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Direct evidence that hypoxia triggers the cardioprotective response of ischemic preconditioning in a dog double-circuit cardiopulmonary bypass model

Yan Deng[a,b,1], Guo Chen[a,1], Ronghua Zhou[a], Wei Wu[c], Zhen You[d], Wei Meng[e], Linhui Yang[a], Yanhua Qiu[a], Jin Liu[a,*], Tao Li[a,b,**]

[a] Laboratory of Anesthesiology and Translational Neuroscience Center, West China Hospital, Sichuan University, Chengdu, China
[b] West China–Washington Mitochondria and Metabolism Center, West China Hospital of Sichuan University, Sichuan, Chengdu, China
[c] Department of Anesthesiology, Chengdu Military General Hospital, Chengdu, China
[d] Department of Hepato-Bilio-Pancreatology, West China Hospital, Sichuan University, Chengdu 610041, China
[e] Department of Thoracic and Cardiovascular Surgery, West China Hospital, Sichuan University, Chengdu 610041, China

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ABSTRACT

Aims: It has been widely accepted that ischemic preconditioning (IPC) exhibits a promising and reproducible cardioprotective effect against ischemia/reperfusion (I/R) injury. However, the actual trigger that amplifies the molecular signaling and protects I/R heart is still unclear.

Main methods: To separate the factors involved in IPC, we established a dog double-circuit cardiopulmonary bypass (CPB) model, which consists of a systemic circuit and a coronary circuit. Forty-two male adult beagle dogs were randomly allocated into 7 groups: sham, I/R, IPC, hypoxia preconditioning (HPC), accumulated metabolite preconditioning (MPC), oxygenated or deoxygenated erythrocytes preconditioning (OxyEPC and DeoxyEPC). After pretreatment, dogs were subjected to 2 h-cardiac arrest and 2 h-reperfusion.

Key findings: There were no differences in the cardiac function and hemodynamic parameters at baseline among groups. Like IPC, the hypoxia-related pretreatments HPC and DeoxyEPC improved post-arrest left ventricular systolic/diastolic performance and reduced pulmonary vascular resistance. The cardiac oxygen (O_2) utilization was also greatly elevated in these hypoxia-related pretreatment groups, as evidenced by increased cardiac O_2 consumption (VO_2) and O_2 extraction index (O_2EI) and suppressed lactate level. Besides, we did not observe improvement of these parameters in the MPC and OxyEPC groups. Further study indicated that these hypoxia-related pretreatments were associated with the attenuation of pro-inflammatory cytokines release and the elevation of complex I-supported mitochondrial respiration.

Significance: With a dog double-circuit CPB model, we demonstrated that hypoxia is the actual trigger to initiate the cardioprotective effect of IPC in vivo, which was related to reduced cardiac inflammation and ameliorated complex-I supported mitochondrial function.

1. Introduction

Ischemic preconditioning (IPC) has been widely accepted to provide a promising and reproducible protective effect against subsequent ischemia/reperfusion (I/R) injury [1,2]. The cardioprotective effect of IPC was first identified in 1986 [1]. The anesthetized open-chest dogs were exposed to four episodes of 5-min coronary artery occlusion and 5-min reperfusion before the onset of a 40-min sustained occlusion of the coronary artery. IPC significantly reduced the myocardial infarct size after I/R [1]. Over the last 3 decades the underlying molecular mechanisms have been investigated comprehensively and our understanding of IPC has increased exponentially. Release of extracellular signaling molecules such as adenosine, bradykinin and opioids, activation of protein kinase C (PKC) and PI3K/Akt/MEK1/2/ERK1/2 cascades, as well as production of moderate reactive oxygen species (ROS) are believed to contribute to IPC-evoked intrinsic protective response

* Corresponding author.
** Correspondence to: T. Li, West China–Washington Mitochondria and Metabolism Center, West China Hospital of Sichuan University, Sichuan, Chengdu, China.
E-mail addresses: scujinliu@foxmail.com (J. Liu), scutaoli1981@scu.edu.cn (T. Li).
[1] These authors contribute equally to this study.

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[3–5]. However, it is worth pointing out that most of these mechanistic studies did not distinguish IPC from hypoxia preconditioning (HPC). In most of the in vivo animal experiments the pretreatment were conducted by left anterior descending artery (LAD) occlusion, which is IPC, while the in vitro cell culture studies were completed by hypoxia or oxygen (O_2) glucose deprivation, which is HPC. This discrepancy raised a question that whether these altered molecular signaling pathways in the cell studies really reflect the situation in animals? As we know, ischemia is a multifactor process including hypoxia, accumulation of metabolites and red blood cells (RBC), and even change of shear force from blood stream. Whether these factors work synergistically or only one factor is responsible to the protective effect of IPC remains unclear.

Cardiopulmonary bypass (CPB) is required in the vast majority of cardiac surgeries, which is a technique that temporarily takes over the function of the heart and lungs during surgery, maintaining the circulation of blood and the O_2 content of the patient's body [6]. Cardiac I/R injury during CPB is the major cause of death and poor prognosis of patients after cardiac surgery [7]. As the population ages, this problem is likely to increase given that elderly hearts are more susceptible to I/R injury [8,9]. Thus, strategies that provide protection of the heart are urgently required. More important, CPB model is an ideal and unique model for investigating IPC because it allows treatment on the heart directly without interfering with other parts of the body. In this study, we will introduce a novel double-circuit CPB model, which consists of a standard CPB circuit to support systemic perfusion and a coronary circuit to maintain myocardial perfusion (Fig. 1). By use of this model, we could easily separate the elements involved in ischemia and investigate their effects individually. Therefore, based on a novel dog double-circuit CPB model, this study was designed to identify which factor is the active trigger responsible for IPC-induced cardioprotective effect in vivo.

