Exogenous Hydrogen Sulfide Postconditioning Protects Isolated Rat Hearts From Ischemia/Reperfusion Injury Through Sirt1/PGC-1α Signaling Pathway

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Summary

Sirt1 is a highly conserved nicotinamide adenine dinucleotide (NAD^+) dependent histone deacetylase which plays an important role in heart diseases. Studies performed with Sirt1 activators indicated that it protects cells from ischemia/reperfusion (I/R) injury. The protective effects of H_2S against I/R injury also have been recognized. Hence, the present study was designed to explore whether Sirt1/PGC-1α participates in the protection of exogenous H_2S postconditioning against I/R injury in isolated rat hearts. Isolated rat hearts were subjected to 30 minutes of global ischemia followed by 60 minutes of reperfusion after 20 minutes of equilibrium. During this procedure, the hearts were exposed to NaHS (10 μmol/L) treatment in the absence or presence of the selective Sirt1 inhibitor EX-527 (10 μmol/L). NaHS exerted a protective effect on isolated rat hearts subjected to I/R, as shown by the improved expression of Sirt1/PGC-1α associated with restoration of Sirt1 nuclear localization, cardiac function, decreased myocardial infarct size, decreased myocardial enzyme release, and several biochemical parameters, including up-regulation of the ATP and SOD levels, and down-regulation of the MDA level. However, treatment with EX-527 could partially prevent the above effects of NaHS postconditioning. These results indicate that H_2S confers protective effects against I/R injury through the activation of Sirt1/PGC-1α. (Int Heart J 2016; 57: 477-482)

Key words: Hydrogen sulfide, Gasotransmitter, EX-527, Cardioprotection

Ischemic heart disease is known to be one of the major causes of death in the world and it can directly lead to myocardial infarction.[1)] The reestablishment of blood flow to the ischemic region is vital for the protection of ischemic myocardium from irreversible necrosis. However, paradoxically, the recanalization of blood may also cause additional damage to the heart independent of the ischemic insult, which is termed "reperfusion injury".[2)] As a prerequisite therapy for ischemic myocardial salvage, studies on reducing reperfusion injury are very urgent.

Hydrogen sulfide (H_2S) was previously known as a noxious gas, but now it is known as an endogenously produced gaseous mediator in various mammalian tissues.[3)] As a gasotransmitter, H_2S can rapidly travel through cell membranes without using specific transporters to directly activate intracellular targets. Recently, many studies have shown that H_2S plays important roles in alleviating ischemia/reperfusion (I/R) injury. The mechanisms of the protection include anti-apoptotic,[4)] mitochondrial protection,[5)] anti-inflammatory,[6)] and resistance to oxidative stress.[7)] Despite a significant amount of research being conducted, the protective mechanisms of H_2S in I/R-injured rats are still not entirely clear.

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Silent information regulator protein 1 (Sirt1) is a highly conserved pro-survival protein whose change in activity is associated with aging,[8)] and many diseases such as neurodegenerative diseases,[9)] cancer,[10)] and myocardial infarction.[11)] Recently, accumulating evidence suggests that activation of Sirt1 attenuates I/R injury by regulating several signaling elements, including peroxisome proliferator activated receptor gamma coactivator-1α (PGC-1α),[12)] FOXO1,[13)] and P53.[14)] Although recent research has reported that H_2S protects against apoptosis under oxidative stress through the Sirt1 pathway in an oxidative stress model,[7)] it is still unknown whether the Sirt1 signaling pathway participates in the protection of exogenous hydrogen sulfide postconditioning against I/R injury in isolated rat hearts. Therefore, the purpose of this study was to explore the role of Sirt1 in H_2S-mediated protection against I/R injury.

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Methods

Animals and reagents: Male Sprague-Dawley rats (8-10 weeks, 250 ± 30 g) were purchased from the Shang Hai Slac Laboratory Animal Co. Ltd. All animal experiments were conducted in compliance with the Guide for the Care and Use of Laboratory Animals published by the China National Institutes of Health and were approved by the Animal Ethics Committees of Jiangnan University, China. Rats were housed in an air filtered, temperature conditioned (20-25°C) and light controlled (12 hours light/dark cycle) room with a relative humidity of 50-55%. The rats had free access to standard commercial pellets and water.

