The cystathionine γ-lyase/hydrogen sulfide pathway mediates the trimetazidine-induced protection of H9c2 cells against hypoxia/reoxygenation-induced apoptosis and oxidative stress

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Abstract

Objective: Trimetazidine is a piperazine-derived metabolic agent. It exerts cardioprotective effects against myocardial ischemia/reperfusion (I/R) injury. In addition, studies confirm that the cystathionine γ-lyase (CSE)/hydrogen sulfide (H₂S) pathway serves a beneficent role in attenuating myocardial I/R injury. However, the underlying role of the CSE/H₂S pathway in the trimetazidine-induced protection against myocardial I/R injury remains elusive. Therefore, this study investigated whether trimetazidine ameliorates hypoxia/reoxygenation (H/R)-induced H9c2 cardiomyocyte injuries in an in vitro cell model of myocardial I/R injury, by enhancing the CSE/H₂S pathway.

Methods: The H9c2 cell viability was determined with a cell counting Kit-8.

Results: Trimetazidine significantly increased the cell viability and decreased lactate dehydrogenase (LDH) release in H/R-treated H9c2 cells. Additionally, trimetazidine increased the H₂S levels and the CSE mRNA and protein levels, promoting the CSE/H₂S pathway under H/R conditions. The inhibition of the CSE/H₂S pathway, induced by transfection with specific siRNA against human CSE (si-CSE), eliminated the trimetazidine-induced upregulation of cell viability, downregulation of LDH release, increase of caspase-3 activity and apoptosis regulator BAX expression, and the decrease of apoptosis regulator Bcl-2 expression, which suggests involvement of the CSE/H₂S pathway in trimetazidine-induced cardioprotection. Furthermore, trimetazidine mitigated the H/R-induced increase in reactive oxygen species production and NADPH oxidase 2 expression, and decrease in superoxide dismutase activity and glutathione level, in H9c2 cells. These effects were also reversed by si-CSE.

Conclusion: This study revealed that the CSE/H₂S pathway mediates the trimetazidine-induced protection of H9c2 cardiomyocytes against H/R-induced damage by inhibiting apoptosis and oxidative stress. (Anatol J Cardiol 2019; 22: 102-11)

Keywords: trimetazidine, myocardial ischemia/reperfusion injury, cystathionine γ-lyase/hydrogen sulfide pathway, apoptosis, oxidative stress

Introduction

Myocardial ischemia/reperfusion (I/R) injury is associated with adverse cardiovascular outcomes following cardiac surgery, circulatory arrest or myocardial ischemia. It is one of the major causes of morbidity and mortality threatening human health (1, 2). Although apoptosis cascades, oxidative stress, mitochondrial dysfunction, and inflammation are recognized as the key drivers for I/R-induced myocardial tissue damage, no drugs that can abate myocardial I/R injury are being tested in clinical trials (3, 4). Therefore, the development of effective interventions and strategies to prevent myocardial I/R injury is of great clinical significance. Emerging evidence demonstrates that trimetazidine [1-(2, 3, 4-trimethoxybenzyl) piperazine dihydrochloride] is an agent with anti-ischemic properties that have been experimentally confirmed in various models, including cell culture, isolated organs, and in vivo (5-7). However, the mechanism that is responsible for trimetazidine-mediated cardioprotection against the pathogenesis of I/R injury remains unclear.

Hydrogen sulfide (H₂S), along with nitric oxide and carbon monoxide, is a well-recognized gasotransmitter capable of modulating numerous physiological processes (8). Endogenous generation of H₂S is mainly mediated by the enzyme cystathionine-γ-lyase (CSE) in the cardiovascular system (9). A growing body of evidence demonstrates that the CSE/H₂S pathway is part of a cardioprotective mechanism, playing a key role in in vivo and in vitro models of myocardial I/R disease (10, 11). In addition, a number of studies have revealed that H₂S mediates cardioprotection via the inhibition of myocardial inflammation, apoptosis,
oxidative stress, and mitochondrial dysfunction in myocardial I/R injury, and that the promotion of \( \text{H}_2\text{S} \) generation and overexpression of CSE decrease the severity of the myocardial I/R injury (12-14). These findings indicate that enhancement of the CSE/\( \text{H}_2\text{S} \) pathway is beneficial in I/R injury treatment. However, it is not known whether the CSE/\( \text{H}_2\text{S} \) pathway is also involved in the cell-protective effect of trimetazidine against myocardial I/R injury.

