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Repeated remote ischemic conditioning attenuates left ventricular remodeling via exosome-mediated intercellular communication on chronic heart failure after myocardial infarction

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ABSTRACT

Background: Remote ischemic conditioning (RIC) by repeated treatment of transient limb ischemia is a clinically applicable method for protecting the heart against injury at the time of reperfusion. In this study, we investigated the effects of repeated RIC on cardiac dysfunction after myocardial infarction (MI).
Methods and results: At 4 weeks after MI, rats were separated into the untreated (UT) group or the RIC-treated group. RIC treatment was performed by 5 cycles of 5 min of bilateral hindlimb ischemia and 5 min of reperfusion once a day for 4 weeks. Despite comparable MI size, left ventricular (LV) ejection fraction (LVEF) was significantly improved in the RIC group compared with the UT group. Furthermore, the LVEF in the RIC group was improved, although not significantly, after treatment. RIC treatment also prevented the deterioration of LV diastolic function. MI-induced LV interstitial fibrosis in the boundary region and oxidant stress were significantly attenuated by RIC treatment. MicroRNA-29a (miR-29a), a key regulator of tissue fibrosis, was highly expressed in the exosomes and the marginal area of the RIC group. Even in the differentiated C2C12-derived exosomes, miR-29a expression was significantly increased under hypoxic condition. As well as miR-29a, insulin-like growth factor 1 receptor (IGF-1R) was highly expressed both in the exosomes and remote non-infarcted myocardium of the RIC group. IGF-1R expression was also increased in the C2C12-derived exosomes under hypoxic conditions.
Conclusions: Repeated RIC reduces adverse LV remodeling and oxidative stress by MI. Exosome-mediated intercellular communication may contribute to the beneficial effect of RIC treatment.

1. Introduction

Chronic heart failure (CHF) is a clinical syndrome representing the end-stage of a number of different cardiac diseases. It causes exercise intolerance, impairs quality of life, and has been associated with high morbidity and mortality. Although the existing pharmacological therapies, such as inhibitors of the renin–angiotensin–aldosterone system and β-adrenoreceptor blockers improved outcomes in CHF [1–4], they are still not optimal and cannot fully prevent progressive cardiac remodeling and dysfunction. Recently, several non-pharmacological therapies such as cardiac rehabilitation and ventilatory support have been developed [5,6]. However, these therapies are associated with tolerability issues, and not all CHF patients benefit from them. Therefore, there is a need to develop a novel therapy that is easy to perform and is well tolerated.

The cardioprotective effect of remote ischemic preconditioning was originally reported by Przykelenk et al. [7]. They reported that ischemic preconditioning in the left circumflex coronary artery attenuated ischemia-reperfusion (IR) injury by subsequent occlusion of the left anterior descending coronary artery (LAD). Thereafter, some reports have shown that ischemic preconditioning [8,9] and postconditioning [10,11] of the extremities can protect the heart from IR injury. Thus, remote ischemic conditioning (RIC) may be one of the therapeutic strategies for protecting organs or tissue against IR injury. Briefly repeated nonlethal ischemia and reperfusion of a remote organ or tissue increase heart tolerability to acute IR injury. Although various studies supported

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the beneficial effect of RIC against acute myocardial infarction (MI), it is still unclear whether RIC is beneficial for CHF.

In this study, we hypothesized that the efficacy of RIC can expand not only to IR injury in the acute phase of MI but to left ventricular (LV) dysfunction in the chronic phase of MI. Our results showed that RIC treatment improved LV dysfunction and attenuated LV interstitial fibrosis in an experimental CHF model. These results may have clinical implications for the treatment of patients with evolving LV dysfunction.

2. Methods

2.1. Animals and experimental design

All procedures were performed in accordance to Osaka City University animal care guidelines, which conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The 8-week-old male Wistar rats weighing 260–290 g were purchased from CLEA Japan, Inc (Osaka, Japan).