Reagents: Sodium hydrosulfide (NaHS), EX-527 (a Sirt1 inhibitor), and 2, 3, 5-triphenyl tetrazolium chloride (TTC) were purchased from Sigma-Aldrich (USA). Mouse monoclonal anti-Sirt1 antibody and rabbit polyclonal anti-PGC-1α antibody were obtained from abcam (USA). Rabbit polyclonal anti-β-actin antibody was purchased from Bioworld Technology (USA). Lactate dehydrogenase (LDH), creatine kinase (CK), superoxide dismutase (SOD), malondialdehyde (MDA), and adenosine triphosphate (ATP) assay kits were purchased from Jiancheng Biological Engineering Institute (Nanjing, China).

Isolated heart preparation: Male SD rats were anesthetized with sodium pentobarbital (80 mg/kg) together with anti-coagulation by heparin (500 IU/kg) though intraperitoneal injection. After performing a midline sternotomy, the hearts were rapidly excised and placed in ice-cold modified Krebs-Henseleit (K-H) buffer solution to induce cardiac arrest. Next, the hearts were perfused retrogradely at a constant flow rate of 12 mL/minute with a modified K-H buffer containing (in mM: NaCl 118.5, $NaHCO_3$ 24.8, D-glucose 11, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, $CaCl_2 \cdot 2H_2O$ 2.25, pH 7.4). The perfusate buffer was saturated with a 95% O_2 and 5% CO_2 gas mixture at 37°C before use. A latex balloon was inserted in the left ventricle via the left atrium, inflated to 5-10 mmHg, and then connected to a PowerLab System (AD Instruments, Australia) to monitor the cardiac function parameters. All of the indicators were dynamic real-time monitored by a physiological signal acquisition system. Each heart was allowed to stabilize for a period of 20 minutes before application of the experimental protocols. Hearts were excluded from further study if after stabilization they failed to develop steady sinus rhythm or the left ventricle developed pressures that were less than 80 mmHg.

Experimental protocols: Sixty male SD rats were randomly assigned to 5 groups. The dose and route of administration of NaHS were as previously described.[15] After a 20-minute equilibration period, the hearts were subjected to the following specific protocol. 1) Control group (n = 12): The isolated hearts were perfused with standard K-H buffer solution for 110 minutes and with no intervention throughout the whole process. 2) I/R group (n = 12): After stabilization, the hearts were exposed to 30 minutes of global no-flow ischemia and then 60 minutes of reperfusion. 3) I/R + NaHS postconditioning group (I/R + NP group, n = 12): The procedure was similar to that for group 2, except that the reperfusion was initiated with 4 cycles of K-H buffer for 15s containing 10 μM NaHS following by 15s of K-H buffer only. 4) I/R + EX-527 group (I/R + EX group, n = 12): Administration of 10 μM EX-527 in DMSO (< 0.1%) for 10 minutes at the end of equilibration and 5 minutes at the onset of reperfusion. In order to completely block the effects of Sirt1, the selective Sirt1 inhibitor EX-527 was given 10 minutes before ischemia. 5) I/R + EX-527 + NaHS group (I/R + EX + NP group, n = 12): In addition to NaHS postconditioning, the selective Sirt1 inhibitor EX-527 (10 μM) in DMSO was administered for 10 minutes at the end of equilibration and 5 minutes at the onset of reperfusion.

Determination of infarct size: The myocardial infarct size was determined by TTC staining. At the end of reperfusion (60 minutes), the hearts were quickly removed from the Langendorff apparatus. After eliminating the right atrium and ventricle, they were frozen at -80°C for 5-10 minutes. Frozen hearts were sliced into uniform sections of 2-3 mm thickness (5 slices/heart) and the slices were incubated in 1% TTC for 20 minutes at 37°C to demarcate the viable and non-viable myocardium. The stained slices were scanned using an EPSON K200 Series scanner. Infarct area was quantified with ImageJ software and is presented as the percentage of total area of the left ventricle (%).

Measurement of CK and LDH level: The coronary effluent was collected immediately before ischemia and at the end of reperfusion for quantification of cardiac enzyme release. The levels of LDH and CK were measured with a colorimetric assay according to the manufacturer's protocol.

Analysis of MDA, ATP, and SOD levels in myocardial tissue: At the end of reperfusion, the myocardial tissue was stored at -80°C until use. The myocardial tissue was homogenized in ice-cold buffer to prepare a corresponding concentration homogenate. The levels were then measured using commercially available kits.

