Nitric Oxide 67 (2017) 10–25

Contents lists available at ScienceDirect

Nitric Oxide

journal homepage: www.elsevier.com/locate/yniox

Hydrogen sulfide attenuates cardiac injury in takotsubo cardiomyopathy by alleviating oxidative stress

Zhihong Zhang [a], Sheng Jin [a], Xu Teng [a, b], Xiaocui Duan [a, b], Yuhong Chen [a], Yuming Wu [a, c, d, *]

[a] Department of Physiology, Hebei Medical University, Shijiazhuang 050017, China
[b] Hebei Key Lab of Laboratory Animal Science, Department of Laboratory Animal Science, Hebei Medical University, Shijiazhuang, China
[c] Hebei Collaborative Innovation Center for Cardio-Cerebrovascular Disease, Shijiazhuang 050017, China
[d] Key Laboratory of Vascular Medicine of Hebei Province, Shijiazhuang 050017, China

ARTICLE INFO

Article history:
Received 21 January 2017
Received in revised form
23 March 2017
Accepted 21 April 2017
Available online 24 April 2017

Keywords:
Takotsubo cardiomyopathy
Hydrogen sulfide
Cardiac function
NADPH oxidase
Reactive oxygen species
Oxidative stress

ABSTRACT

Takotsubo cardiomyopathy (TCM) is characterized by transient left ventricular apical ballooning with the absence of coronary occlusion, which is an acute cardiac syndrome with substantial morbidity and mortality. It was reported that reduced endogenous hydrogen sulfide (H_2S) levels may be related to various heart diseases. The present study investigated the mechanism by which H_2S administration modulates and protects cardiac function in TCM rats. In order to establish a TCM model, Sprague Dawley (SD) rats were injected with a single dose of β-adrenergic agonist isoprenaline (ISO). We found that ISO induced cardiac dysfunction, which was characterized by a significant decrease in left ventricular systolic pressure (LVSP), maximum contraction velocity (+dp/dtmax), maximum relaxation velocity (−dp/dtmax) and increased left ventricular end-diastolic pressure (LVEDP). Accordingly, we found that plasma and heart tissue H_2S levels in TCM rats decreased significantly, and cardiac cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST) expression were lower. Moreover, cardiac dysfunction in TCM was associated with oxidative stress response and reactive oxygen species (ROS) formation. NADPH Oxidase 4 (NOX_4) and p67 protein expressions significantly increased in TCM cardiac tissues. In addition, Sodium hydrosulfide (NaHS) ameliorated ISO-induced cardiac dysfunction and reversed ISO-induced oxidative stress. This study revealed that H_2S exerted cardioprotective effects by reducing NADPH oxidase, which reduced ROS formation and prevented oxidative stress. Our study provided novel evidence that H_2S is protective in myocardial dysfunction in TCM rats and could be a therapeutic target for alleviating β-adrenergic system overstimulation-induced cardiovascular dysfunction.

1. Introduction

Takotsubo cardiomyopathy (TCM), also known as stress-induced cardiomyopathy or broken heart syndrome is triggered by acute endogenous catecholamine discharge [1,2]. TCM is characterized by wall motion abnormalities, such as acute, transient apical and mid-ventricular akinesia with either a preserved or hypercontractile basal segment, in the absence of a coronary lesion [3,4]. The wall motion abnormality resolves over a period of days to weeks [5].

The mechanisms behind TCM are not clear but likely related to beta-adrenergic agonist stimulation and acute endogenous catecholamine discharge, these are the main etiologies of this syndrome, both of which could trigger an oxidative stress response and reactive oxygen species (ROS) formation, which leads to further deterioration of cardiac function [6–9].

Abbreviations: TCM, takotsubo cardiomyopathy; H_2S, hydrogen sulfide; ISO, isoprenaline; LVSP, left ventricular systolic pressure; +dp/dtmax, maximum contraction velocity; −dp/dtmax, maximum relaxation velocity; LVEDP, left ventricular end-diastolic pressure; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; CSE, cystathionine γ-lyase; CBS, cystathionine β-synthase; 3-MST, 3-mercaptopyruvate sulfurtransferase; ROS, reactive oxygen species; NOX_4, NADPH Oxidase 4; NaHS, Sodium hydrosulfide; MDA, malondialdehyde; GSH, glutathione; SOD, superoxide dismutase; H_2O_2, hydrogen peroxide; CK, creatine kinase; CK-MB, creatine kinase-MB; LDH, lactate dehydrogenase; AST, aspartate transaminase.

* Corresponding author. Department of Physiology, Institute of Basic Medicine, Hebei Medical University, 361 Zhongshan East Road, Shijiazhuang 050017, China. Tel./fax: +86 311 8626 5619.
E-mail address: wuyum@yahoo.com (Y. Wu).

http://dx.doi.org/10.1016/j.niox.2017.04.010
1089-8603/

Although the overall prognosis is good, there is a finite acute mortality rate among TCM patients (1%–4.2%) [10–12], and pre-hospital TCM patient mortality is unknown but should not be underestimated. Furthermore despite recovery of left ventricular (LV) function and absence of stenotic coronary artery disease, patient mortality from TCM after hospital discharge is also remarkably poor and worse than an aged-matched healthy population [13,14]. TCM prognosis with regard to mortality was worse than in control subjects without coronary artery disease and similar to patients with acute coronary syndrome, emphasizing an urgent need for studies on optimal TCM treatment [10].

