Hydrogen sulfide exhibits cardioprotective effects by decreasing endoplasmic reticulum stress in a diabetic cardiomyopathy rat model

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Abstract. Endoplasmic reticulum (ER) stress is critical in the occurrence and development of diabetic cardiomyopathy (DC). Hydrogen sulfide (H_2S) has been found to be the third gaseous signaling molecule with anti-ER stress effects. Previous studies have shown that H_2S acts as a potent inhibitor of fibrosis in the heart of diabetic rats. This study aimed to demonstrate whether H_2S exhibits protective effects on the myocardium of streptozotocin (STZ)-induced diabetic rats by suppressing ER stress. In this study, diabetic models were established by intraperitoneal (i.p.) injection of 40 mg/kg STZ. The STZ-treated mice were divided into three groups, and subsequently treated with normal saline, 30 μmol/kg or 100 μmol/kg NaHS, i.p., respectively, for 8 weeks. The extent of myocyte hypertrophy was measured using hematoxylin and eosin-stained sections and collagen components were investigated using immunostaining. The expression of glucose-regulated protein (Grp78), C/EBP-homologous protein (CHOP) and caspase-12 in the heart tissue of each group was detected by western blot analysis. It was demonstrated that H_2S could improve myocardial hypertrophy and myocardial collagen deposition in diabetic rats. In addition, it could reduce the expression of Grp78, caspase-12 and CHOP. In conclusion, these findings demonstrate that H_2S suppresses STZ-induced ER stress in the hearts of rats, and it may serve as a novel cardioprotective agent for DC.

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Introduction

In diabetic patients, a wide range of structural reconfiguration has been observed, such as cardiomyocyte hypertrophy, ventricular dilation, prominent interstitial fibrosis (1,2), diastolic and systolic dysfunction, and left ventricular hypertrophy (3,4). This disease process is termed diabetic cardiomyopathy (DC), which is a heart muscle-specific disease without other vascular pathology (5,6). Investigation revealed that patients with diabetes mellitus (DM) were more likely to suffer from coronary artery disease, hypertension and mortality following myocardial infarction (7,8), while DC was one of the most common serious cardiovascular complications of DM (9). Cellular metabolic abnormalities and defections of organelles were shown to participate in the pathology of DC (10-14), which is a chronic and complex process associated with impaired calcium homeostasis, increased lipid uptake, myocardial insulin resistance, glucotoxicity, increased oxidative stress and activation of the renin-angiotensin system (15). Furthermore, the pathological role of endoplasmic reticulum (ER) stress in DC has been noted in a number of studies (16-18).

The endoplasmic reticulum (ER) is central in lipid synthesis, calcium homeostasis, and the folding and maturation of membrane and secretory proteins (19). The normal functions of the ER can be disturbed by various conditions, such as glucotoxicity, ER-Ca^{2+} disequilibrium, ischemia and hypoxia, free radicals, hyperhomocysteine, increased protein synthesis and gene mutation, which results in ER stress (ERS) (20-25). ERS occurs in a number of pathological conditions, such as diabetic kidney disease (26). ERS is known to be involved in a number of complex homeostatic signaling pathways among which the unfolded protein response (UPR) is most commonly recognized (27). The expression of glucose-regulated protein 78 (Grp78) can be activated by the UPR, which is known to be a safeguard for normal function of the ER. Grp78 is an ER resident protein, which reacts to accumulated proteins. Moderate ER stress can relieve injury triggered by stress, while severe and chronic ERS result in apoptosis and induce a number of diseases. Apoptotic processes can be initiated by caspase-12-dependent pathways and CHOP-dependent pathways (28,29), which are ER-specific pathways. Recently, a number of studies have demonstrated the crucial role of ER stress in the development of DC (30,31). Therefore, it was

hypothesized that downregulating ER stress in diabetic rats could prevent the development of DC.