Real-time PCR analysis: Total RNA was extracted using an RNAprep pure Tissue Kit (Tian gen Biotechnology, China) and converted to cDNA using a cDNA synthesis kit (Takara Biotechnology, Japan). Real-time PCR was carried out in a Light Cycler480 °C apparatus (Roche) using an SYBR Green PCR Master Mix (Takara Biotechnology, Japan). The 3 kits were used according to the manufacturers' instructions. Light Cycler480 optical system software (SW1.5.1) was used to analyze the fluorescence intensity. The primers of Sirt1 were 5'-CACCGAGGAACTACCTGAT-3' (forward) and 5'-CATCCCAGCCTCCGTTAT-3' (reverse). PGC-1α primers were 5'-CCTCCATGCCTGACGGCACC-3' (forward) and 5'-GAGCTGAGTGTTGGCTGGCG-3' (reverse). The primer sequences of GAPDH were 5'-GGATGGAATTGTGAGGGAGA-3' (forward) and 5'-GTGGACCTCATGGCCTACAT (reverse). The primers utilized for the real-time PCR were devised in accordance with the GenBank Nucleotide Sequence Database. The PCR conditions were: initial denaturation of one cycle at 95°C for 1 minute, followed by amplification of 40 cycles at 95°C for 5 seconds and 60°C for 30 seconds. GAPDH was used as an endogenous reference and the results were calculated using the arithmetic formula "2-ΔΔCT".

Western blot analysis: Total proteins of cardiac tissue were extracted using PIPA lysis buffer (Beyotime Biotechnology, China). The protein concentration of each sample was quantified using a BCA protein assay kit (Thermo Scientific, USA). Equal amounts of proteins were boiled with 1/5 of SDS sample buffer and separated with 10%SDS-PAGE (30 minutes, 80 V;60 minutes,100 V) and electrophoretically transferred to a PVDF membrane (Millipore; 200 mA, 2 hours). After blocking for 1 hour at room temperature in 5% skim milk, mem-

Table. Effects of NaHS and EX-527 on the Hemodynamic Parameters of I/R-Injured Hearts

	Group	Baseline	Reperfusion	
			30 minutes	60 minutes
HR (beats/minute)	Control	250.47 ± 18.77	236.84 ± 25.44	241.97 ± 20.02
	I/R	271.47 ± 27.59	246.87 ± 24.10	254.52 ± 24.64
	I/R + NP	255.66 ± 20.86	245.16 ± 25.91	253.48 ± 31.06
	I/R + EX	258.07 ± 25.05	229.41 ± 35.82	267.32 ± 26.67
	I/R + EX + NP	256.13 ± 23.52	264.55 ± 23.16	252.09 ± 27.85
LVEDP (mmHg)	Control	6.49 ± 1.50	8.23 ± 1.62	8.60 ± 1.74
	I/R	6.74 ± 1.62	49.44 ± 3.12*	45.10 ± 10.72*
	I/R + NP	6.43 ± 2.13	33.23 ± 5.04$^{\#}$	33.74 ± 5.73$^{\#}$
	I/R + EX	6.17 ± 1.63	45.42 ± 6.31$^{\&}$	45.85 ± 6.52$^{\&}$
	I/R + EX + NP	6.22 ± 1.26	45.62 ± 6.04$^{\&}$	42.93 ± 6.59$^{\&}$
LVDP (mmHg)	Control	108.54 ± 7.29	106.89 ± 13.54	102.42 ± 9.79
	I/R	107.45 ± 9.08	42.23 ± 10.84*	43.95 ± 13.72*
	I/R + NP	104.66 ± 11.29	58.74 ± 16.32$^{\#}$	62.49 ± 12.82$^{\#}$
	I/R + EX	109.41 ± 12.34	40.76 ± 12.30$^{\&}$	48.58 ± 9.19$^{\&}$
	I/R + EX + NP	103.65 ± 7.43	44.19 ± 10.29$^{\&}$	45.76 ± 13.16$^{\&}$
$+dP/dt_{max}$ (mmHg/second)	Control	2986.13 ± 246.78	2983.78 ± 230.63	2887.14 ± 229.80
	I/R	2812.59 ± 304.74	1161.32 ± 150.37*	1271.54 ± 190.01*
	I/R + NP	2793.87 ± 285.08	1550.44 ± 222.63$^{\#}$	1660.54 ± 243.01$^{\#}$
	I/R + EX	2871.07 ± 312.77	967.89 ± 223.15$^{\&}$	1195.00 ± 229.19$^{\&}$
	I/R + EX + NP	2818.31 ± 250.17	1110.14 ± 279.10$^{\&}$	1258.46 ± 228.11$^{\&}$
$-dP/dt_{min}$ (mmHg/second)	Control	-2072.32 ± 175.40	-1944.42 ± 232.19	-1885.76 ± 225.76
	I/R	-2011.13 ± 192.28	-849.13 ± 216.49*	-936.08 ± 205.48*
	I/R + NP	-1959.82 ± 103.57	-1206.92 ± 267.15$^{\#}$	-1223.76 ± 204.20$^{\#}$
	I/R + EX	-2065.44 ± 212.50	-853.84 ± 282.28$^{\&}$	-887.548 ± 228.11$^{\&}$
	I/R + EX + NP	-2047.58 ± 174.61	-904.85 ± 221.55$^{\&}$	-953.84 ± 173.28$^{\&}$

