Heart, Lung and Circulation (2015) **24,** 602–610
1443-9506/04/$36.00
http://dx.doi.org/10.1016/j.hlc.2014.11.018

ORIGINAL ARTICLE

Cardioprotective Effect of Hydrogen-rich Saline on Isoproterenol-induced Myocardial Infarction in Rats

Lei Jing [a,b,d,1], Yun Wang [a,1], Xiao-Min Zhao [a,b*], Bing Zhao [a,b,e], Ji-Ju Han [a], Shu-Cun Qin [a], Xue-Jun Sun [c]

[a]Key Laboratory of Atherosclerosis in Universities of Shandong (Taishan Medical University), Taian, 271000, China
[b]Department of Pharmocology, Taishan Medical University, Taian, 271000, China
[c]Department of Diving Medicine, the Second Military Medical University, Shanghai, 200433, China
[d]Department of Pharmacy, Dongping County People's Hospital, Dongping, 271500, China
[e]Department of Pharmacy, Boshan District Hospital of Zibo, Boshan, 255200, China

Received 30 July 2014; received in revised form 18 November 2014; accepted 24 November 2014; online published-ahead-of-print 4 December 2014

Background Infusion with hydrogen gas-saturated saline has recently been reported to exert antioxidant and anti-inflammatory activity that may protect against organ damage induced by oxidative stress. Therefore because oxidative stress plays a significant role in the pathophysiology of myocardial infarction (MI), the aim of our study was to investigate whether hydrogen-rich saline has cardioprotective effects against isoproterenol-induced MI in rats.

Methods An acute MI model was induced in male Wistar rats by subcutaneous injection of isoproterenol. Different doses of hydrogen-rich saline (5, 7.5, and 10 mL/kg body weight i.p.) or Vitamin C (250 mg/kg body weight i.g.) were administered to the rats. Oxidative stress indices including levels of myocardial marker enzymes, inflammatory cytokines, membrane-bound myocardial enzymes and histopathological changes were measured.

Results Compared with those in isoproterenol-MI group, hydrogen-rich saline decreased malondialdehyde and 8-hydroxy-desoxyguanosine concentrations, enhanced superoxide dismutase and Na^+-K^+-ATPase activity, lowered Ca^{2+}-ATPase activity and decreased interleukin-6 and tumour necrosis factor-α levels in the serum and/or cardiac tissue of rats. Hydrogen-rich saline pretreatment also diminished infarct size, improved left heart function, and ameliorated pathological changes of the left heart.

Conclusion From these results, hydrogen-rich saline exerts cardiovascular protective effects against isoproterenol-induced MI at least in part via interactions which evoke antioxidant and anti-inflammatory activities.

Keywords Hydrogen • Inflammation • Isoproterenol • Myocardial infarction • Oxidative stress

Introduction

As an acute condition, myocardial infarction (MI) occurs as the result of unbalanced coronary artery supply and myocardial demand [1]. It is still the main cause of death worldwide, although therapeutic modalities and clinical care have improved [2]. Extensive previous studies have shown that the production of toxic, reactive oxygen species (ROS), such as hydrogen peroxide, hydroxyl radicals and superoxide radicals in ischaemic tissue, induce damage to myocardial cells, leading to oxidative damage to membrane lipids, proteins and DNA [3]. To date the major challenge remains in

*Corresponding author at: Key Laboratory of Atherosclerosis in Universities of Shandong, Taishan Medical University, Taian, 271000, China.
Tel.: +86 538 6225275; fax: +86 538 6225275, Email: zhaoxiaominty@hotmail.com

[1] These two authors contributed equally to the study.

finding a clinically practical and efficacious agent to limit this post-MI related injury.

Isoproterenol (ISO) is a potent mixed beta-adrenergic agonist that at high acute dose has been reported to cause severe myocardial stress and induce infarct-like necrosis [4]. Isoproterenol-induced MI in a rat model replicates the acute myocardial necrosis, which is followed by increased release of cardiac enzymes, accumulation of lipid peroxidases, and impaired cardiac function [5]. The pathophysiological and morphologic alterations in this model mimic those of human MI [6]. Thus, to evaluate MI originating from oxidative stress, we considered the ISO-induced MI to be the most suitable.