Hydrogen sulfide (H_2S) is a toxic gas with a pungent rotten egg smell. It is produced naturally in mammalian tissues and exhibits various biological and physiological effects (32-35). A large number of experiments showed that H_2S had the anti-ER stress, anti-apoptosis, anti-inflammatory and anti-oxidant effects (36-39). In the nervous system, H_2S acts as a neuroprotectant and may exhibit pharmacological effects in patients with Parkinson's disease and Alzheimer's disease (40-45). A recent study found that H_2S had antidepressant-like and anxiolytic-like effects (46). In the cardiovascular system, H_2S was shown to relax smooth muscles, regulate blood pressure (47-49) and prevent atherosclerosis (39,50-52), which resulted in the prevention of ischemia-reperfusion injury in myocardial cells (53-55). In DM, H_2S has been shown to improve insulin resistance and protect β-cells in the pancreas (56,57). A previous study has also shown that H_2S acts as a potent inhibitor of fibrosis in the heart of diabetic rats (58). Thus, these studies suggested that H_2S may exhibit a cardioprotective effect in the pathophysiology of DC. However, whether H_2S can prevent the pathological process of DC by suppressing ERS has not yet been demonstrated. Thus, the present study aimed to demonstrate whether H_2S exhibits protective effects on the myocardium of streptozotocin (STZ)-induced diabetic rats by suppressing ERS.

Materials and methods

Animals. Fifty adult male Sprague-Dawley (SD) rats, weighing 280-300 g, were purchased from the SJA Lab Animal Center of Changsha (Changsha, China). The animals were maintained in accordance with institutional policies, and all experiments were performed with approval of the University of China Committee on the Use and Care of Animals of University of South China (Hengyang, China).

Drugs and reagents. Sodium hydrosulfide (NaHS) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Streptozotocin (STZ) was purchased from MP Biomedicals Company (Santa Ana, CA, USA). Specific monoclonal anti-GRP78 antibody was purchased from Epitomics Inc. (Burlingame, UK). Specific monoclonal anti-CHOP antibodies were purchased from Proteintech Group, Inc. (Chicago, IL, USA). Specific monoclonal anti-caspase-12 antibodies were obtained from Sigma-Aldrich. Anti-rabbit and anti-rat IgG secondary antibodies were purchased from Proteintech Group, Inc. Cell lysis buffer for western blot analysis, Enhanced Chemiluminescence Reagent kit, Bicinchoninic acid assay (BCA) Protein Assay kit and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) Gel Preparation kit were purchased from Beyotime Institute of Biotechnology (Shanghai, China).

Study design. Prior to the experiment, rats were adapted to the experimental environment for one week. During this period, SD rats were kept under a 12 h light/dark cycle at a constant temperature (23±1˚C) and humidity (60%). They had access to standard rat chow and normal water *ad libitum*. NaHS, acting as a H_2S donor, was dissolved in stroke-physiological saline solution (also termed 'physiological saline'; purchased from Ruji Biological Technology Development Co., Ltd., Shanghai, China) and filtered through a 0.2 mm device (Pall Corporation, Port Washington, NY, USA). STZ was dissolved in sodium citrate buffer (pH 4.4; the citric acid and trisodium citrate were purchased from Sinpharm Chemical Reagent Co., Ltd (Shanghai, China). One week later, DM was induced in SD rats by a single intraperitoneal (i.p.) 40 mg/kg STZ injection after an overnight fast. Instead of normal water, 5% glucose solution (Kelun Pharmaceutical Co., Ltd., Hunan, China) was administered to STZ-treated rats for 24 h after injection in order to prevent death caused by hypoglycemic shock. At 72 h following the STZ injection, blood samples were collected from the tail vein to measure blood glucose levels. Rats with fasting blood glucose levels >16.7 mmol/l were considered successful DM models and were used for further investigation (59). The normal rats who did not accept the treatment of STZ were randomly divided into two groups: The control group (treated with normal saline injection every day), and the H_2S-c2 group (normal rats treated with a high concentration of NaHS, c2=100 μmol/kg; received i.p., daily). By contrast, the rats who accepted the treatment of STZ were divided into three groups: the STZ group (diabetic rats group, treated with normal saline injection every day), the STZ+H_2S-c1 group (diabetic rats treated with a low concentration of NaHS; c1=30 μmol/kg) and the STZ+H_2S-c2 group (diabetic rats treated with a high concentration of NaHS; c2=100 μmol/kg). The rats had free to access to food and water during the experiment, and feeding conditions were consistent with the acclimatization period. Eight weeks later, rats in the five groups were weighed. Rats were anesthetized by i.p. injection of chloral hydrate (350 mg/kg). Thoracic cavities were opened, and the hearts of rats were lavaged with ice-cold normal saline, then removed and weighed. Three rats were randomly selected from the five groups, their myocardial tissues were fixed in 10% formalin for immunohistochemical examination. The remaining hearts were preserved at -80˚C prior to further analysis. This study including animal care was supervised and approved by the Animal Ethics Committee of the University of South China.