To the best of our knowledge, this study is the first to examine the effects of trimetazidine on the CSE/\( \text{H}_2\text{S} \) pathway in hypoxia/reoxygenation (H/R)-treated H9c2 cells (an in vitro cell model of myocardial I/R injury). The aim was to determine whether the enhancement of the CSE/\( \text{H}_2\text{S} \) pathway, induced by trimetazidine, is a potential novel therapeutic approach to prevent myocardial I/R injury.

**Methods**

**Cell culture**

The embryonic rat heart-derived H9c2 cell line was purchased from the American Type Culture Collection (CRL1446; Manassas, VA, USA) and maintained in Dulbecco’s modified Eagle’s medium (DMEM; cat. no. C11995500BT) supplemented with 10% (v/v) fetal bovine serum (cat. no. 10270-106) (both from Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and 100 mg/ml penicillin/streptomycin (cat. no. ST488; Beyotime Institute of Biotechnology, Shanghai, China) at 37°C in a humidified atmosphere containing 5% \( \text{CO}_2 \). The medium was replaced every 2–3 days. The cells were sub-cultured or subjected to subsequent experimental procedures at 70%–80% confluence.

**H/R injury model establishment and cell treatment**

To establish an in vitro model of H/R injury, following cell growth at 70% confluence, the cell culture medium was changed to serum-free low-glucose DMEM and the cells were placed into a tri-gas incubator containing 94% \( \text{N}_2 \), 5% \( \text{CO}_2 \), and 1% \( \text{O}_2 \) (HF 100; Heal Force Bio-meditech Holdings, Ltd., Shanghai, China) for 6 h, which was treated as the hypoxia process. Subsequently, reoxygenation was initiated by incubating the cells in complete DMEM at 37°C with 5% \( \text{CO}_2 \) for 12 h. The cells in the control group were cultured under normoxic conditions. The cell culture supernatant from each sample was collected, and 100 μl supernatant was transferred to a 96-well plate and incubated with 100 μl freshly prepared reaction mixture for 30 min at 37°C. The optical density value at the wavelength of 450 nm was measured with a multi-detection microplate reader (VICTOR2 1420; PerkinElmer, Inc., Waltham, MA, USA), and the results were presented as a percentage compared to the control group. All experiments were repeated at least three times.

**Lactate dehydrogenase activity detection**

The activity of lactate dehydrogenase (LDH) released from the cytosol of damaged cells into the supernatant, an indicator of cytotoxicity, was determined using the LDH cytotoxicity detection kit (cat. no. 11644793001; Roche Applied Science, Penzberg, Germany) according to the manufacturer’s instructions. Briefly, following the aforementioned treatment of H9c2 cells, the culture supernatant from each sample was collected, and 100 μl supernatant was transferred to a 96-well plate and incubated with 100 μl freshly prepared reaction mixture for 30 min at 37°C. Following the addition of 50 μl stop solution, the absorbance at 490 nm was measured using a microplate spectrophotometer (Epoch 2; BioTek Instruments, Inc., Winooski, VT, USA).

**H \(_2\text{S} \) content measurement**

The levels of \( \text{H}_2\text{S} \) in H9c2 cells were determined by methylene blue, as described by Chunyu et al. (15) with modifications (16). In brief, cells were homogenized in ice-cold PBS, and the assay mixture (500 μl), including cell homogenate (450 μl), L-cysteine (10 mM; 20 μl), pyridoxal 5’-phosphate (2 mM; 20 μl), and saline (10 μl), was incubated at 37°C for 20 min in tightly sealed Eppendorf vials. Then, 1% zinc acetate (250 μl) was injected to trap formed \( \text{H}_2\text{S} \), followed by the addition of 10% trichloroacetic acid (250 μl) to precipitate the protein and stop the reaction. Subsequently, N,N-dimethyl-p-phenylenediamine sulfate (20 mM; 133 μl) and FeCl\(_3\) (30 mM; 133 μl) were added; and the mixture was
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Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay

