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Chronic intermittent hypobaric hypoxia attenuates radiation induced heart damage in rats

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ABSTRACT

Aims: Radiation-induced heart damage (RIHD) is becoming an increasing concern for patients and clinicians due to the use of radiotherapy for thoracic tumor. Chronic intermittent hypobaric hypoxia (CIHH) preconditioning has been documented to exert a cardioprotective effect. Here we hypothesized that CIHH was capable of attenuating functional and structural damage in a rat model of RIHD.

Main methods: Male adult Sprague-Dawley rats were randomly divided into 4 groups: control, radiation, CIHH and CIHH plus radiation. Cardiac function was measured using Langendorff perfusion in *in vitro* rat hearts. Cardiac fibrosis, oxidative stress and endoplasmic reticulum stress (ERS) was assessed by quantitative analysis of protein expression.

Key findings: No significant difference between any two groups was observed in baseline cardiac function as assessed by left ventricular end diastolic pressure (LVEDP), left ventricular developing pressure (LVDP) and the derivative of left ventricular pressure (±LVdp/dt). When challenged by ischemia/reperfusion, LVEDP was increased but LVDP and ±LVdp/dt was decreased significantly in radiation group compared with controls, accompanied by an enlarged infarct size and decreased coronary flow. Importantly, CIHH dramatically improved radiation-induced damage of cardiac function and blunted radiation-induced cardiac fibrosis in the perivascular and interstitial area. Furthermore, CIHH abrogated radiation-induced increase in malondialdehyde and enhanced total superoxide dismutase activity, as well as downregulated expression levels of ERS markers like GRP78 and CHOP.

Significance: CIHH pretreatment alleviated radiation-induced damage of cardiac function and fibrosis. Such a protective effect was closely associated with suppression of oxidative stress and ERS responses.

1. Introduction

Radiation induced heart damage (RIHD) is defined as structural and functional heart damage resulting from exposure to high dose radiation [1,18]. RIHD can affect almost all facets of the heart, including but not restricted to the pericardial sac, coronary arteries, myocardium, and heart valves [10]. It is hence necessary to explore the mechanisms involved in the pathophysiology of RIHD and to introduce new strategy against RIHD.

Most of the early work focuses on clinical symptoms and manifestations, rather than understanding cardiac homeostatic processes in response to radiation. Here, we propose basic issues of RIHD to be further elucidated. For example, a single variable dose of radiation results in cardiac structural or functional alterations in animal models 4–6 months after exposure to irradiation [20,28], but little is known as to how early the damage of cardiac function commences. Emphasis is laid on this issue because early clinical intervention may be more beneficial to patients. Moreover, a prominent pathological manifestation of RIHD is fibrosis that leads to deterioration of cardiac function [7,14]. Radiation-stimulated myocardial fibrosis is accompanied by endoplasmic reticulum stress (ERS) in cultured cardiac fibroblasts [11]. Under pathophysiologic conditions, ERS responses and oxidative stress are integrated and amplified to facilitate the development of disease [8]. Whether oxidation stress, ERS responses, and fibrosis occurred at very early stage of RIHD remains unexplored. In addition, the pathogenesis of RIHD is largely unknown, and a preventive intervention is unavailable as well.

Accumulating evidence demonstrates that CIHH protects heart against ischemia/reperfusion (I/R) or hypoxia/reoxygenation injuries. For example, CIHH promotes the recovery of cardiac function from I/R, prevents apoptosis of cardiomyocytes, reduces myocardial infarct and fibrosis, and antagonizes arrhythmia [9,22,30]. Multiple mechanisms have been suggested to contribute to the cardiac protection of CIHH, such as induction of heat-shock proteins [32], increase in coronary

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flow and myocardial capillary angiogenesis [31], activation of adenosine triphosphate sensitive potassium channels and inhibition of mitochondrial permeability transition pores [5], maintaining of cellular calcium homeostasis [17], and enhancement of myocardial antioxidation [33]. Recently, we found that CIHH protects liver through inhibition of ERS in a rat model of metabolic syndrome [29]. However, no study to date illustrates whether CIHH exerts a protective effect on RIHD in animal experiments or clinical trials.

Here, we asked whether CIHH pretreatment prior to irradiation alleviated RIHD in a rat model of RIHD and sought to reveal the putative cellular mechanism. Our data indicated that CIHH attenuated functional and structural damage of rat hearts subjected to irradiation, most likely *via* inhibition of oxidation stress and ERS responses, providing a potentially clinical strategy to attenuate RIHD.

2. Materials and methods

2.1. Animals

A total of 48 adult male Sprague-Dawley rats aged 8 ~ 10 weeks, obtained from the Experimental Animal Center of Hebei Medical University (Shijiazhuang, China), were randomly divided into four groups: control, CIHH (simulated 5000 m altitude, 6 h per day for 4 weeks), radiation (irradiation with a single fraction of 20.0 Gy) and CIHH plus radiation. Rats in radiation group were sacrificed for experiment two weeks after irradiation. In CIHH plus radiation group, rats were exposed to CIHH for 28 days prior to radiation. Rats in all groups were sacrificed for experiment at similar time point. The program was approved by Animal Care and Ethical Committee of Hebei Medical University. Twelve rats were chosen in each group, six rats for Langendorff perfusion *in vitro* and the other six for histopathological or molecular biological trials. Animals were maintained on a 12:12 light to dark cycle with free access to food and water.