The results are expressed as the mean ± SD of 8 rat hearts per group. HR indicates heart rate; LVEDP, left ventricular end-diastolic pressure; LVDP, the left ventricular developing pressure; $+dP/dt_{max}$, maximum derivatives of the ventricular pressure; $-dP/dt_{min}$, minimum derivatives of the ventricular pressure. $^{*}P < 0.05$ versus control group; $^{\#}P < 0.05$ versus I/R group; $^{\&}P < 0.05$ versus I/R + NaHS group.

branes were incubated overnight at 4°C with the indicated primary antibodies (Sirt1, 1:1000; PGC-1α, 1:1000; β-actin, 1:3000). After washing 3 times, the membranes were incubated with horseradish peroxidase conjugated secondary antibody (1:1000) for 2 hours at room temperature on a shaker. β-Actin was used to monitor the amounts of samples applied. The intensity of each band was determined using Image J software.

Immunohistochemistry: At the end of reperfusion, the heart was sectioned and small pieces of the heart were immersion fixed for 24 hours in 10% formalin solution.[16] Next, all of the samples were processed using a standard histological procedure.[12] After blocking in 5% BSA for 30 minutes, sections were incubated with anti-Sirt1 antibody (1:200) and anti-PGC-1α antibody (1:300) at 4°C overnight. Sections were then incubated in biotinylated secondary antibody for 30 minutes and DAB for 60 seconds to visualize the immunolabeling. Finally, the sections were counterstained with hematoxylin, mounted, and covered with cover slips. Images were acquired with a light microscope (Nikon ECLIPSE 80i, Olympus, Japan) at 40·.

Statistical analysis: All data are expressed as the mean ± standard deviation (SD). The results were analyzed by one-way ANOVA followed by the Student-Newman-Keuls test. A $P < 0.05$ was considered statistically significant (SPSS 19 software).

Results

Effects of NaHS and EX-527 on cardiac function during ischemia and reperfusion: No significant differences in baseline were observed among the experimental groups (Table). All other hemodynamic parameters showed a significant difference compared to the control group at 30 and 60 minutes reperfusion ($P < 0.05$) except heart rate. Compared to the I/R group, the I/R + NP group showed significant increases in LVDP and $\pm dP/dt_{max}$ and a significant decrease in LVEDP ($P < 0.05$). However, compared to the I/R group, the addition of EX-527 (a selective inhibitor of Sirt1) reversed the beneficial effects of NaHS on the hemodynamic parameters ($P > 0.05$).

Effects of NaHS and EX-527 on myocardial tissue injury: There was a significant increase in infarct size in the I/R group compared with the control group (control: 16.5% ± 4.3% versus I/R: 48.7% ± 6.2%, $P < 0.01$, Supplemental Figure 1A). NaHS postconditioning produced a significant reduction in myocardial infarct size (I/R + NP: 27.1% ± 5.2% versus I/R: 48.7% ± 6.2%, $P < 0.05$). However, the addition of EX-527 reversed the cardiac protection provided by NaHS postconditioning (I/R + EX + NP: 43.8% ± 3.5% versus I/R + NP: 27.1% ± 5.2%, $P < 0.05$).