Total RNA was extracted from cells using RNAeasy Total RNA kit (cat. no. DP419; Tiangen Biotech Co., Ltd., Beijing, China), following the manufacturer’s protocol. The cDNA was prepared using the PrimeScript™ RT Master Mix kit (RR0036A, Takara, Osaka, Japan), and qPCR was carried out with the SYBR Green PCR Master Mix (RR036A, Takara, Osaka, Japan) using the ABI 7500 Real-time PCR System (ABI, Carlsbad, CA, USA), according to the manufacturers’ instructions. The final volume of the PCR reaction mixture (20 μl) contained SYBR Premix Ex Taq II (10 μl), primers (1 μl each), cDNA (1 μl), and RNase-free H₂O (8 μl); and the cycling parameters for amplification were as follows: a denaturation step at 95˚C for 15 s, followed by 45 cycles at 95˚C for 10 s, 60˚C for 20 s, and 72˚C for 30 s. The GAPDH expression was used as the internal control. The primer sequences were as follows: CSE forward, 5'-GGCATTCCGGTTTTGAAATGCT-3' and reverse, 5'-TGACTTTCAACAGCCACACCCA-3'; and GAPDH forward, 5'-CACCCCTGTTGCTGTAGCCAAA-3' and reverse, 5'-TGACTTTCAACAGCCACACCCA-3'. Relative mRNA levels were calculated with the Data Assist Software version 3.0 (Applied Biosystems/Life Technologies) according to the 2⁻ΔΔCt method.

Caspase-3 activity assay

The activity of caspase-3 was determined using a colorimetric assay kit (cat. no. 878-BC; R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer’s protocol. Briefly, H9c2 cells were lysed in the lysis buffer supplied in the kit, and equal amounts of protein were incubated with the reaction buffer including dithiothreitol as substrate for caspase-3 at 37˚C for 2 h in the dark. The absorbance at 405 nm was measured using a microplate reader (Thermo Scientific, New York City, NY, USA). Each experiment was independently repeated three times.

Reactive oxygen species accumulation detection

The intracellular reactive oxygen species (ROS) production was determined with a 2',7'-dichlorofluorescin diacetate (DCFH-DA) ROS assay kit (cat. no. HY-D0940; Molecular Probes; Thermo Fisher Scientific, Inc.), following the manufacturer’s protocol. The H9c2 cells were treated for a certain period of time, and then were harvested, washed, and resuspended in DCFH-DA solution (10 μM). The mixture was incubated at 37˚C for 20 min in a dark room and the results were analyzed by an FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) using a 488 nm excitation filter and a 525 nm emission filter. The data are expressed as percentage of fluorescence intensity relative to the control cells. A fluorescence microscope (Olympus Corporation, Tokyo, Japan) captured the images.

Lipid peroxidation marker (MDA) content measurement

The MDA content was determined using the MDA assay kit (Bioxytech LPO-586; GT Biopharma, Westlake Village, CA, USA) following the manufacturer’s protocol. Following the aforementioned treatment, the cells were washed with PBS, harvested by scraping in ice-cold PBS, and then centrifuged at 3000 x g for 10 min at 4˚C to remove cell debris. The MDA content in the supernatant was measured by recording the absorbance at 586 nm using the Epoch 2 spectrophotometer. Total MDA levels (nM) were calculated based on a standard curve and normalized to total protein levels.

Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activity determination

Following the aforementioned treatment for 24 h, H9c2 cells were harvested, homogenized, and centrifuged at 10,000 x g for 12 min. The lysates were stored at −70˚C until they were required for the enzyme activity assay. The SOD activity in the H9c2 cells was measured using an SOD assay kit (cat. no. S311; Dojindo Molecular Technologies, Inc.). GSH-Px activity in H9c2 cells was measured using a colorimetric assay kit (cat. no. E20130114019; Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Both assays were performed according to the protocols of the manufacturers of the kits.

