Journal of Surgical Research **164,** 175–181 (2010)
doi:10.1016/j.jss.2010.08.050

ASSOCIATION FOR ACADEMIC SURGERY

Ischemic Postconditioning Does Not Provide Cardioprotection from Long-Term Ischemic Injury in Isolated Male or Female Rat Hearts[1]

Daniel S. Lee, B.S.,* Gregory E. Steinbaugh, Ph.D.,* Ricardo Quarrie, M.D.,* Fuchun Yang, M.D.,†,‡ M. A. Hassan Talukder, M.B.B.S., Ph.D.,† Jay L. Zweier, M.D.,† and Juan A. Crestanello, M.D.*,[2]

*Division of Cardiothoracic Surgery, Department of Surgery, and Davis Heart and Lung Research Institute, Ohio State University, Columbus, Ohio; †Division of Cardiovascular Medicine, Department of Internal Medicine, and Davis Heart and Lung Research Institute, Ohio State University, Columbus, Ohio; and ‡The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, People's Republic of China

Submitted for publication May 20, 2010

Background. **Ischemic postconditioning (PoC) is a cardio-protective strategy in which initial reperfusion is interrupted by episodes of ischemia. It is unclear whether PoC can be achieved in the Langendorff perfused rat heart model. We investigated (1) whether postconditioning occurs in Langendorff perfused rat heart and (2) whether there is a gender-specific response to PoC.**

Materials and Methods. **Male/female rat hearts (n = 8/group) were subjected to 30 min of equilibration, 30 min of ischemia, and 120 min of reperfusion (Control). PoC was induced by 6 cycles (PoC 6c10s), 3 cycles (PoC 3c10s), or 2 cycles (PoC 2c10s) of 10 s reperfusion/10 s ischemia. Rate pressure product (RPP) and infarct size were measured. Male rats (n = 7/group) were subjected *in vivo* to 30 min left coronary ligation followed by 24 h of reperfusion (Control) or PoC 6c10s and 24 h of reperfusion.**

Results. **Recovery of RPP was 18% ± 4% in male Control *versus* 17% ± 2% for 6c10s, 16% ± 1% for 3c10s, and 15% ± 3% for 2c10s. Female Control hearts recovered 25% ± 3% of their RPP *versus* 21% ± 2% for 6c10s. Infarct size was 25% ± 3% for male Control *versus* 26% ± 3% for 6c10s, 30% ± 2% for 3c10s, 28% ± 1% for 2c10s, and 30% ± 2% for female Control *versus* 29% ± 2% in 6c10s. *In vivo* infarct size for Control and PoC 6c10s was 44% ± 3% and 28% ± 5%, respectively ($P < 0.05$).**

Conclusions. **In the Langendorff perfused rat hearts, none of the PoC protocols improved myocardial tolerance to ischemia reperfusion injury nor decreased infarct size; however, *in vivo* postconditioning did confer protection. The lack of protection in the isolated hearts was not gender specific.** Published by Elsevier Inc.

Key Words: **ischemic postconditioning; cardioprotection; ischemic injury; rat heart myocardium; infarct.**

INTRODUCTION

Ischemic postconditioning (PoC) was first proposed by Zhao and colleagues [1] as an alternative method to ischemic preconditioning (IPC) [2] to improve myocardial tolerance to ischemia-reperfusion (IR) injury. PoC is a protection strategy in which restoration of myocardial perfusion after ischemia is established in a staged fashion with multiple brief episodes of ischemia interrupting the initial reperfusion period. PoC is an "after the fact" protection strategy and may have greater clinical application in particular in reperfusion after myocardial infarction or cardiac surgery.

PoC has been shown to decrease infarct damage *in vivo* [3–6]. However, there are reports that show no benefit from postconditioning *in vivo* [5, 7]. The isolated crystalloid perfused heart is a time proven model for the study of ischemia reperfusion injury and its mechanisms [2]. It is unclear whether PoC protection can be achieved in the isolated rat heart. There also has been controversy in terms of gender-specific protection associated with PoC. It has been demonstrated that females of different species have improved tolerance to IR injury. Mechanisms underlying this gender-specific tolerance to IR injury are thought to involve the sex hormone estrogen [8–11].

[1] Presented at the 5th Annual Academic Surgical Congress, San Antonio, Texas, February 5, 2010.

[2] To whom correspondence and reprint requests should be addressed at Division of Cardiothoracic Surgery, Ohio State University, 410 W 10th Ave, N816 Doan Hall, Columbus, OH 43210-1218. E-mail: Juan.Crestanello@osumc.edu.

0022-4804/$36.00
Published by Elsevier Inc.

The purpose of this study was to investigate the effect of postconditioning on the crystalloid perfused isolated rat hearts and to determine whether there is gender specific response to PoC in the isolated rat heart. PoC was induced by different protocols in male and female rat hearts and mechanical function parameters and myocardial infarct size were measured. The PoC protocols utilized did not improve myocardial tolerance to ischemia reperfusion, nor decrease myocardial infarct size in crystalloid perfused isolated male and female rat hearts. We speculate that humoral or blood factors may play an important role on the induction of PoC.