Hydrogen sulfide (H_2S), a recently characterized gasotransmitter, is produced in the body from L-cysteine via the activity of three enzymes: 1)cystathionine γ-lyase (CSE); 2) cystathionine β-synthase (CBS); and 3)3-mercaptopyruvate sulfurtransferase (3-MST) [15–17]. A role for H_2S in a wide range of physiological and pathological cardiovascular processes, including blood pressure reduction, vasorelaxation, cardioprotection, and inhibition of atherosclerosis has been confirmed [18–21]. In the heart, H_2S is mainly produced via CSE, and it has been reported to provide cardioprotection in various cardiac injury models through its ability to potentiate antioxidant defense, decrease the ROS levels and preserve mitochondrial function [22–24]. In the cardiovascular system, exogenous H_2S administration has been reported to be cardioprotective in various disease models. For example, sodium hydrosulfide (NaHS) treatment was shown to significantly decrease the severity and duration of ischemia/reperfusion(I/R)-induced arrhythmias and increase the viability of cardiomyocytes in isolated perfused rat hearts [25].

Therefore, the present study was designed to investigate any functional and structural changes in TCM rat hearts, to examine whether H_2S can ameliorate the cardiac function in a TCM model and to address the precise mechanism(s) underlying this effect. Understanding these mechanisms may provide new therapeutic targets for TCM patients.

2. Materials and methods

2.1. Drugs and chemicals

Isoprenaline (ISO) and NaHS were obtained from Sigma (Sigma–Aldrich, St Louis, MO, USA). NaHS was freshly prepared before use. Dihydroethidium (DHE) was purchased from Beyotime Biotechnology (Beyotime Biotechnology, Shanghai, China) and dissolved in dimethyl sulfoxide (DMSO). Detection kits for malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and glutathione (GSH) concentrations, and superoxide dismutase (SOD) activity were purchased from Jiancheng BioEngineering (Nanjing, China).

2.2. Animals and experimental protocol

All protocols and procedures used in this study were reviewed and approved by the Institutional Animal Ethics Committee of Hebei Medical University and in accordance with the Guide for the Care and Use of Laboratory Animals (1985, NIH). Male Sprague-Dawley (SD) rats (250g–300g) were provided by Vital River Laboratories, Beijing, China. The animals were given free access to a laboratory diet and water, and housed under constant environmental conditions (12 h light/dark cycle) in a temperature controlled (25 °C) facility.

TCM models were induced by administering intraperitoneally (i.p.) a single dose of the β-adrenergic agonist, ISO(50 mg/kg) [26,27]. In order to better observe NaHS cardioprotective effects on TCM models, we administered NaHS single-dose pretreatment and NaHS long-term pretreatment. (1): NaHS single dose pretreatment: 32 rats received i.p. NaHS pretreatment at doses of 10, 50, 100, or 200 μmol/kg (n = 8 in each group) 5 min before ISO injection. The reason for the dose-titration experiment was to study the dose–response relationship and determine the minimal dose of NaHS to elicit the best cardiac protective effects in TCM rats. The dose of 100 μmol/kg was chosen for subsequent experiments. (2): NaHS long-term pretreatment: 16 rats received NaHS pretreatment (100 μmol/kg/day, i.p.) or saline (0.6 ml/kg/day, i.p.) for 30 days(n = 8), and then received a single dose of ISO (50 mg/kg, i.p.).

2.3. Measurement of cardiac function

The rats were anaesthetized with urethane (1.5 g/kg, i.p.). A PE-50 catheter filled with heparin saline (500 U/ml) was carefully inserted into the left ventricle from the right carotid artery and then connected to a pressure transducer coupled with Power Lab (ML4818) Data Acquisition System (AD Instruments Australia) to record the left ventricular end diastolic pressure (LVEDP), left ventricular systolic pressure (LVSP), maximum contraction velocity (+dp/dtmax), maximum relaxation velocity (−dp/dtmax), and heart rate. After 20 min equilibration, cardiac parameters were consecutively recorded for 150 min after ISO injection. Then, the blood and heart tissues were collected for other measurements.

2.4. Measurement of echocardiography

To test left ventricular function, two-dimensional echocardiography was performed using a Vevo2100 ultrasound device (VisualSonics Inc.). Rats were anaesthetized with isoflurane (2%), and M-mode images of the left ventricle were recorded. All measurements were averaged for five consecutive cardiac cycles. Left ventricular ejection fraction(LVEF) and left ventricular fractional shortening (LVFS) were measured to evaluate heart function. The rats in each group were monitored by echocardiography before treatment and for the first 150 min post treatment.

2.5. Measurement of H_2S levels

Tissue and plasma H_2S levels were measured according to the previously described methods [28]. Briefly, heart tissues were homogenized in ice-cold Tris-HCl (100 mmol/l, pH 8.5) and centrifuged at 12,000 g for 20 min at 4 °C. The supernatant was immediately used to measure H_2S, and proteins in the supernatant were quantified using the BCA reagent (Generay Biotechnology, Shanghai, China). Thirty microliters of supernatant or plasma were mixed with 80 μl monobromobimane (MBB, Sigma–Aldrich) and 10 μl 0.1% ammonia with shaking for 1 h at room temperature for derivatization of sulfide. MBB reacts with sulfide to produce sulfide-dibimane (SDB). SDB is more hydrophobic than most physiological thiols and can be separated by gradient elution and analyzed by high-performance liquid chromatography. The reaction was then terminated with 10 μl 20% formic acid and centrifuged at 15,000 g for 10 min. The supernatants were stored at −80 °C until H_2S measurements were done. H_2S concentrations were determined using a curve generated with sodium sulfide (0–40 μmol/L) standards, and the H_2S concentrations in plasma was expressed as μmol/L. H_2S concentrations in heart tissues were divided by the protein concentrations and expressed as μmol/g of protein.

2.6. Determination of activities of SOD and contents of H_2O_2, MDA, GSH in plasma and cardiac tissues

Blood samples were centrifuged at 3500 × g for 10 min. The frozen cardiac tissue samples were weighed and homogenized in