Western blot analysis

Following the aforementioned treatment for 24 h, the protein from the H9c2 cells in the different experimental groups was extracted using radioimmunoprecipitation assay buffer supplemented with complete protease inhibitor cocktail (cat. no. 11838170001; Roche Applied Science) at 4˚C. The mixture was incubated for 30 min, and the cell lysates were centrifuged at 14,000 x g for 10 min at 4˚C. The amount of protein was determined using the Pierce BCA protein assay (cat. no. 23229; Thermo Fisher Scientific, Inc.). Equal amounts of protein (30 μg) were separated by 10% SDS-PAGE and transferred onto polyvinylidene fluoride membrane (cat. no. ISEQ00010; Merck KGaA, Darmstadt, Germany). The membranes were blocked with 5% non-fat milk for 2 h at room temperature, and incubated overnight at 4˚C with the following primary antibodies: anti-apoptosis regulator BAX (cat. no. ab32503), anti-apoptosis regulator Bcl-2 (cat. no. ab32124) (both rabbit monoclonal; 1:1,000; Abcam, Cambridge, UK), anti-CSE (cat no. 30068), and anti-GAPDH (cat. no. 5174) (both rabbit polyclonal; 1:2,000; Cell Signaling Technology Europe, B.V., Leiden, The Netherlands). Following washing with Tris-buffered saline containing Tween-20 three times (5 min each), the membrane was incubated at 37˚C for 2 h with horseradish-peroxidase-conjugated anti-rabbit (cat. no. 323-005-024) or anti-mouse (cat. no. 223-005-024) IgG (both 1:40,000; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA). The protein bands were visualized using the Pierce enhanced chemiluminescence western blot substrate (cat. no. 32134; Thermo Fisher Scientific, Inc.) and quantified as a ratio to GAPDH using
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Statistical analysis
Data are presented as the mean±standard error of the mean from three independent experiments. The normal distribution of the data was evaluated by the Shapiro–Wilk tests. The Tukey test was applied for comparing of group means. Statistical significance was determined using one-way analysis of variance followed by the variance homogeneity test. P<0.05 was considered to indicate a statistically significant difference.

Results
Trimetazidine increases cell viability and decreases LDH activity in H/R-treated H9c2 cells. To determine the trimetazidine-induced protection of H9c2 cells against injury following H/R treatment, the cell viability and LDH activity were detected. As observed in Figure 1, the cell viability (Fig. 1a) and LDH activity were significantly lower (Fig. 1b), in the H/R-treated group compared with the control. However, trimetazidine treatment notably reversed these effects, in particular at 10 μM. Therefore, 10 μM trimetazidine was the concentration of choice in the subsequent experiments. These results suggest that trimetazidine protects H9c2 cells against H/R-induced injury.

Trimetazidine blocks the H/R-induced inhibition of the CSE/H₂S pathway in H9c2 cells. To determine whether the CSE/H₂S pathway is involved in the protective effects of trimetazidine against myocardial H/R injury, we investigated the effects of trimetazidine on the levels of H₂S and CSE. As demonstrated in Figure 2, H/R treatment markedly decreased the H₂S level in H9c2 cells, whereas trimetazidine pretreatment mitigated this effect (Fig. 2a). The results from the RT-qPCR and western blot analyses revealed that compared with the control cells, CSE mRNA (Fig. 2b) and protein (Fig. 2c, 2d) levels, respectively, were significantly downregulated in the H/R-treated cells. However, these effects were also avoided by trimetazidine pretreatment. These results indicate that trimetazidine enhances the CSE/H₂S pathway under H/R conditions in H9c2 cells.

CSE knockdown attenuates trimetazidine-induced protection of H9c2 cells against H/R-induced injury. To further confirm the role of the CSE/H₂S pathway in the cardioprotective action of trimetazidine, H9c2 cells were transfected with si-CSE to induce CSE knockdown. As expected, the western blot analysis results revealed that the expression of the CSE protein was suppressed following si-CSE transfection (Fig. 3a, 3b). Subsequently, it was demonstrated that the CSE knockdown induced by si-CSE transfection notably inhibited the trimetazidine-induced increase in cell viability (Fig. 3c) and decrease in LDH activity (Fig. 3d) in H/R-treated H9c2 cells, whereas transfection with si-CSE or si-scramble alone did not affect cell viability or LDH activity. These results suggest that the CSE/H₂S pathway mediates the inhibitory effect of trimetazidine on the H/R-induced toxicity in H9c2 cells.

CSE knockdown alleviates the trimetazidine-induced decrease of apoptosis in H/R-treated H9c2 cells. The apoptosis of cardiomyocytes is another important indicator of myocardial I/R injury (17). To determine whether trimetazidine protects against cardiomyocyte apoptosis through the CSE/H₂S pathway, we investigated the effects of CSE knockdown on apoptosis. Caspase-3 is an important factor in cell apoptosis (18), and BAX and Bcl-2 are pro- and anti-apoptotic proteins, respectively (19). Trimetazidine mitigated the H/R-induced upregulation of caspase-3 activity in H9c2 cells, while this effect was reversed by si-CSE transfection (Fig. 4a). Western blot analysis (Fig. 4b) demonstrated that trimetazidine markedly decreased BAX expression (Fig. 4c) and increased Bcl-2 expression (Fig. 4d) in H/R-treated H9c2 cells. However, these effects of trimetazidine were mitigated by si-CSE. These results suggest that the CSE/
H₂S pathway contributes to the trimetazidine protection of H9c2 cells against H/R-induced apoptosis.