Body weight, heart weight/body weight (HW/BW) and blood glucose assay. The body weight of the rats, and their blood glucose levels, were measured immediately prior to the STZ injection, and also subsequently, prior to their sacrifice. The following formula was used to analyze HW/BW: HW/BW = (heart weight / body weight) x 100. Tail vein blood was tested for the blood glucose levels and it was analyzed during the acclimatization period, 72 h after STZ injection and before the end of experiment.

Histopathology and immunohistochemistry. Myocardium samples from rats were fixed using 4% paraformaldehyde (Sinopharm Chemical Reagent Co.), dehydrated with alcohol, embedded in paraffin (Sinopharm Chemical Reagent Co.) and cut into 5 μm sections. Some of the samples were stained using a hematoxylin and eosin (H&E) staining kit (Beyotime Institute of Biotechnology) and observed under a microscope (Motic BA210; Motic Medical Diagnostic Systems, Co.,

Ltd., Xiamen, China) at a magnification of ×200. Several of the sections were incubated overnight with rabbit polyclonal anti-collagen I or rabbit polyclonal anti-collagen III. Subsequently, after washing three times (10 min each wash) in 0.1 M Tris buffer, the sections were flat-mounted, placed on coverslips, and images were captured using microscopy (Motic BA210; Motic Medical Diagnostic Systems, Co., Ltd.). The extent of myocyte hypertrophy was measured using H&E-stained sections; collagen components were displayed by immunostaining (60).

SDS-PAGE and western blot analysis. Cell lysis buffer (20 mM Tris, pH 7.5; 150 mM NaCl and 1% Triton X-100) containing protease inhibitors (sodium pyrophosphate, β-glycerophosphate, EDTA, $Na_3VO_{4\ and}$ leupeptin) was added to heart tissues and homogenized on the ice. Then, lysates were centrifuged at 12,000 rpm (7,992 g) for 30 min at 4˚C to attain the supernatant. Protein concentrations were quantified using a BCA protein assay kit. Protein (15 μg) was used for electrophoresis using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [the SDS-PAGE gel preparation kit (cat. no. P0012A) was purchased from Beyotime Institute of Biotechnology, and the concentration (%) was made up according to the molecular weight of the proteins], and transferred to a polyvinylidene difluoride membrane (Merck Millipore, Billerica, MA, USA). It was then blocked in Tris-buffered saline with Tween (TBST) (50 mM Tris-HCl, pH 7.5; 150 mM NaCl; and 0.05% Tween-20) containing 5% skimmed milk for 2 h. The primary antibodies used for western blot analysis were diluted in TBST buffer (5% skimmed milk) at the following concentrations: The monoclonal antibodies used were: Monoclonal anti-GRP78 antibody (1:2,000; cat. no. Ab108613, purchased from Epitomics, Inc., Burlingame, CA, USA), specific monoclonal mouse anti-CHOP antibody (1:1,000; cat. no. 60304-1-Ig, purchased from ProteinTech Group, Inc., Chicago, IL, USA), specific rabbit monoclonal anti-caspase-12 antibody (1:2,000, cat. no. SBP2325, purchased from Sigma-Aldrich, Inc., St. Louis, MO, USA) and mouse anti-β-actin monoclonal antibody (1:2,000; cat. no. 60008-1-Ig, purchased from ProteinTech Group, Inc.). The secondary antibodies used were as follows: Peroxidase-conjugated Affinipure goat anti-rabbit immunoglobulin G (IgG)(H+L) (1:8,000; cat. no. SA00001-2, purchased from ProteinTech Group, Inc.) and peroxidase-conjugated Affinipure goat anti-Mouse IgG(H+L) (1:8,000, cat. no. SA00001-1, purchased from ProteinTech Group, Inc.). Rabbit polyclonal anti-collagen I (1:400, cat no. BA0235) and rabbit polyclonal anti-collagen III (1:400, cat no. BA0326) were purchased from Wuhan Boster Biological Technology, Ltd. (Wuhan, China). The membranes were incubated with primary antibody overnight at 4˚C. The following day, the membranes were washed with TBST buffer three times (for 15 min each time), and incubated with secondary antibody for 2 h at normal temperature. Subsequently, the membrane was washed again. Finally, the blot was visualized using an enhanced chemiluminescence reagent kit and the optical density was quantified using the Molecular Imager VersaDoc MP 5000 system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). β-actin was used to ensure that equal protein was loaded in every sample. All values were normalized by setting the optical density of the control group to 1.0.