To further determine the extent of I/R-induced myocardial injury, the levels of LDH and CK in coronary effluent

were measured. A 20-minute equilibration perfusion did not lead to significant enzyme release in any group. At the end of reperfusion, compared to the control group, the levels of LDH and CK in coronary effluent increased significantly in the I/R group ($P < 0.05$, Supplemental Figure 1B and 1C). NaHS postconditioning significantly limited the release of LDH and CK from coronary effluent compared with the I/R group ($P < 0.05$). However, when administered with EX-527, it abolished the protection ($P < 0.05$).

Effects of exogenous H_2S and EX-527 on levels of Sirt1 and PGC-1α mRNA: According to previous reports, Sirt1 activators changed the expression of Sirt1 in a time or dose dependent manner.[12,17] Since the dose and duration of NaHS were constant in our study, we first measured the level of Sirt1 mRNA to explore the effects of exogenous H_2S on Sirt1 expression as well as PGC-1α which has been reported to be a major target of Sirt1. Compared with the control group, I/R increased the levels of Sirt1 and PGC-1α mRNA, although the increases were not statistically significant (Supplemental Figure 2A and 2B). Also, the level of Sirt1 mRNA was further augmented with exogenous H_2S treatment after I/R ($P < 0.05$). However, the addition of EX-527did not completely reverse the increase of Sirt1 mRNA by NaHS postconditioning ($P > 0.05$), while EX-527 abolished the effect of exogenous H_2S on PGC-1α mRNA ($P < 0.05$).

Effects of exogenous H_2S and EX-527 on expression of Sirt1 and PGC-1α: In order to further determine the role of Sirt in NaHS postconditioning, we examined the expression levels of Sirt1 and PGC-1α. Compared to the control group, there was a noticeable loss of Sirt1 in the I/R group (Supplemental Figure 2C and 2D). In contrast, pretreatment with NaHS significantly increased Sirt1 expression, and this effect was attenuated by pretreatment with EX-527. PGC-1α was minimally expressed in the control group, but was induced after I/R and further augmented with NaHS treatment after I/R. However, this effect was reversed by the addition of Ex-527. These results indicated that H_2S might directly regulate the protein level of Sirt1 and PGC-1α under I/R injury.

Effects of exogenous H_2S and EX-527 on the location of Sirt1: The importation of Sirt1 to the nucleus was found to be essential for its cytoprotective effects against oxidative stress.[18] It is intriguing to speculate that nuclear translocation of Sirt1 may have a role in the effects of NaHS postconditioning associated with the increase of Sirt1 expression. Hence, we examined the localization of Sirt1 by immunohistochemistry. In the control group, Sirt1 was predominantly located within the nucleus and cytoplasmic staining was very light (Supplemental Figure 3A). In the I/R group, the percentage of Sirt1-positive nuclei was significantly decreased, which was replaced by more intense cytoplasmic staining. NaHS treatment markedly restored the nuclear Sirt1 and also increased the cytoplasmic staining. However, this effect was reversed by Ex-527 (Supplemental Figure 3B).

Effects of NaHS and EX-527 treatment on ATP, MDA, and SOD levels: We continued to test the effect of NaHS on the levels of ATP, MDA, and SOD with or without the Sirt1 inhibitor. Compared with the control group, the I/R group had decreased ATP and SOD levels and an increased MDA level ($P < 0.05$). In the I/R + NP group, the ATP and SOD levels were increased and the MDA level was decreased ($P < 0.05$ versus I/R group). This effect was reversed by the addition of Ex-527 (Supplemental Figure 4).

Discussion

Although both H_2S and Sirt1 have been shown to play a similar role in ischemia/reperfusion injury, there is no information about the interaction between them. Therefore, the aim of this study was to test the hypothesis that H_2S protected the hearts against I/R injury through a Sirt1-mediated pathway. The results from the current study suggest that NaHS could alleviate myocardial injury induced by I/R in association with improved Sirt1/PGC-1α expression, Sirt1 nuclear location, cardiac function, reduced infarct size, and the leakage of myocardial enzymes (LDH, CK). Improved cardiac function was indicated by significant increases in LVDP and $\pm dP/dt_{max}$ and a significant decrease in LVEDP ($P < 0.05$). However, at none of the different time points was there a significant difference among groups in heart rate, a finding which was similar to that of Behmenburg, *et al*.[19] It might be possible that 30 minutes of global no-flow ischemia is not sufficient to cause serious sinoatrial node injury and that a constant flow rate provides sufficient heart perfusion to help maintain heart pumping. These effects were largely reversed by the addition of Ex-527, a specific inhibitor of Sirt1. The dosage of Ex-527 was selected based on previous reports[20-22] and our preliminary experiments.

