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Original Contribution

Methane attenuates myocardial ischemia injury in rats through anti-oxidative, anti-apoptotic and anti-inflammatory actions

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ABSTRACT

Myocardial infarction (MI) remains the most frequent cardiovascular disease with high mortality. Recently, methane has been shown protective effects on small intestinal ischemia–reperfusion injury. We hypothesized that methane-rich saline (MS) could protect the myocardium again MI via its anti-oxidative, anti-apoptotic and anti-inflammatory effects. In experiment 1, tetrazolium chloride staining and detection of myocardial enzymes and oxidative and inflammatory parameters were performed at 12 h after MI to determine the optimal dose at which intraperitoneal MS exerted the best protective effects on MI. In experiment 2, rats were treated with 10 ml/kg MS. Myocyte apoptosis was detected 72 h after MI, and cardiac function and myocardial remodeling were evaluated 4 weeks after MI. Results showed different dose of MS reduced infarct area, decreased myocardial enzymes, inhibited inflammation and oxidative stress following MI. The optimal dose of MS was 10 mg/kg. Moreover, treatment with 10 mg/kg MS for 3 days significantly reduced myocyte apoptosis, improved cardiac function and inhibited myocardial remodeling (reduced anterior wall thickness, attenuated myocyte hypertrophy, and decreased myocardial collagen). MS protects the myocardium of MI rats via its anti-oxidative, anti-inflammatory, anti-apoptotic and anti-remodeling activities. Thus, MS provides a novel and promising strategy for the treatment of ischemic heart diseases.

1. Introduction

Myocardial infarction (MI) caused by coronary artery occlusion is the most common cardiovascular disease and a leading cause of death worldwide. MI is a fatal and acute disease of the cardiovascular system that threatens human health. According to the latest statistics, ~620,000 individuals in the United States have a new coronary attack and 295,000 experience a recurrent attack annually [1]. Although myocardial protection has improved during recent decades, the application of western medicine (such as angiotensin II receptor antagonists and calcium channel blockers) remains limited because of their side effects [2]. Thus, it is imperative to develop strategies for the treatment of MI without significant or with only mild side effects.

After MI, the loss of blood flow results in the death of cardiomyocytes in the ischemic area, which diminishes cardiac

Abbreviations: AW, left ventricular anterior wall thickness; CK, creatine kinase; CK-MB, MB isoenzyme of creatine kinase; cTnI, cardiac troponin I; GSH, glutathione; GSSG, glutathione disulfide; H&E, hematoxylin and eosin; HR, heart rate; IL-1β, interleukin-1β; LAD, left anterior descending coronary artery; LDH, lactate dehydrogenase; LV, left ventricular; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; LVIDd, left ventricular diastolic inner chamber dimensions; LVIDs, left ventricular systolic inner chamber dimensions; LVP, left ventricular pressures; MDA, malondialdehyde; MI, myocardial infarction; MPO, myeloperoxidase; MS, methane-rich saline; OD, optical density; PBS, phosphate-buffered saline; PVDF, polyvinylidene difluoride; ROS, reactive oxygen species; SD, standard deviation; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α; TTC, tetrazolium chloride; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling; XO, xanthine oxidase; 8-OHdG, 8-hydroxy-2'-deoxyguanosine

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contractility and impedes angiogenesis and myocardial repair. In addition, the accelerated generation of reactive oxygen species (ROS) is a major cause of cell apoptosis and inflammation. Following ischemia, the accumulated ROS, particularly hydroxyl radicals, not only destroy the structure of cells but activate the mitochondrial apoptotic pathway [3]. The inflammatory responses activated after MI further aggravate the myocardial necrosis and cell apoptosis to eventually cause infarction expansion, hypertrophy of the non-infarct myocardium, myocardial fibrosis, and left ventricular (LV) dilatation. Together these processes are known as LV remodeling, which is an important pathology related to LV dysfunction.

Methane exerts protective effects on small intestinal ischemia–reperfusion by reducing ROS production and inhibiting inflammatory response in a dog model [4]. However, the therapeutic effects of methane in an MI model are unclear. In this study, methane-rich saline (MS) was prepared and used to treat rats with MI, with the aim of investigating the therapeutic effects of methane and its potential mechanism of action.

2. Materials and methods

2.1. Animals

A total of 342 adult male Sprague–Dawley rats weighing 200–250 g were purchased from the Experimental Animal Center of the Second Military Medical University, Shanghai, China. Animals were housed in polypropylene cages and given ad libitum access to food and water with a natural day/night cycle in accordance with the guidelines of the Animal Care and Use Committee of the Second Military Medical University. All animal manipulations were performed according to the recommendations of the Committee of the Care and Use of Laboratory Animals at the Second Military Medical University.

2.2. Methane saline preparation

Methane was dissolved in normal saline and then pressured at 0.4 MPa for 8 h using our professional apparatus (Shanghai Yangyuan Pressure Vessel Co., Ltd.). The MS was stored at 4 °C and made freshly once a week to guarantee that the concentration of methane was maintained at a high level. The concentration of methane in the saline was measured using gas chromatography (Gas Chromatography-9860, Qiyang, Shanghai, China).