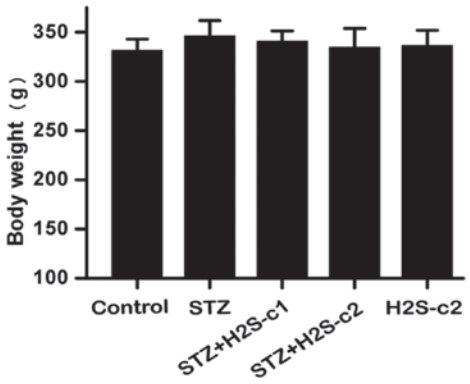

Figure 1. Body weight before streptozotocin injection.

Statistical analysis. Data are presented as the mean ± standard error values. Potential differences between groups with different treatments were determined using one-way analysis of variance. P<0.05 was considered to indicate a statistically significant difference. All analysis was performed using SPSS software package 18.0 (SPSS Software, Inc., Chicago, IL, USA).

Results

H_2S has no effect on BW, HW, HW/BW and plasma glucose concentration. The body weight of the rats prior to the STZ injection was determined, and no significant differences among the five groups were identified (Fig. 1). The BW, HW, HW/BW and plasma glucose concentration were detected before the end of the experiment. The results revealed that the BW and HW in the Control group were significantly higher compared with those in the STZ group (P<0.001), the STZ+H_2S-c1 group (P<0.001) and the STZ+H2S-c2 group (P<0.001). No significant differences in BW and HW were identified among the STZ, the STZ+H2S-c1 and the STZ+H_2S-c2 groups. Blood glucose levels in the STZ, STZ+H_2S-c1 and STZ+H_2S-c2 groups were significantly higher compared with that in the Control group (P<0.001), although no significant differences were identified when making comparisons among the STZ, STZ+H_2S-c1 and STZ+H_2S-c2 groups (Table I).

H_2S can reduce myocardial fibrosis caused by STZ-induced high plasma glucose concentrations. Myocardial hypertrophy was analyzed by hematoxylin and eosin staining. The results show that the myocardial tissue structure was tightly organized in the control and H_2S-c2 groups, whereas it was notably looser with a less-ordered structure in the STZ group. However, these pathological changes were improved following treatment with H_2S in the STZ+H_2S-c1 and STZ+H_2S-c2 groups (Fig. 2).

H_2S can reduce myocardial fibrosis caused by high plasma glucose concentration. Myocardial collagen fibrosis was detected by immunostaining. Results showed that type I collagen (Fig. 3A) and type III collagen (Fig. 3B) expression was markedly increased, accompanied by disordered arrangement in the

Table I. Effect of H_2S on HW, BW, HW/BW and BG.

