Equivalent Cardioprotection Induced by Ischemic and Hypoxic Preconditioning

Xujin Xiang1 Haixia Lin2 Jin Liu1 Zeyan Duan1

1 Department of Anesthesiology, West China Hospital, Sichuan University, Chengdu, China
2 Laboratory of Anesthesiology and Critical Care Medicine, West China Hospital, Sichuan University, Chengdu, China


Introduction

Ischemic/reperfusion (I/R) injury is closely related to organ transplantation, stroke, cardiovascular surgery, and disease. Myocardial I/R injury is the major reason of death associated with heart disease.1 Ischemic preconditioning (IPC), consisting of single or multiple episodes of sublethal ischemia and reperfusion, markedly reduces myocardial infarct size and is the most effective endogenous protective approach against I/R injury. However, clinical application of IPC is limited for practical reasons and high risks.2 New ways against I/R injury are urgently required.

Hypoxic preconditioning (HPC) is a phenomenon that sublethal hypoxia increases cellular resistance against following lethal I/R injury. The clinical application of HPC may be much easier and safer than that of IPC, for hypoxia was not accompanied by blood flow cessation. HPC has been verified in various tissues. However, most studies have been focused on prolonged HPC, which requires hours to weeks, or delayed HPC, in which the protective effect occurs approximately 24 hours after the preconditioning stimulus.3–6 Early cardioprotection, which occurs immediately after the preconditioning induced by short duration HPC, would be of great value clinically. Only several studies demonstrated that one-cycle short duration HPC exerted early cardioprotection.7–9 Cai et al reported that 30 minutes after preconditioning of mice with 5 cycles of 6-minute 6%O2 and 6-minute 21%O2, the hearts were not protected against I/R injury.10 It has not been known that whether multiple-cycle short duration HPC could produce early protective effects in

Abstract

Objective We aimed to compare cardioprotection induced by various hypoxic preconditioning (HPC) and ischemic preconditioning (IPC) protocols.

Methods Isolated rat hearts were randomly divided into 7 groups (n = 7 per group) and received 3 or 5 cycles of 3-minute ischemia or hypoxia followed by 3-minute reperfusion (IPC33 or HPC33 or IPC53 or HPC53 group), 3 cycles of 5-minute ischemia or hypoxia followed by 5-minute reperfusion (IPC35 group or HPC35 group), or 30-minute perfusion (ischemic/reperfusion group), respectively. Then all the hearts were subjected to 50-minute ischemia and 120-minute reperfusion. Cardiac function, infarct size, and coronary flow rate (CFR) were evaluated.

Results Recovery of cardiac function and CFR in IPC35, HPC35, and HPC53 groups was significantly improved as compared with I/R group (p < 0.01). There were no significant differences in cardiac function parameters between IPC35 and HPC35 groups. Consistently, infarct size was significantly reduced in IPC35, HPC35, and HPC53 groups compared with ischemic/reperfusion group.

Conclusion Multiple-cycle short duration HPC exerted cardioprotection, which was as powerful as that of IPC.

Keywords
► ischemic preconditioning
► hypoxic preconditioning
► reperfusion injury

Cardiovascular

229

© 2013 Georg Thieme Verlag KG
Stuttgart · New York

ISSN 0171-6425.
isolated rat hearts. Therefore, we investigated the early effects of various cycles and the number and hypoxic duration of each cycle on isolated rat hearts subjected to I/R injury and compare the effects with cardioprotection by IPC.

**Materials and Methods**

**Animal and Rat Langendorff Model**

Hearts from adult male Sprague-Dawley rats (220–300 g) anesthetized with pentobarbital sodium (50 mg/kg intraperitoneally) were mounted in a Langendorff model (Radnoti, Monrovia, California, United States) and perfused with Krebs-Henseleit buffer (in mM: 118.0 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, 25.0 NaHCO₃, 11.1 glucose, and 0.125 EDTA-2Na, pH: 7.35 to 7.45) at 37°C and a constant pressure of 100 cm H₂O. Oxygenated KH buffer was continuously bubbled with 95% O₂ + 5% CO₂. Hypoxic Krebs Henseleit (KH) buffer was bubbled with 95% N₂ + 5% CO₂.

**Experimental Protocol**

After being stabilized with oxygenated KH buffer for 20 minutes, 49 isolated hearts were randomly divided into 7 groups (n = 7 per group) and received 3 cycles of 3-minute global ischemia or hypoxic KH buffer followed with 3-minute oxygenated KH buffer reperfusion (IPC33 or HPC33 group), 5 cycles of 3-minute global ischemia or hypoxic KH buffer followed with 3-minute oxygenated KH buffer reperfusion (IPC53 group or HPC53 group), 3 cycles of 5-minute global ischemia or hypoxic KH buffer followed with 5-minute oxygenated KH buffer reperfusion (IPC35 group or HPC35 group), or 30-minute perfusion with oxygenated KH buffer (I/R group), respectively. Then all the hearts were subjected to 50-minute ischemia and 120-minute oxygenated KH buffer reperfusion. Cardiac left ventricle (LV) function, infarct size, and coronary flow rate (CFR) were evaluated. The experiment protocol was shown in Fig. 1.

