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Brief left ventricular pressure overload reduces myocardial apoptosis

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ABSTRACT

Background: Both apoptosis and necrosis contribute to cell death after myocardial ischemia and reperfusion. We previously reported that brief left ventricular pressure overload (LVPO) decreased myocardial infarct (MI) size. In this study, we investigated whether brief pressure overload reduces apoptosis and the mechanisms involved.

Materials and methods: MI was induced by a 40-min occlusion of the left anterior descending coronary artery and 3-h reperfusion in male anesthetized Sprague–Dawley rats. Brief LVPO was achieved by two 10-min partial snarings of the ascending aorta, raising the systolic left ventricular pressure 50% above the baseline value. Ischemic preconditioning was elicited by two 10-min coronary artery occlusions and 10-min reperfusions.

Results: Brief LVPO and ischemic preconditioning significantly decreased MI size ($P < 0.001$). Brief pressure overload significantly reduced myocardial apoptosis, as evidenced by the decrease in the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling-positive nuclei ($P < 0.001$), little or no DNA laddering, and reduced caspase-3 activation ($P < 0.01$). Moreover, brief pressure overload significantly increased Bcl-2 ($P < 0.001$) and decreased Bax ($P < 0.001$) and p53 ($P < 0.01$). Akt phosphorylation was significantly

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increased by brief pressure overload ($P < 0.001$), whereas c-Jun N-terminal kinase phosphorylation was significantly decreased ($P < 0.001$). Hemodynamics, area at risk, and mortality did not differ significantly among groups.

Conclusions: Brief left LVPO significantly reduces myocardial apoptosis. The underlying mechanisms might be related to modulation of Bcl-2 and Bax, inhibition of p53, increased Akt phosphorylation, and suppressed c-Jun N-terminal kinase phosphorylation.

1. Introduction

Apoptosis and necrosis are two distinct forms of cell death induced by myocardial ischemia and reperfusion [1]. Myocardial apoptosis has been reported to play important roles in the pathophysiology of myocardial infarction (MI), heart failure, and dilated cardiomyopathy [2]. Inhibition of apoptosis mediates myocardial protection, and ischemic preconditioning (IPC) provides powerful myocardial protection [3]. Accumulating evidence shows that inhibition of apoptosis contributes to IPC protection [4]. Pharmacologic preconditioning also confers myocardial protection via the inhibition of apoptosis [5].

We previously reported that brief left ventricular pressure overload (LVPO) by partial snaring of the ascending aorta significantly reduced MI size [6]. Chronic pressure overload has been reported to activate apoptosis and is associated with the development of hypertrophy and heart failure. Okada *et al.* [7] revealed an increase of cardiomyocyte apoptosis in the mice 4 wk after transverse aortic constriction. Condorelli *et al.* [8] reported that cardiomyocyte apoptosis was found 18 wk after transverse aortic constriction in the rats. The effects of brief LVPO, which we use as a preconditioning maneuver, on apoptosis, however, are not known. Therefore, we conducted this study to investigate whether brief LVPO reduces apoptosis. The roles of Bcl-2, Bax, p53, Akt, and c-Jun N-terminal kinase (JNK) were also investigated in the present study. Our findings might serve to elucidate the mechanisms through which brief LVPO reduces myocardial apoptosis induced by ischemia and reperfusion and provides a rationale for the development of therapeutic strategies to protect myocardium in cardiac surgery or catheter-based treatment for ischemic heart disease.

2. Material and methods

This study was approved by the Animal Experiment Committee of Taipei Veterans General Hospital. Animals were cared for humanely in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Academic Press, 1996).

2.1. Animal preparation

The techniques that we used in the present study for animal preparation have been previously reported [6]. Briefly, male Sprague–Dawley rats (250–300 g) were anesthetized with intraperitoneal pentobarbiturate (40 mg/kg). After tracheotomy, each rat was intubated and ventilated. The descending aorta was cannulated for arterial pressure monitoring. Electrocardiography leads were placed on limbs. After median sternotomy, a 4-0 silk suture was passed around the proximal part of the left anterior descending coronary artery. The ends of the silk suture were threaded through a small vinyl tube to form a snare. A ribbon tape was passed around the ascending aorta. A microtip-manometer (Millar, Houston, TX) was inserted into the left ventricle to measure the left ventricular pressure. The body temperature was monitored using a rectal thermometer and maintained at 37°C with heating pads throughout the experiments.

2.2. Experimental protocol

After achieving hemodynamic stability for 20 min, one hundred ten rats were divided randomly into four groups (Table 1). Group 1 (sham group) received the same surgical procedures without any pretreatment, coronary artery occlusion (CAO) or reperfusion. Group 2 (control group) did not receive any pretreatment. For group 3 (LVPO group), two episodes of brief LVPO were induced via partial snaring of the ribbon tape around the ascending aorta to raise the systolic left ventricular pressure 50% above baseline value for 10 min. The two episodes of brief LVPO were interspersed with a 10-min recovery. For group 4 (IPC group), IPC was induced via two episodes of 10-min CAO. The two episodes of CAO were interspersed with a 10-min reperfusion. Ten minutes after the previously mentioned treatments, a 40-min CAO was induced in the rats in group 2, group 3, and group 4 by pulling the snare around the left anterior descending coronary artery. Successful occlusions were verified by observing the development of ST-segment elevation and changes in the QRS complex on the electrocardiograms and cyanotic changes in the myocardium in the occluded area. After 40 min of CAO, the snare was released for reperfusion for 3 h. Reperfusion was confirmed by refilling the coronary artery and visualizing a reactive

Table 1 – Mortality and exclusions.

Group	Treatment	Number	Mortality			Number included
			VF	HF	Other	
1	Sham	18	0	0	0	18
2	Control	31	1	1	1	28
3	LVPO	31	1	1	1	28
4	IPC	30	0	1	1	28

HF = heart failure; VF = ventricular fibrillation.
No significant differences in the mortality rate were observed among the groups. Pearson chi-square *P* value was 0.581.