Parameter	Control	STZ	STZ+H_2S-c1	STZ+H_2S-c2	H_2S-c2
BW (g)	437.71±64.75	269.86±20.41[b]	285.57±34.60[b]	280.14±12.06[b]	471.29±24.16
HW (g)	1.46±0.17	1.04±0.10[b]	1.08±0.13[b]	1.07±0.13[b]	1.52±0.96
HW/BW (x100%)	0.34±0.02	0.39±0.04[a]	0.38±0.05[a]	0.38±0.05[a]	0.32±0.01
BG (mmol/l)	7.26±0.58	28.5±2.55[b]	24.5±6.96[b]	25.2±5.54[b]	8.12±2.06

HW, heart weight; BW, body weight; BG, blood glucose. [a]P<0.05 and [b]P<0.001, compared with the control group. STZ, streptozotocin, H_2S, hydrogen sulfide.

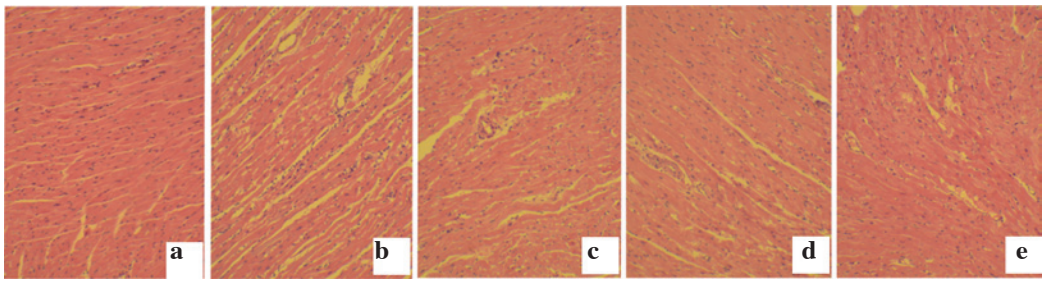

Figure 2. Effect of H_2S on the myocardial hypertrophy caused by hyperglycemia. (a) Control group; (b) STZ group; (c) STZ+H_2S-c1 group; (d) STZ+H_2S-c2 group; and (e) H_2S-c2 group. H_2S, hydrogen sulfide; STZ, streptozotocin. The cells were stained with hematoxylin and eosin (x200).

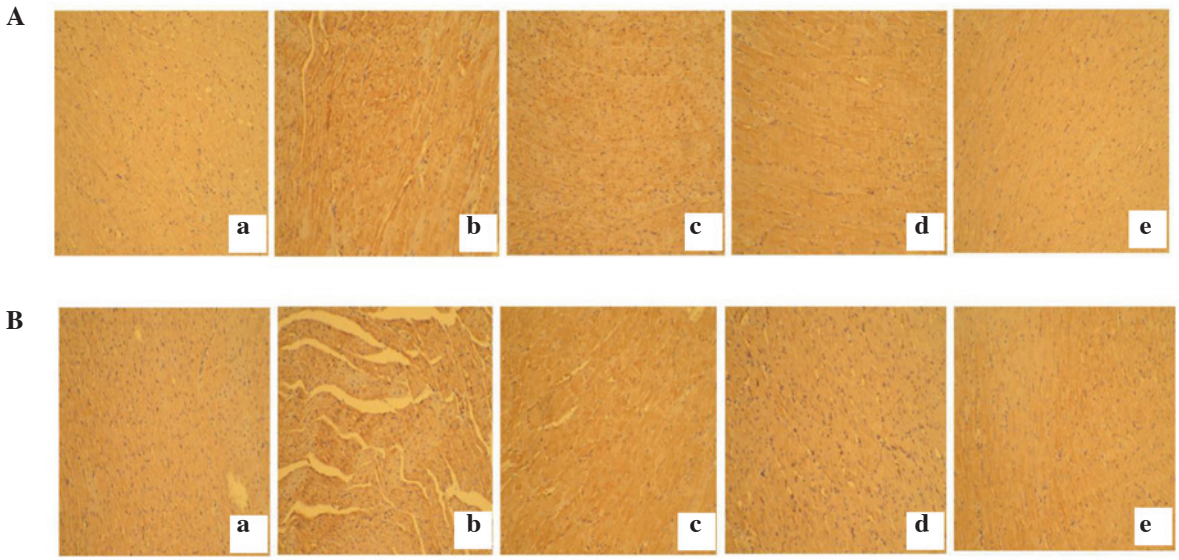

Figure 3. Effect of H_2S on the myocardial collagen fibrosis in the heart tissue of diabetes mellitus. Immunostaining of (A) collagen I and (B) collagen III after treatment of the rats with NaHS (30 μmol/kg and 100 μmol/kg, ip) for 8 weeks (magnification, x40). (a) Control group; (b) STZ group; (c) STZ+H_2S-c1 group; (d) STZ+H_2S-c2 group; (e) H_2S-c2 group. H_2S, hydrogen sulfide; STZ, streptozotocin.