As a protective molecule, Sirt1 regulates a wide array of cellular processes that are crucial to cell survival, apoptosis, cell growth, cell senescence, and metabolism.[10,23] In myocardial ischemia/reperfusion injury, the activation of Sirt1 also plays a key role in cardioprotection.[24,25] In the present study, we measured the effect of NaHS on the expression of Sirt1 with or without Ex-527 by Western blot and real-time PCR analysis. As expected, we found that Sirt1 was up-regulated by exogenous H_2S postconditioning. Although the increase of Sirt1 mRNA by NaHS postconditioning was not completely abolished by EX-527 ($P > 0.05$), it still abolished the effects of H_2S postconditioning to a certain extent. Since this was the first application of EX-527 to this animal model, the dosage and duration may not be entirely appropriate. In addition, Revollo, *et al* demonstrated that a synergistic system seems to have evolved in the regulation of Sirt1, whereby the levels of factors that impact the activity of Sirt1 coordinately shift with Sirt1 expression levels.[26] Overinhibition of Sirt1 activity may also increase the level of Sirt1 mRNA which made the significance unclear. However, the exact mechanism of this phenomenon remains to be clarified.

Sirt1 expresses in all mammalian cells and was originally identified as a nuclear protein.[27] However, recent studies also showed that subcellular localization of Sirt1 differs from cell to cell and that it is a nucleocytoplasmic shuttling protein.[28] Meanwhile, the importation of Sirt1 to the nucleus was demonstrated to be essential for its cytoprotective effects.[18] Therefore, we examined the localization of Sirt1 by immunohistochemistry. In contrast to previous reports,[29] we found that Sirt1 is predominantly localized in the cardiomyocyte nuclei, which is a finding similar to that of Tong, *et al*.[12,30] One reason to explain this difference in findings is that our experiment protocol is different from that of Tanno, *et al* and slight injury caused by continuous perfusion may promote the translocation of Sirt1.

H_2S improved Sirt1 nuclear location associated with the increase of Sirt1 expression. These results confirmed that Sirt1 plays an important role in H_2S-mediated protection against I/R injury. Two clinical studies have reported that a change in Sirt1 level contributes to myocardial infarction pathogenesis[11] and normal human cardiomyocytes predominantly expressed Sirt1 in their cytoplasm.[18] These two may make humans more susceptible to ischemia/reperfusion injury. The results of previous studies have shown that the activity of Sirt1 can be modulated by higher levels of NAD^+,[31] members of the FOXO family of transcription factors,[32] peroxisome proliferator-activated receptors (PPARs), phosphorylation, and ubiquitylation.[26] In order to utilize the effects of H_2S, we should next focus our attention on understanding the molecular mechanisms through which H_2S postconditioning changes Sirt1 expression and nuclear location.

Oxidative stress plays an important role in the pathogenesis of ischemic/reperfusion injury. Since the majority of superoxide is generated from the mitochondrial respiratory chain, mitochondria are directly damaged by oxidative stress.[33] Sirt1 can preserve mitochondrial function though post-translational modification of mitochondrial proteins and regulating the activity of antioxidant enzymes.[31,34] PGC-1α is not only a target of Sirt1 but also a marker of mitochondrial biogenesis.[35] Thus, we use PGC-1α protein expression and ATP level as indicators of mitochondrial status. MDA, a cytotoxic product of lipid peroxidation, is a biomarker for oxidative stress and reflects the extent of ROS.[36] SOD scavenges the superoxide anion radical by catalyzing its dismutation to oxygen and hydrogen peroxide.[37] Likewise, these two are used as an indicator of cellular redox status. The PGC-1α, ATP, and SOD levels were increased and the MDA level was decreased in the I/R + NP group ($P < 0.05$ versus I/R group). These effects were reversed by Ex-527 treatment. These data indicated that the effects of Sirt1 on the protection of mitochondrial function and the regulation of antioxidant enzymes activity may be involved in the H_2S-mediated protection against I/R injury (Supplemental Figure 5). In conclusion, the present findings have established that Sirt1/PGC-1α participated in the H_2S-mediated protection against I/R injury in isolated rat hearts.

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Supplemental Files

Supplemental Figure 1, 2, 3, 4, 5
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