2.2. CIHH pretreatment

Rats were treated with intermittent hypoxia before irradiation. The protocol has been described previously in detail [12,33]. Briefly, exposure to intermittent hypoxia was accomplished by placing unrestrained rats in a custom-made hypobaric chamber to mimic 5000 m altitude ($P_B = 404$ mm Hg, $P_aO_2 = 84$ mm Hg). Rats were subjected to intermittent hypoxia between 9:00 A.M. and 3:00 P.M. for 28 consecutive days. Between 3:00 P.M. and 9:00 A.M., rats were exposed to room air. The animals were fed *ad libitum*. In order to avoid disadvantage of CIHH to rats, the ascending speed was limited at no >500 m/s and the ultimate altitude was stabilized at 5000 m all the time. The other group rats were raised in the same environment as CIHH rats except hypobaric hypoxia exposure.

2.3. Irradiation treatment

After 28 days of CIHH acclimatization, rats were anesthetized with 10% chloral hydrate (0.35 ml/100 g) and were irradiated with a single fraction of 20.0 Gy from a precise type medical high-energy linear accelerator (Elekta Corporation, Sweden) operated at 6MV X-ray and with a dose rate of 1.87 Gy/min. Radiation was delivered locally to rat hearts using parallel opposed fields (anterior: posterior 1:1) with a diameter of 19 mm, while the rest part of rat body was shielded with lead plates to avoid maximally irradiation.

2.4. Langendorff perfusion in isolated rat hearts

The protocol has been described previously [17]. Briefly, rats were heparinized (1000 IU/kg, i.p.) and anesthetized with 10% chloral hydrate (0.35 ml/100 g). The hearts were excised rapidly in ice-cold perfusion buffer solution at 4 °C. After dissection, isolated hearts were mounted to an *in vitro* Langendorff apparatus *via* the aorta and perfused continually at a constant perfusion pressure (80 mm Hg) using a modified Krebs-Henseleit (K-H) solution bubbled with 95% O_2 and 5% CO_2 at 37 °C to yield a pH value of 7.40. The ingredients of K-H buffer were as follows (in mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2, and glucose 11.0. A water-filled latex balloon-tipped catheter was introduced into the left ventricle through left atrium. The left ventricular end diastolic pressure (LVEDP) was adjusted to 3–10 mm Hg. The distal end of the catheter was connected to a pressure transducer (model Gould P23Db, AD Instrument Ltd., Australia) to monitor the left ventricular pressure. Cardiac function, assessed by LVEDP, Left ventricular developing pressure (LVDP) and the derivative of left ventricular pressure (LVdp/dt), were consecutively recorded using Chart software (version 8, AD Instrument Ltd., Australia) for data acquisition and analysis. The coronary flow (CF) was determined by measuring the volume of the coronary sinus effluent in the perfusate. To examine post-ischemia/reperfusion (I/R) level of LVEDP, LVDP, LVdp/dt and CF, isolated hearts from each group were challenged by 30 min of global ischemia, followed by 60 min of reperfusion. After that, measurements of above parameters were then carried out again. In the experiment concerning measurement of myocardial infarct size, the reperfusion time was set 120 min. At the end of reperfusion, rat hearts were removed quickly and frozen at −20 °C. The frozen hearts were cut into thin slices (5 mm), which were perpendicular to the septum from the apex to the base. Then slices were incubated in sodium phosphate buffer containing 3% (wt./vol.) 2,3,5-triphenyl-tetrazolium chloride (TTC) for 10 min to visualize the unstained infarct regions. The infarct myocardium without staining by TTC displayed pale. The image was visualized using a digital microscope (DM6000B, Leica Company, Germany) and analyzed by Image Processing System (Motic Med 6.0, Xiamen, China). The extent of infarct myocardium was expressed as the percentage of the infarct size in relative to the ventricular size.

2.5. Histopathological evaluations

Rats were anesthetized with 10% chloral hydrate (0.35 ml/100 g) and hearts were rapidly removed from mediastinum in each group. Total collagen accumulation was determined by preparing tissue sections with Masson's trichrome stain. As a result, the myocyte, nucleus and collagen were stained as red, black and green, respectively. Collagen volume fraction (CVF) was applied for semi-quantitative analysis of myocardial collagen *via* Image Processing System (Motic Med 6.0, Xiamen, China). Interstitial CVF was calculated as the area occupied by the green dyed tissue, divided by the total myocardial area under direct vision. For each animal, 5 microscopic fields were examined, and the average of CVF was computed.

2.6. Determination of cardiac oxidative stress by ELISA

After anesthetization, rat hearts were harvested, snap frozen, and crushed in liquid nitrogen. The tissue was then homogenized in cold lysis buffer for determining activity of myocardial total superoxide dismutase (T-SOD) and content of mylondialdehyde (MDA) by enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Firstly, prepared 10% heart homogenate with normal saline (0.85%) as solvent; Secondly, determined protein content of each group by bicinchoninic acid (BCA) assay; Thirdly, calculated the activity of T-SOD and content of MDA in cardiac homogenate with the absorbance valve at 550 nm and 532 nm, respectively. All the protocols were determined according to the ELISA kits instructions (Nanjing Jiancheng Bioengineering Institute, China). Moreover, when testing T-SOD activity, care was taken to look for the best sample quantity by pre-experiment in terms of the instruction. T-SOD activity was based on the generation of superoxide anions by xanthine and xanthine oxidase reaction system, which oxidized hydroxylamine into nitrite, detection the absorbance value at 550 nm of indirect nitrite by color development and