2.3. Preparation of rat MI model

Rats were anesthetized by the intraperitoneal injection of chloral hydrate (300 mg/kg), intubated, and ventilated using a small-animal ventilator (SAR-830, CWE, Ardmore PA, USA) at 78 breaths/min and a tidal volume of 12 ml/kg. After a left thoracotomy at the fourth intercostal space, the heart was exposed, the pericardium was opened, and a 5–0 silk suture was used to ligate the left anterior descending coronary artery (LAD). Rats in the sham group underwent the same surgical procedures except that the LAD was not ligated.

2.4. Experimental protocols

This study was divided into two experiments, as shown in Fig. 1. The aim of experiment 1 was to determine the optimal dose of MS that achieved the best therapeutic effects. Rats were assigned randomly into five groups: sham (sham, $n=25$), MI (MI, $n=30$), MI+0.6 ml/kg MS (0.6, $n=30$), MI+2.5 ml/kg MS (2.5, $n=30$), and MI+10 ml/kg MS groups (10, $n=30$). Rats in the sham group underwent surgical procedures, but the LAD was not ligated, and they were then injected intraperitoneally with normal saline. Rats in the MI group underwent MI and were then injected intraperitoneally with normal saline. Rats in the 0.6, 2.5, and 10 ml/kg MS groups underwent MI and were then injected intraperitoneally with 0.6 ml/kg, 2.5 ml/kg and 10 ml/kg MS, respectively, at 30 min after LAD ligation. Twelve hours later, rats were sacrificed, and the hearts were harvested, frozen, and stored at −80 °C for further analyses.

The aim of experiment 2 was to investigate the mechanisms underlying the protective effects of MS on MI. Rats were assigned randomly into three groups: sham (sham, $n=35$), MI (MI, $n=45$), and MI+10 ml/kg MS groups (Methane, $n=45$). Rats in the sham group underwent heart exposure and were treated intraperitoneally with normal saline (10 ml/kg), but the LVD was not ligated. Animals in the MI group underwent MI and then normal saline (10 ml/kg) was injected intraperitoneally. Rats in the MS group underwent MI and were then injected intraperitoneally with 10 mg/kg MS. MS or normal saline were injected at 30 min after LAD ligation and thereafter once daily for three consecutive

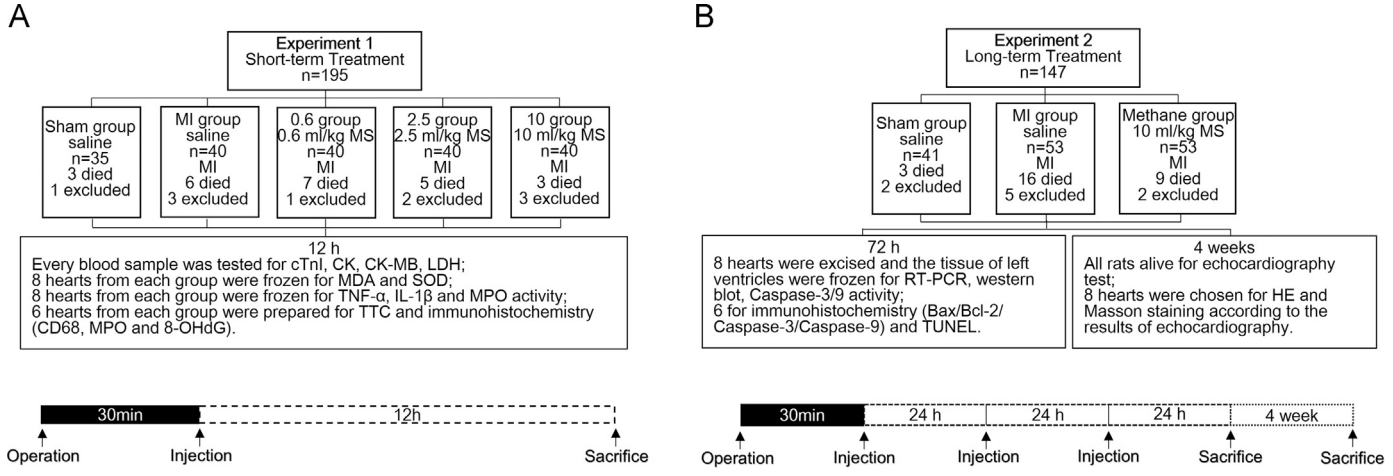

Fig. 1. Animal groups and treatments. In experiment 1 (A), animals were divided into five groups: sham, MI, and three MS groups. Rats in the MS groups were injected intraperitoneally with different concentrations of MS (0.6, 2.5, and 10 ml/kg) 30 min after MI. Cardiac enzymes, oxidative parameters, inflammatory parameters, and the infarct area were measured 12 h after MI. In experiment 2 (B), animals were divided into three groups: sham, MI, and 10 ml/kg MS groups. Rats in the MS group were injected intraperitoneally with MS once a day for 3 consecutive days. Apoptosis-related parameters were detected 72 h after MI, and cardiac function and heart remodeling were evaluated 4 weeks after surgery.