The rats were intubated and were under mechanical ventilation with adequate anesthesia by pentobarbital (50 mg·kg^{-1}, intraperitoneally). Then, MI was induced by permanent ligation of the left coronary artery, as described previously [12,13]. Excluding the suturing of the coronary artery, the same surgical procedure was performed on a control group of rats.

At 4 weeks after MI induction, blood pressure (BP) and heart rate (HR) of the rats by the tail cuff method (BP98A; Softron, Tokyo, Japan) were measured and transthoracic echocardiography was performed to assess cardiac function. Basing on the echocardiographic findings, MI-induced rats were divided into two groups: the RIC-treated (RIC) group and untreated (UT) group (Fig. 1A). The sham-operated rats were used as the control group.

After 4 weeks of treatment, the BP and HR of the rats were measured. Under anesthesia, cardiac function was assessed by echocardiography. Immediately after echocardiography, the rat abdomen was cut open, and a blood sample was collected from the inferior vena cava. The hearts were then immediately excised, and the ventricle was separated from the atrium and was weighed.

The infarct size was calculated as the ratio of the scar area to the entire cardiac muscle area. The ventricle was separated into the upper and lower portions, and then the upper portion of the left ventricle was divided into the marginal zone and non-infarcted zone; the specimens obtained were then immediately frozen in liquid nitrogen and stored at −80 °C until use. The lower portion was fixed in 10% formaldehyde overnight and embedded in paraffin.

2.2. RIC treatment

The rats in all groups were adequately anesthetized with pentobarbital, and the bilateral hindlimbs of rats in the RIC group were subjected to repeated transient ischemia: 5 cycles of 5 min of bilateral hindlimb ischemia and 5 min of reperfusion, by tourniquets [11]. RIC treatment was performed once a day for 4 weeks (Fig. 1B).

2.3. Echocardiographic study

Transthoracic echocardiography was performed using a Xario ultrasound device (Toshiba Medical Systems, Tokyo, Japan) with a 6-MHz cardiac transducer, according to previously described methods [12,13]. In brief, rats were anesthetized with pentobarbital. A two-dimensional short-axis view of the left ventricle was obtained at the level of the papillary muscles. The LV ejection fraction (LVEF) was calculated by measuring the LV end-diastolic volume (LVEDV) and the LV end-systolic volume (LVESV), by using a modified Simpson's method. Pulsed wave Doppler spectra (early rapid filling [E] wave and atrial contraction [A] wave) of mitral inflow velocities were recorded from the apical 4-chamber view, with the sample volume placed near the tips of the mitral leaflets and adjusted to the position at which velocity was maximum and the flow pattern was laminar, and the ratio of E wave velocity to A wave velocity (E/A) was calculated. The early velocity of the mitral annulus (e′) was determined by tissue Doppler imaging. The ratio of the E wave velocity to e′ wave velocity (E/e′) was calculated.

2.4. Histological assessment and evaluation of oxidative stress

The area of interstitial fibrosis in the marginal area of the infarct was measured, as described previously [13–15]. In brief, 4-μm-thick sections were cut and stained with hematoxylin–eosin stain and Sirius red stain. The fibrosis area was calculated as the ratio of the sum of the total area of interstitial fibrosis to the sum of the total connective tissue area plus the area of cardiomyocytes in the marginal area of the LV. Each field was analyzed using image-analyzing software (Micro Analyzer, Japan Poladigital, Tokyo, Japan).

The serum levels of derivatives of reactive-oxygen metabolites (d-ROMs) were measured by the Free Radical Elective Evaluator (Diacron International, Grosseto, Italy) using commercial assay kits (Diacron International).

2.5. Exosome purification

Exosomes were purified from serum or culture media by the ultracentrifugation method, as previously reported [16]. In brief, each serum sample was centrifuged for eliminating debris and cellular components at 2000 ×*g* for 30 min at 4 °C, and then at 10,000 ×*g* for 30 min at 4 °C. Next, supernatants were ultracentrifuged at 10,000 ×*g* for 3 h at 4 °C. After washing with phosphate buffered saline (PBS), exosome pellets were dissolved in PBS and stored at −80 °C until use. The amount of exosome protein was quantified by BCA assay (ThermoScientific, Waltham, MA, USA).