MATERIALS AND METHODS

Isolated Heart Preparation

Male and female Sprague-Dawley rats weighing between 275 and 350 g (Harlan Laboratories, Indianapolis, IN) were housed in a quiet environment and fed a standard diet. Rats were anaesthetized with sodium pentobarbital (60 mg/kg i.p.) (Ovation Pharmaceuticals, Deerfield, IL) and heparin sodium (500 U i.p.) (APP Pharmaceuticals, LLC, Schaumburg, IL) in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication no. 86-23, revised 1996), the Public Health Service Policy on Humane Care and Use of Laboratory Animals (National Institutes of Health, revised 2002), and the Animal Welfare Act (United States Drug Administration, revised 2007). Hearts were excised and perfused in a Langendorff apparatus at a constant aortic pressure of 76 mm Hg with oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit buffer containing (in mM) 118 NaCl, 4.6 KCl, 1.17 KH_2PO_4, 1.17 $MgSO_4$, 1.16 $CaCl_2$, 23 $NaHCO_3$, and 5.3 glucose, pH 7.4, at 37°C. A latex balloon was inserted in the left ventricle through the mitral valve and attached to a pressure transducer (COBE, Lakewood, CO) for monitoring of ventricular function. Left ventricular end diastolic pressure was set at 10 mm Hg. Hearts were maintained at 37°C throughout the experiment by immersion in a water-jacketed non-gassed perfusate bath. Global non-flow ischemia was created by a stopcock located immediately above the aorta. Heart rate and left ventricular developed pressure (LVDP: peak systolic minus end diastolic pressure) were recorded throughout the experiment. Rate pressure product (RPP) was calculated by multiplying the heart rate and left ventricular developed pressure. Coronary flow was measured using a TS410 transmit time tubing flowmeter (Transonic Systems Inc., Ithaca, NY). Data were continuously recorded using a PowerLab Chart ver. 4.2 (AD Instruments Inc., Milford, MA) and a Dell GenuineIntel x86 Family 6 Model Stepping 6 computer (Dell Computer Corp., Round Rock, TX).

Postconditioning Protocols

Hearts ($n = 8$/group) were assigned to the Control or Postconditioning groups (Fig. 1). In the Control group, hearts were subjected to 30 min of equilibration, followed by 30 min of global normothermic ischemia, and 120 min of reperfusion. In the postconditioning groups, postconditioning was induced after 30 min of equilibration, and 30 min of global normothermic ischemia, by either (1) six cycles of 10 s reperfusion and 10 s ischemia (PoC 6c10s), (2) three cycles of 10 s reperfusion and 10 s ischemia (PoC 3c10s), and (3) two cycles of 10 s reperfusion and 10 s ischemia (PoC 2c10s). Hearts were then reperfused for a total of 120 min.

Infarct Size Measurement

Infarct measurements were made using 2,3,5-triphenyltetrazolium chloride (TTC, Fluka, Milwaukee, WI). Briefly, ventricles were removed from the Langendorff apparatus at end reperfusion and placed at –20°C for 30 min for hardening. Ventricles were cut with a rat heart slicer (Zivic Instruments, Pittsburgh, PA) into 2 mm thick pieces. Slices were incubated with TTC (45 mM in PBS) solution at 37°C for 15 min and then stored overnight in 10% formalin solution (Sigma-Aldrich, Inc., St. Louis, MO) for color enhancement. Infarct size measurements were taken using Metamorph ver. 7.1.2.0 software (MDS Analytical Technologies, Toronto, CN) for clear visualization of infarct (white) *versus* healthy (red). Percent (%) infarct of slice was calculated by determining the infarct area (white) of the heart slice divided by total area of the heart slice. The following formula was used to determine total infarct size for each heart: the sum of % infarct of slice × weight of slice (in g) divided by the sum of weight of slice (in g).

In vivo Postconditioning Protocol and Measurement of Myocardial Infarction

Male Sprague-Dawley (Harlan, Indianapolis, IN) rats aged 8–10 wk were randomly allocated into 2 groups ($n = 7$/group): Control, subjected to 30 min of ischemia and 24 h of sustained reperfusion; and postconditioning (PoC 6c10s), subjected to 30 min of ischemia followed by six cycles of 10 s reperfusion and 10 s ischemia, and then 24 h of sustained reperfusion. Animals were provided standard rat chow and water *ad libitum*.

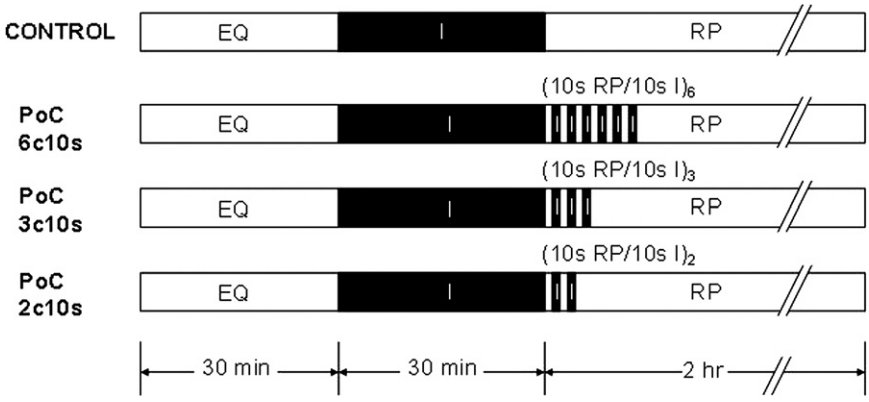

FIG. 1. Postconditioning protocols for isolated hearts. Control hearts were subjected to 30 min of equilibration (EQ), 30 min of global normothermic ischemia for 30 min (I), and 90 min of reperfusion (RP). Postconditioning was induced by 6 cycles of 10 s reperfusion and 10 s of ischemia (PoC 6c10s), 3 cycles of 10 s reperfusion and 10 s of ischemia (PoC 3c10s), or 2 cycles of 10 s reperfusion and 10 s of ischemia (PoC 2c10s).