanu MM, Yellon DM. PTEN, the Achilles' heel of myocardial ischaemia/reperfusion injury? Br J Pharmacol 2007; 150: 833-838.

[18] Wu DN, Pei DS, Wang Q, Zhang GY. Down-regulation of PTEN by sodium orthovanadate inhibits ASK1 activation via PI3-K/Akt during cere-

bral ischemia in rat hippocampus. Neurosci Lett 2006; 404: 98-102.

[19] Yin X, Wang X, Fan Z, Peng C, Ren Z, Huang L, Liu Z, Zhao K. Hyperbaric oxygen preconditioning attenuates myocardium ischemia-reperfusion injury through upregulation of heme oxygenase 1 expression: PI3K/Akt/Nrf2 pathway involved. J Cardiovasc Pharmacol Ther 2015; 20: 428-438.

[20] Kida K, Yamada M, Tokuda K, Marutani E, Kakinohana M, Kaneki M, Ichinose F. Inhaled hydrogen sulfide prevents neurodegeneration and movement disorder in a mouse model of Parkinson's disease. Antioxid Redox Signal 2011; 15: 343-352.

[21] Peake BF, Nicholson CK, Lambert JP, Hood RL, Amin H, Amin S, Calvert JW. Hydrogen sulfide preconditions the db/db diabetic mouse heart against ischemia-reperfusion injury by activating Nrf2 signaling in an Erk-dependent manner. Am J Physiol Heart Circ Physiol 2013; 304: H1215-24.

[22] Kang B, Hong J, Xiao J, Zhu X, Ni X, Zhang Y, He B, Wang Z. Involvement of miR-1 in the protective effect of hydrogen sulfide against cardiomyocyte apoptosis induced by ischemia/reperfusion. Mol Biol Rep 2014; 41: 6845-6853.

[23] Pan TT, Neo KL, Hu LF, Yong QC, Bian JS. H2S preconditioning-induced PKC activation regulates intracellular calcium handling in rat cardiomyocytes. Am J Physiol Cell Physiol 2008; 294: C169-77.

[24] Luan HF, Zhao ZB, Zhao QH, Zhu P, Xiu MY, Ji Y. Hydrogen sulfide postconditioning protects isolated rat hearts against ischemia and reperfusion injury mediated by the JAK2/STAT3 survival pathway. Braz J Med Biol Res 2012; 45: 898-905.

[25] Osipov RM, Robich MP, Feng J, Liu Y, Clements RT, Glazer HP, Sodha NR, Szabo C, Bianchi C, Sellke FW. Effect of hydrogen sulfide in a porcine model of myocardial ischemia-reperfusion: comparison of different administration regimens and characterization of the cellular mechanisms of protection. J Cardiovasc Pharmacol 2009; 54: 287-297.

[26] Schwartzbauer G, Robbins J. The tumor suppressor gene PTEN can regulate cardiac hypertrophy and survival. J Biol Chem 2001; 276: 35786-35793.

[27] Keyes KT, Xu J, Long B, Zhang C, Hu Z, Ye Y. Pharmacological inhibition of PTEN limits myocardial infarct size and improves left ventricular function postinfarction. Am J Physiol Heart Circ Physiol 2010; 298: H1198-208.