STZ group compared with the control group. These changes were reversed by H_2S intervention.

H_2S reduces ERS caused by high plasma glucose concentrations. High plasma glucose concentrations, caused by the injection of STZ, significantly increased the expression of caspase-12 (P<0.01), GRP-78 (P<0.05) and CHOP (P<0.05) proteins, which are markers of ERS. The expression of these proteins was shown to be decreased in STZ+H_2S-c1 (caspase-12 P<0.05, GRP-78 P<0.05, CHOP P<0.05) and STZ+H_2S-c2 (caspase-12 P<0.001, GRP-78 P<0.05, CHOP P<0.05) groups, and the expression of GRP-78 and CHOP was not significant different between the control, H_2S-c2, STZ+H_2S-c1 and STZ+H_2S-c2 groups. However, the expression of caspase-12 protein was lower in STZ+H_2S-c2 and H_2S-c2 groups compared with the STZ+H_2S-c1 group (STZ+H_2S-c2 P<0.05, H_2S-c2 P<0.01), and was also lower in the H_2S-c2 group compared with the control group (P<0.05).

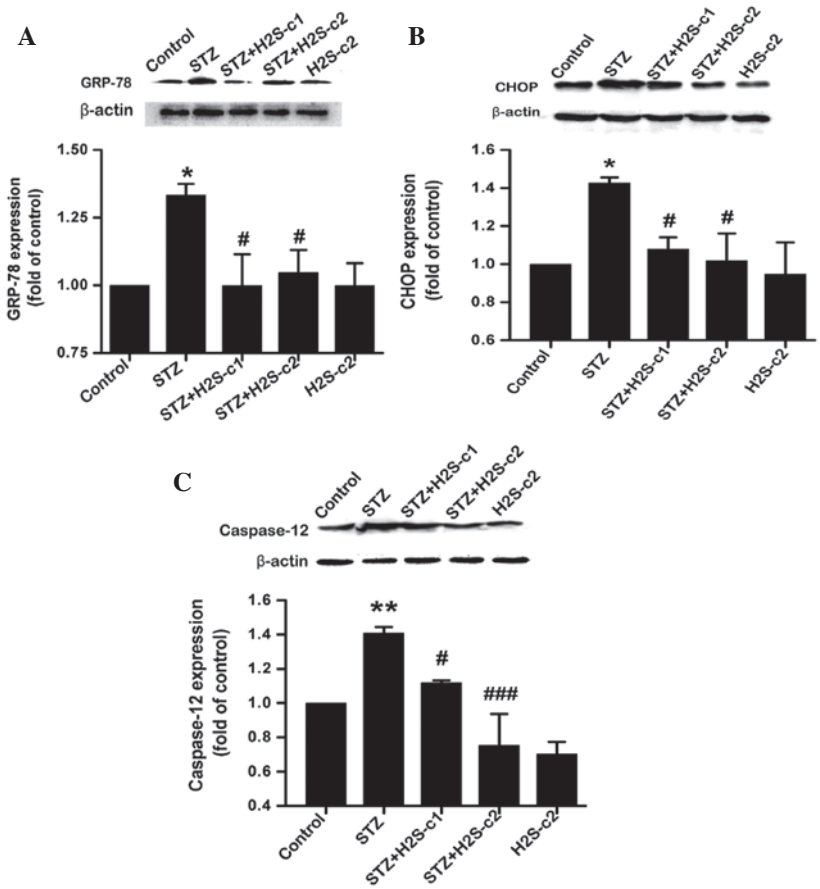

Figure 4. Effect of H_2S on the expression of Grp78, CHOP and caspase-12 in heart tissue. Western blot analysis of the protein expression of (A) Grp78, (B) CHOP and (C) caspase-12. β-actin served as a control. Bars indicate the mean ± standard error from at least 3 independent experiments. *P<0.05 and **P<0.01 compared with the control group; $^{\#}$P<0.05 and $^{\#\#\#}$P<0.001 compared with the STZ group. H_2S, hydrogen sulfide; STZ, streptozotocin; Grp78, glucose-regulated protein; CHOP, C/EBP-homologous protein.