2.6. RNA and MicroRNA expression analysis

Total RNA and microRNA were extracted from tissues or exosomes with Isogen II (Nippon Gene, Toyama, Japan). The concentration and quality of RNA were assessed by the Nano Drop 2000 (Thermo, Waltham, MA, USA). To quantify the gene expression levels,

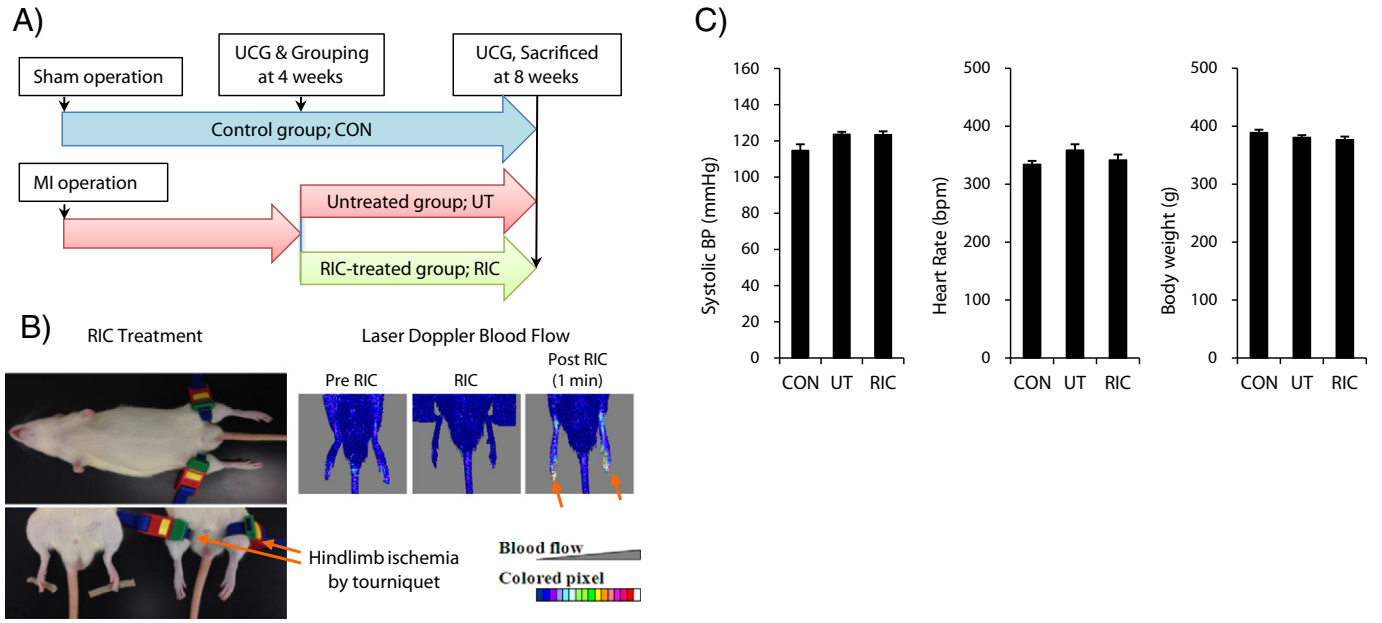

Fig. 1. Experimental protocols and hemodynamics. (A) The protocol of the present study is shown. At 4 weeks after the induction of MI, the rats were divided into the untreated group and RIC-treated group. (B) Transient ischemia was produced in the bilateral hindlimbs of the rats by tourniquets (left panel). A decrease in blood flow during RIC treatment was confirmed by a laser Doppler. Blood flow in the hindlimbs was more increased after the tourniquets were released (right panel, arrow). (C) Hemodynamic status and body weight of each group. All values are mean ± SEM (n = 11 to 17). UCG, ultrasound cardiography; MI, myocardial infarction; CON, control group; UT, untreated group; RIC, remote ischemic conditioning group; BP, blood pressure.