**Cardiac LV Function and CFR Measurement**

A water-filled latex balloon connected to a pressure transducer (Model SP844; MEMSCAP, Durham, North Carolina, United States) was inserted into the left ventricular cavity via the left auricle to measure cardiac LV function. Left ventricular developed pressure (LVDP, in mm Hg), its maximal and minimal rates of rise ([dP/dt]max and [dP/dt]min, in mm Hg/s), and heart rate (HR) were measured at the end of stabilization (baseline), and at various times, during reperfusion. Coronary flow rate (mL/minute) was also estimated at these time points. Recoveries of LVDP, ([dP/dt]max, [dP/dt]min, HR, and CFR were, respectively, calculated as percentages of baseline values.

**Infarct Size Assessment**

At the end of 120 minutes of reperfusion, hearts were sliced into 2-mm transverse sections from apex to base. The slices were incubated at 37°C with 1% 2,3,5-triphenyltetrazolium chloride (TTC, Sigma–Aldrich, St. Louis, Missouri, United States) in phosphate buffer (pH = 7.4) for 15 minutes and then fixed in a 4% paraformaldehyde phosphate buffer for 24 hours.
hours. Subsequently, the slices were scanned (MRS-800P48U, MICROTEK, Shanghai, China). The infarct (white) area was assessed by planimetry using Image Pro Plus 5.0 software (Media Cybernetics, Silver Spring, Maryland, United States) and expressed as a percentage of total slice area.

**Statistical Analysis**

All values are expressed as means ± SE, the recovery of LVDP, (dp/dt)max, (dp/dt)min, HR, and CFR were analyzed by repeated-measures analysis of variance and using least significant difference procedure for post hoc test. Infarct size was...
tested with one-way analysis of variance followed by least significant difference procedure for post hoc test. All p values less than 0.05 were considered statistically significant (version 16.0, SPSS, Chicago, Illinois, United States).

Results

Recovery of Cardiac LV Function and CFR
During reperfusion, recovery of LVDP, (dp/dt)max, (dp/dt) min, and CFR in IPC35, HPC35, and HPC53 groups were significantly higher than I/R group (p < 0.01). There was no significant difference in recovery of LVDP, (dp/dt)max, (dp/dt) min, or CFR between IPC35 and HPC35 groups. Recovery of LVDP, (dp/dt)max, and CFR did not differ significantly between HPC53 and HPC35 groups. Only recovery of (dp/dt) min in HPC53 group was lower than that in HPC35 group (p < 0.05) (Fig. 2). There was no significant difference in HR among all the groups (data not shown; Fig. 2E).

Infarct Size
Infarct size was significantly reduced in IPC35, HPC35, and HPC53 groups (14.15 ± 2.45%, 15.00 ± 3.17%, and 19.47 ± 3.54%, respectively) in comparison with I/R group (49.11 ± 5.99%, p < 0.01). There was no significant difference in infarct size among IPC35, HPC35, and HPC53 groups (Fig. 3).

Discussion

In this study, our results demonstrated that multiple-cycle short duration HPC, which was performed by 3 cycles of 5-minute hypoxia followed with 5-minute reperfusion or 5 cycles of 3-minute hypoxic followed with 3-minute reperfusion, exerted early cardioprotection on isolated rat hearts subjected to I/R injury. The protection by multiple-cycle short duration HPC was comparable with that of IPC. Our finding was evidenced by improved recoveries of cardiac LV function and CFR and reduced myocardium infarct size.

Several studies have demonstrated that one-cycle IPC and HPC exerted similar cardioprotection. However, it has been unknown whether multiple-cycle HPC and IPC elicited similar cardioprotection. We demonstrated that early cardioprotection of multiple-cycle HPC was as powerful as that of IPC. In a whole-body hypoxia model, 30-minute intermittent hypoxia (repetitive cycles of 40-second hypoxia with 5 or 10% O2 followed by 20-second normoxia) did not induce delay cardioprotection in rat hearts. However, we indicated that 30-minute intermittent HPC (3 cycles of 5-minute hypoxia followed with 5-minute reperfusion or 5 cycles of 3-minute hypoxic followed with 3-minute reperfusion) exerted early cardioprotection on isolated rat hearts, and HPC may be intrinsic mechanisms that could be rapidly triggered involved in early cardioprotection by short duration HPC. A further understanding of these mechanisms could contribute

Fig. 2  Effects of IPC and HPC on cardiac infarct size after prolonged ischemia and reperfusion. (A) Histology picture. Sections of hearts were stained with triphenyltetrazolium chloride. (B) Infarct sizes are expressed as a percentage of the total slice area. Data are expressed as mean ± SE, n = 7. *p < 0.05, **p < 0.01 compared with I/R group. Abbreviations: HPC, hypoxic preconditioning; IPC, ischemic preconditioning.

Fig. 3  Effects of IPC and HPC on cardiac infarct size after prolonged ischemia and reperfusion.
to minimizing early cardioprotection provided by short duration HPC and developing new treatments against I/R injury in the future.

In conclusion, using an isolated rat heart model, our results demonstrated that multiple-cycle short duration HPC exerted early cardioprotection, which was as powerful as that of IPC. Further studies are needed to investigate the underlying mechanisms.

**Funding**

This study was supported by grants from the National Nature Science Foundation of China (81070117).

**References**