Discussion

DM is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin and/or insulin resistance. The long-standing hyperglycemia of DM is associated with long-term damage, dysfunction and failure of various organs, particularly the heart (61). In the current study, the DM model was established, and the histological changes were evaluated using H&E staining to identify the existence of myocardial hypertrophy and with Masson's trichrome staining to identify the existence of myocardial fibrosis. In order to evaluate the ER stress, western blotting was used to test the expression of GRP-78, CHOP and caspase-12 protein. The present study shows that H_2S acts as a protective agent in rats with DC. The key findings were as follows: Hyperglycemia increased ERS levels, leading to myocardial hypertrophy and myocardial fibrosis in rats. Moreover, H_2S reduced the ERS caused by hyperglycemia, thus inhibiting myocardial hypertrophy and improving myocardial fibrosis.

The main pathophysiological effects of DC were myocardial fibrosis, cardiomyocyte hypertrophy and cardiac remodeling, leading to systolic and diastolic dysfunction (62). Hyperglycemia is the primary agent responsible for the occurrence and development of DC, which can lead to the progression of complex and chronic processes, including abnormal cellular metabolism and gene expression, even leading to cell death. The death of myocytes has been considered to be an important consequence of DC. As a type of permanent cell, myocytes are not capable of proliferation, and a decrease in the number of myocytes can cause systolic and diastolic dysfunction in the heart (63). The remaining cardiomyocytes embark upon a pathway of compensatory hypertrophy. Apoptosis is an important pathway for cell death, and a previous study demonstrated that myocardial apoptosis was increased notably in an STZ-induced DM model (64). Moreover, the effect of myocardial apoptosis caused by hyperglycemia was also demonstrated *in vitro* (65,66). The pathophysiology of apoptosis was closely associated with ERS. New evidence demonstrated that hyperglycemia induced apoptosis by activating ERS (67,68). In addition, in diabetic heart tissue, the ER was found to be swollen and dilated following ultrastructural analysis, suggesting abnormalities in the ER in a hyperglycemic environment (69-71). Recently, an increasing number of studies have investigated the correlation between ER stress and DC (18,30,31). H_2S was found to be the third gaseous signaling molecule, which is involved in neuromodulation, neuroprotection, vasodilatation, cardioprotection and regulation of the inflammatory response. It is known that H_2S affects the development of DM and its complications, such as protects β-cells in the pancreas, improves insulin resistance (56,57), prevents diabetic kidney from reconstruction (72) and alleviates myocardial damage in DM rat (73). It is encouraging that H_2S functions as an antioxidant, and as an

anti-apoptotic and anti-ERS compound. It was hypothesized that H_2S could protect against DC by inhibiting ERS.