2. Materials and methods

All animal experimental procedures were performed in accordance with the policies of the Animal Care and Use Committee of Sichuan University, and conformed 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). A total of 46 adult male beagle dogs, weighing 8–10 kg, were supplied by the Experimental Animal Center of West China Hospital, Sichuan University in Chengdu, China. The animals were housed at a constant temperature (23 ± 1 °C) on a 12-h light/dark cycle with free access to food and water. Four dogs were excluded from the experiment because failure to cannulate aorta or excessive blood loss during surgery.

2.1. Dog double-circuit cardiopulmonary bypass model

As illustrated in the Fig. 1A, a dog double-circuit CPB model was composed of a standard extracorporeal circuit to support systemic perfusion [10,11] and a coronary circuit to maintain myocardial perfusion. In brief, after induction (4 mg/kg propofol, 0.1 mg/kg midazolam and 5 μg/kg fentanyl) and muscle relaxation (1 mg/kg scoline), dogs were intubated with a Fr. 7.5 endotracheal tube and mechanically ventilated using an air/O_2 mixture (1:4) with tidal volume 10 mL/kg (Datex-Ohmeda Excel 210, Soma Technology, Cheshire, Connecticut, USA). Each group received a continuous infusion of fentanyl at 0.3 μg/kg/min and vecuronium bromide at 0.2 mg/kg/h during surgery. Anesthesia was maintained with 150 μg/kg/min propofol. After heart exposure through a mid-sternal incision and heparinization (3 mg/kg), the ascending aorta and the right atrial appendage were cannulated and connected to a blood reservoir (Kewei Medical Ltd. Guangdong, China), a rolling pump (StÖckert II, Munich, Germany), a membrane oxygenator (1500 mL/min, Kewei Medical Ltd.) and an arterial filter (Kewei Medical Ltd.). The CPB circuit was primed with 200 mL Lactate Ringer's solution containing 5% sodium bicarbonate (10 mL/L), 20% mannitol (2.5 mL/L), furosemide (0.5–1.0 mg/L), dexamethasone (5 mg/L), heparin (10 mg/L) and 10% potassium chloride (5 mL/L), 200 mL 6% hydroxyethyl starch 130/0.4 and 200 mL allogeneic fresh blood from a donor dog. For the coronary circuit, another cannula was inserted into the root of aorta and connected to a blood reservoir, a rolling pump and a membrane oxygenator. The coronary circuit was primed with half amount of the solution described above.

2.2. Experimental protocol

To explore which factor triggers the protective response of IPC, 42 beagle dogs were randomly allocated into 7 groups: sham group, I/R control group, ischemia preconditioning group (IPC), hypoxia preconditioning group (HPC), accumulated metabolite preconditioning group (MPC), oxygenated erythrocytes preconditioning group (OxyEPC) and deoxygenated erythrocytes preconditioning group

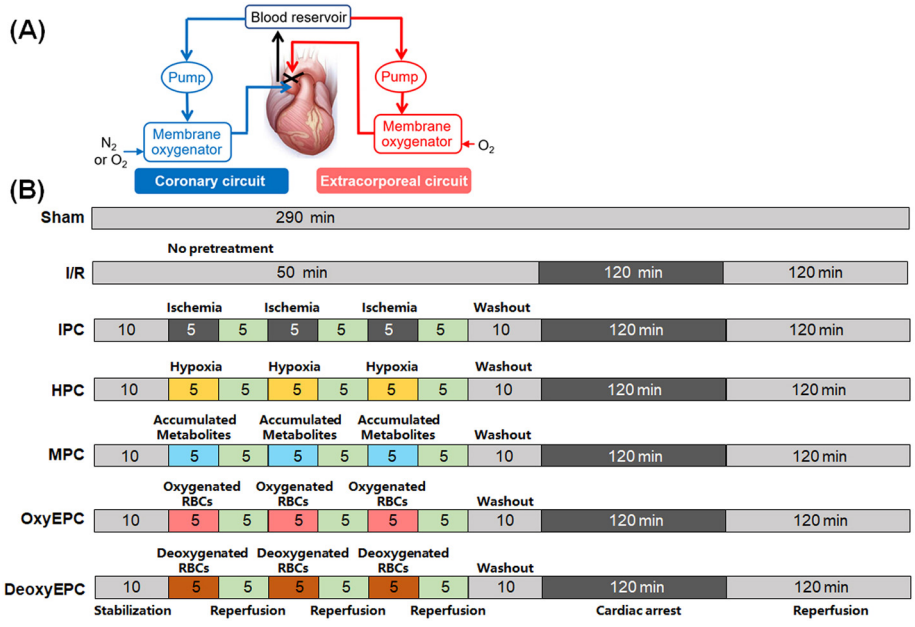

Fig. 1. A schematic diagram of the experimental protocol. A, A dog double-circuit CPB model was composed of a standard extracorporeal circuit to support systemic perfusion and a coronary circuit to maintain myocardial perfusion. B, Forty-two male adult beagle dogs were randomly allocated into 7 groups (n = 6 per group): Sham group, I/R group, IPC group, HPC group, MPC group, OxyEPC group and DeoxyEPC group. The preconditioning protocol was composed of 3 cycles of 5-min pretreatment and 5-min reperfusion. After 2 h of cardiac arrest, hearts were reperfused by aortic declamping. After 30 min of reperfusion, the dogs were weaned from CPB and observed for 90 min. The animal received sham operation without CPB and cardiac arrest was used as sham control.