Hydrogen gas (H_2) has been used to prevent decompression sickness [7]. In 2007, Ohsawa et al. found that hydrogen could be used as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals [8]. Several other studies have shown that H_2 had therapeutic antioxidant activity and fought against organ damage, such as neonatal cerebral hypoxia-ischaemia, pulmonary hypertension, lung, hepatic or myocardial injury induced by ischaemia/reperfusion [9–13]. Taking convenience and safety into account, we used hydrogen-rich saline instead of hydrogen gas. This study was therefore designed to explore possible pharmacological effects, as well as the mechanism of hydrogen-rich saline, on ISO-induced MI in rats.

Materials and Methods

Animals

Male Wistar rats, weighing 220 ± 20 g, were supplied by the Experimental Animal Center of Shandong University of Traditional Chinese Medicine (Shandong, China). Rats were acclimated for five days before any operation. All rats received humane care according to the Chinese Academy of Science's Guide for the Care and Use of Laboratory Animals.

Drugs and Materials

The method for the preparation of hydrogen-rich saline was described previously [14]. Briefly, hydrogen was dissolved in physiological saline for six hours under high pressure of 0.4 megapascal (MPa) to the supersaturated level. The saturated hydrogen saline was stored in an aluminum bag with no dead volume at 4 °C under atmospheric pressure. To ensure the hydrogen concentration of more than 0.6 mM, hydrogen-rich saline was made every week. Isoproterenol (ISO) was obtained from Sigma-Aldrich (Sigma, St. Louis, MO, USA). Malondialdehyde (MDA), superoxide dismutase (SOD), aspartate aminotransferase (AST), Na^+-K^+-ATPase and Ca^{2+}-ATPase assay reagents were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Interleukin-6 (IL-6), 8-hydroxy-desoxyguanosine (8-OHdG) and tumour necrosis factor-α (TNF-α) enzyme-linked immunosorbent assay (ELISA) kits were obtained from Shanghai Bluegene Biotech Co., Ltd. (Shanghai, China). Creatine kinase isoenzyme (CK-MB) ELISA kits were from R&D systems (Beijing, China). All the other chemicals used were of analytical grade.

Experimental Design

Rats were divided randomly into six groups: Group 1 (n = 14): normal control rats pretreated with physiological saline intraperitoneally [i.p.] before physiological saline was given subcutaneously; Group 2 (n = 22): MI group in which rats pretreated with physiological saline i.p. before ISO was administered; Groups 3 to 5 (n = 22 in each group): rats pretreated with hydrogen-rich saline (5, 7.5, and 10 mL/kg body weight i.p.) before ISO was administered. Group 6 (n = 22): rats pretreated with Vitamin C (250 mg/kg body weight intragastrically [i.g.]) [15] before ISO was administered. ISO was given twice subcutaneously at an interval of 24 hours at a dose of 200 mg/kg body weight in 1 mL 0.9% saline [16,17], and physiological saline was given in the same way in group 1. Haemodynamic studies were performed before animals were sacrificed and cardiac tissues were collected from eight rats in each group for measurements of infarct area. Hearts from an additional six animals in each group were used for histopathological examination, and eight animals were used for preparation of homogenate.

Infarct area was not measured in the normal control group, thus all 14 control animals were assessed for other measures. All animal experiments were approved by the Animal Ethics Committee of the Taishan Medical University.

Haemodynamic Studies

At the end of the experiment (12 h after the second injection of ISO) [17], rats were anaesthetised i.p. with 1% pentobarbital sodium (40 mg/kg), and placed in the supine position. A PE-50 polyethylene tube was advanced into the left ventricle through the right carotid artery. Haemodynamic parameters, including left ventricular systolic pressure (LVSP), the maximal rate of pressure rise (+dP/dt max) and pressure fall (-dP/dt max) of the left ventricular, and left ventricle end-diastolic pressure (LVEDP) were recorded on an MP150 system (BIOPAC, USA).

Measurement of Infarct Area

A triphenyl tetrazolium chloride (TTC) test was applied to evaluate infarct size [18]. After haemodynamic studies, the heart was removed, washed in cold saline to eliminate excess blood, trimmed of excess epicardial fat, sliced transversely into 2 mm thick sections, stained with 1% TTC solution, and scanned; the percentage of infarct size was calculated by dividing the infarct (white) area by the total (white and red) area using the Image-Pro® Plus Version 6.0.

Determination of Biochemical Parameters, Enzymes, and Cytokines

Blood was taken from the abdominal aorta for preparation of serum. Heart homogenate was prepared using cardiac apex