The UPR is activated following ERS to inhibit protein synthesis and promote protein folding. However, following chronic ERS, the UPR activates the apoptotic pathway (74-77). A study by Gunn *et al* demonstrated that activation of the UPR contributes to myocardial apoptosis (78). In addition, cytokines and norepinephrine are vital in the pathophysiology of DC and were shown to stimulate the UPR (79,80). This suggests that ERS is involved in the occurrence and development of DC. Grp78, a regulatory protein of ERS, is important in assembly and folding of proteins, degradation of unfolded protein, calcium homeostasis and control of the activation of transmembrane ER stress sensors. Furthermore, Grp78 serves as a master modulator for the UPR network by binding to the ERS sensors, such as protein kinase R-like ER kinase, inositol requiring 1 (IRE1), and activating transcription factor 6 (ATF6) and inhibiting their activation (81). These results suggested that Grp78 is an activator of ER stress and UPR. Previous studies have demonstrated that two specific ER-related death pathways are involved in the apoptosis pathway. The first is activation of the transcriptional gene for CHOP and IRE1, and ATF6 signaling has been shown to be involved in the activation of ER stress (82,83). A previous study demonstrated that overexpression of CHOP promotes apoptosis (84), while lack of expression of CHOP inhibits apoptosis by downregulating ER stress (85). Furthermore, CHOP lowers the expression of Bcl-2, which is an anti-apoptosis protein (86). The second is the activation of caspase-12. It was demonstrated that pre-caspase-12 is hydrolyzed, and caspase-12 is activated during ERS (87-90). Activated caspase-12 promotes apoptosis by activating caspase-9 and caspase-3 through a non-cytochrome *c*-dependent pathway. The above information suggests that CHOP and caspase-12 are markers of ER stress. In the present study, expression of Grp78, CHOP and casepase-12 was increased in diabetic rats compared with controls. Conversely, H_2S decreased the expression level of these three proteins. In addition, the present study demonstrated that H_2S suppresses the expression of caspase-12 in a concentration-dependent manner, which suggests that the caspase-12-dependent apoptosis pathway is very sensitive to H_2S. These findings indicated that ERS serves as an important pathophysiological mechanism for DC, and H_2S acts as a cardioprotective agent and could reduce apoptosis by inhibiting ERS in the diabetic heart.

There have been large numbers of studies reporting a decrease in BW in diabetic rats compared with normal rats (91-93). However, there is no evidence for the impact of H_2S on the HW and BW of diabetic rats. It was demonstrated that H_2S did not affect the BW or HW in diabetic rats. The potential reasons for the decrease in BW and HW are inefficient use of glucose, and increased consumption of fat and protein. A number of studies have demonstrated that as H_2S improves insulin resistance, inhibits the activity of α-glucosidase and increases hepatic glucokinase activity and glycogen storage, it can reduce blood glucose (94-96). Conversely, the present study shows no significant effect of H_2S on blood glucose. This may be because STZ destroys the pancreatic β-cells, leading to an absolute lack of insulin secretion. Conversely, the present study reveals no significant effect of H_2S on blood glucose. Possible explanations are as follows: On one hand, STZ may destroy the pancreatic β-cells, leading to an absolute lack of insulin secretion. Although it has the effect of improving insulin resistance, H_2S has no role in reducing blood glucose. On the other hand, the animal models and experimental conditions were different.

In the clinic, most diabetic patients are suffering from DC when they go hospital, because it has gone unnoticed in terms of their health. Therefore, there is a need to focus on the effect of H_2S on cardiomyopathy in the heart. One shortcoming of the present study was that H_2S was added as an intervention factor at the same time as when the DM model was established, rather than after DC had developed. In the present study, we have confirmed the cardioprotective effects of H_2S, as it restrained DC. In a subsequent study, diabetic rats will receive i.p. infusions of NaHS after the occurrence of DC, by which means we will be able to investigate clearly the therapeutic action of H_2S on DC. It is widely known that ER stress is not the only factor that impacts DC, other mechanisms, including oxidative stress, autophagy and inflammatory reactions are also involved. These mechanisms interact to stimulate the development of DC. For example, active oxygen, increased under conditions of oxidative stress, may transform gene expression and cause the abnormal functioning of signal transduction pathways; it may also activate ERS to adapt to oxidative damage. Apoptosis occurs when the damage exceeds the regulative capability of the UPR. A previous study showed that DC is associated with suppression of cardiac autophagy, and restoration of cardiac autophagy can prevent DC in DM (97). Future experiments may focus on whether H_2S exhibits a protective effect on DC by affecting oxidative stress and autophagy. In addition, other signaling pathways associated with these mechanisms are also the an area of research.

In conclusion, the present study clearly demonstrate that high blood sugar can promote the development of DC by increasing the ERS. H_2S was shown to inhibit hyperglycemia-induced ERS, resulting in myocardial protection against diabetes. This renders H_2S a potential anti-DC agent.

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