CSE knockdown alleviates the trimetazidine-induced decrease in oxidative stress in H/R-treated H9c2 cells. Oxidative stress serves a major role in myocardial I/R pathology, and the release of intracellular ROS is known as a mediator of the intracellular signaling cascade, which can trigger a series of events, including apoptosis (20). Therefore, we investigated the effects of CSE knockdown on oxidative stress under H/R conditions in trimetazidine-treated H9c2 cells. As observed in Figure 5, trimetazidine markedly attenuated the overproduction of ROS in H9c2 cells, as indicated by the decrease in green fluorescence, while this effect was eliminated by si-CSE transfection (Fig. 5a, 5b). si-CSE also led to the reversal of the trimetazidine-induced decrease in MDA in H/R-treated H9c2 cells (Fig. 5c). In addition, CSE knockdown overturned the trimetazidine-induced down-regulation of NADPH oxidase 2 (Nox2) protein in H/R-treated H9c2 cells (Fig. 5d, 5e). Furthermore, transfection with si-CSE depleted the trimetazidine-induced antioxidant defenses as evidenced by the diminished SOD activity (Fig. 5f) and GSH-Px (Fig. 5g) level, as compared to trimetazidine and H/R co-treatment group. These results indicated that CSE/H₂S pathway mediates trimetazidine-induced the attenuation of oxidative injury in H/R-treated H9c2 cells.

**Discussion**

In this study, the signaling pathways by which trimetazidine triggers anti-apoptosis and antioxidant outcomes in H/R-induced myocardial injury were demonstrated. Specifically, the study fo-
cused on the potential role of the CSE/H$_2$S system in these processes. It was found that trimetazidine significantly increased the cell viability and decreased LDH release in H/R-treated H9c2 cells. Additionally, trimetazidine increased the H$_2$S levels and the CSE mRNA and protein levels, promoting the CSE/H$_2$S pathway under H/R conditions. Overall, the major findings of this study on H9c2 cells are as follows: i) trimetazidine prevents against H/R-induced injury; ii) trimetazidine enhances the CSE/H$_2$S pathway under H/R conditions; iii) the CSE/H$_2$S pathway mediates trimetazidine-induced protection against H/R-induced injury; and iv) the CSE/H$_2$S pathway contributes toward the trimetazidine-induced inhibition of apoptosis and oxidative stress stimulated by H/R. These results confirmed for the first time that the enhancement of the CSE/H$_2$S system mediates the trimetazidine-induced protection of H9c2 cells against H/R injury, expanding our knowledge and understanding of the mechanism of action of trimetazidine and the role of the CSE/H$_2$S pathway in the protection against myocardial I/R injury.

Trimetazidine, a metabolic agent with several properties, is included in the current European Society of Cardiology 2013 guidelines on the management of coronary heart disease. A growing body of evidence supports the theory that trimetazidine exerts significant protective effects against myocardial I/R injury (21-23). In accordance with these findings, this study revealed that trimetazidine notably eliminated H/R-induced cardiomyocyte damage, improving cell viability and LDH activity, reducing the indicators of cell injury (24), and confirming the protective function of trimetazidine against cardiac H/R-induced injury.

It was reported that TMZ protected HF ventricular myocytes from cytosolic Ca (2+) overload and subsequent hypercontrac-
ture, induced by electrical and β-adrenergic (isoproterenol) stimulation (25). This effect was mediated by the ability of TMZ to protect HF myocytes against mitochondrial permeability transition pore (mPTP) opening via attenuation of ROS generation by the mitochondrial electron transport chain and uncoupled mitochondrial nitric oxide synthase (mtNOS) (25). Another study has found that H$_2$S could ameliorate cardiac function (26). Treatment with NaHS inhibited the occurrence of cardiac apoptosis and improved cardiac structure. H$_2$S reduced the expression of the cleaved caspase-3, NOX4, and the leakage of cyt c from the mitochondria to the cytoplasm. Exogenous H$_2$S could maintain the mitochondrial membrane potential and reduce ROS production in the mitochondria. Therefore, H$_2$S reduces oxidative stress due to cardiac hypertrophy through the cardiac mitochondrial pathway (26). TMZ-induced enhancement of autophagy was considered to be related to increased AMP-activated protein kinase (AMPK) phosphorylation and decreased mammalian target of rapamycin (mTOR) phosphorylation (21).

Several studies have demonstrated that the CSE/H$_2$S pathway plays a cardioprotective role in myocardial I/R injury via reducing the extent of ischemic infarction, promoting cell survival, and decreasing cardiomyocyte apoptosis, whereas inhibition of this pathway contributes to the process of myocardial I/R injury (27, 28). The results of this study revealed that H/R treatment notably decreases H$_2$S generation and CSE protein expression in H9c2 cells, which demonstrates the inhibitory effects of H/R injury on the CSE/H$_2$S pathway, in line with the study by Salloum et al. (14), which reported that endogenous CSE/H$_2$S system was inhibited.
in cardiac I/R injury. Additionally, certain studies have revealed the importance of the CSE/H$_2$S pathway in the cardioprotective mechanism of a number of pharmacological or natural compounds, including chelerythrine (12), zofenopril (13), and beetroot juice (14). However, the role of the CSE/H$_2$S axis in the function of trimetazidine has not yet been reported. To the best of our knowledge, this study is the first to demonstrate that trimetazidine pretreatment markedly promotes H$_2$S production and CSE expression. The inhibition of the CSE/H$_2$S pathway, caused by si-CSE transfection, reversed the trimetazidine-induced protection of H9c2 cells against injury triggered by H/R. These results suggest that the CSE/H$_2$S pathway mediates the protective effect of trimetazidine against myocardial I/R injury.

Apoptosis is a genetically programmed form of cell death. Several studies have demonstrated that trimetazidine protects against cardiac I/R injury by decreasing cardiomyocyte apoptosis (5, 29). In this study, trimetazidine pretreatment decreased caspase-3 activity, the most important apoptotic factor, in H/R-
treated H9c2 cells. Anti-apoptotic protein Bcl-2 and pro-apoptotic protein BAX play important pathophysiological roles in myocyte apoptosis following I/R injury (30). This study further revealed that trimetazidine decreased the expression of BAX and increased the expression of Bcl-2, in agreement with previous studies where trimetazidine was found to act protectively against myocardial I/R injury with a lower apoptotic cell death rate (5, 31). Notably, there is accumulating evidence that H$_2$S inhibits the apoptosis of cardiomyocytes induced by myocardial I/R injury, by regulating the Bcl-2/BAX ratio and caspase-3 activity in the myocardium (32, 33). In line with these findings, this study found that the inhibition of the CSE/H$_2$S pathway, induced by si-CSE, blocked the anti-apoptotic effect of trimetazidine during H/R injury. These results suggest that the CSE/H$_2$S pathway is involved in the trimetazidine-induced inhibition of apoptosis in myocardial H/R injury.

Emerging evidence has revealed that oxidative stress causes I/R injury, and excessive ROS production and an oxidant/antioxidant imbalance have long been recognized as major mediators of I/R injury (20). There are reports that H$_2$S exerts cardioprotective effects during I/R injury through enhancing antioxidant enzymes, including SOD and GSH-Px, to scavenge ROS, ultimately resulting in increased cell survival. Accumulating evidence indicates the curative effect of trimetazidine on the oxidative damage associated with myocardial H/R injury (34, 35). However, the role of the CSE/H$_2$S pathway in the protection of trimetazidine against oxidative stress-mediated injury remains unreported. This study is the first in which trimetazidine significantly reversed the H/R-induced increase of ROS and MDA, which is the end product of lipid peroxidation, and the decrease in the activity of antioxidant enzymes SOD and GSH-Px. Furthermore, these changes were markedly attenuated by the inhibition of the CSE/H$_2$S pathway by si-CSE. These results indicate that trimetazidine attenuates the myocardial damage caused by oxidative stress by activating the CSE/H$_2$S pathway.

There were also some limitations in this study. First, our study was performed only in vitro experiment. Further study with in vivo experiment is needed. Second, the mechanism investigated in this study was still not comprehensive. Further study involving the mechanism of the effect of trimetazidine is needed.

Conclusion

In conclusion, this study confirmed that trimetazidine acts protectively against cardiac I/R injury in vitro. The findings highlight the contribution of the CSE/H$_2$S pathway to this process via inhibiting apoptosis and oxidative stress. As no drugs that can abate myocardial I/R injury are being tested in clinical trials, the results of this study may provide a basis for the use of trimetazidine in the treatment of cardiac I/R injury in further clinical trial.

Ethics approval and consent to participate: Not applicable.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.


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