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ORIGINAL ARTICLE

Effects of Different Limb Remote Ischaemic Preconditioning on Ischaemia Reperfusion Injury in an Acute Left Anterior Descending Artery Occlusion Rat Model

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Background The aim is to compare effects of three different protocols of limb remote ischaemic preconditioning (LRIP) on ischaemia reperfusion injury in an acute left anterior descending artery (LAD) occlusion model rat.

Methods Forty adult male Wistar rats were randomly assigned into four groups: group A, control; group B, LRIP in bilateral upper-limb (BUL) IP; group C, LRIP in bilateral lower-limb (BLL) IP; group D, LRIP in bilateral upper and lower limbs (ULL) IP. The 60 min ligation and 180 min reperfusion in LAD were applied to all rats. Limb remote ischaemic preconditioning was performed using 5 min occlusion and 15 min reperfusion (six cycles). Heart rate, blood pressure and electrogastrography (EGG) were recorded. Creatine kinase isoenzyme (CK-MB) level and infarct size were measured.

Results Limb remote ischaemic preconditioning did not significantly affect heart rate, systolic blood pressure and arrhythmia score. However, LRIP significantly increased DBP value and decreased CK-MB levels and infarct size in group B, C, and D. Moreover, LRIP in ULL had a significantly better effect on reducing infarct size than LRIP in BUL and BLL.

Conclusions Limb remote ischaemic preconditioning at limbs could significantly reduce reperfusion injury in the heart. Moreover, LRIP in ULL indicated a better effect in reducing infarct size than LRIP in BUL and BLL.

Keywords Reperfusion injury • Remote preconditioning • Limbs preconditioning • Infarct size

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Introduction

Ischaemia reperfusion injury after myocardial infarction (MI) has been reported to be caused by restoration of blood supply to the heart after a critical period of ischaemia which resulted in parenchymal injury and dysfunction [1]. The early reperfusion injury is caused by an oxidative burst with the generation of a mass of oxygen free radicals [2]. It is likely that myocardial cells have adapted to a low-oxygen or oxygen-free environment following a period of ischaemia [3]. The abrupt restoration of blood supply will result in an excess of oxygen supply that leads to a series of human stress responses and consequent generation of extra heart damage [4,5]. Ischaemic preconditioning (IP) has been a simple and effective method against reperfusion injury [1].

Current IP methods include local and remote IP. However, the clinical applicability of local IP is limited by the need to induce ischaemia in the vulnerable target organ which may aggravate organ injury and dysfunction [6]. Remote IP (RIP) which was demonstrated by Przyklenk in 1993 [7], is characterised by short periods of ischaemia interspersed with reperfusion performed peripherally to the target site. The RIP has been developed as brief ischaemic intervals in a distant organ or limb to resistance ischaemia reperfusion injury [8]. Moreover, similar to the local IP, RIP also has some prevention functions in reducing infarct size [9] and preserving vascular endothelial function [10]. Remote IP in limbs (LRIP) is the commonly used protocol, which has been proved a safe and well-tolerated method with equivalent effect to local IP [11,12]. It was reported that LRIP could reduce the injury of myocardial ischaemia [13,14] and contribute neuroprotective effects [15].

Different preconditioning protocols (upper, lower or all limbs) of LRIP are reported in previous studies [16–18], and may have a different effect on preventing reperfusion injury. However, it is still unknown which is the best protocol for resistance reperfusion injury. Thus, in this study, we compared the effect of three LRIP protocols (upper, lower and all four limbs) on reducing reperfusion injury in an acute left anterior descending artery (LAD) occlusion rat model. It would provide the basis for the clinical application of LRIP.

Materials and Methods

Animals

Forty adult male Wistar rats (weight, 200-300 g) were provided and raised by the Laboratory Animal Centre of the First Affiliated Hospital of Harbin Medical University. All rats were housed individually in a room with a 12 h light-dark cycle. Rats were fed with rodent laboratory chow and water changed three times weekly. The experimental protocol was approved by the Ethics Committee for Animal Experimentation and conducted in accordance with the Guidelines for Animal Experimentation of our laboratory (which was based on the Guidelines in Asian Federation of Laboratory Animal Science [19]). We made all efforts to minimise animal use and suffering in these experiments.

Surgery and the Animal Model

After an overnight fast with unrestricted access to water, the rats were anaesthetised with an intraperitoneal injection of 10% chloral hydrate (Zhongshan Golden Bridge, Beijing, China) at a dose of 0.3 g/kg. Anaesthesia was maintained via supplemental dosage of chloral hydrate (0.1 mg/kg intraperitoneal) as needed. Then the 40 rats were randomly assigned into four groups with 10 rats in each group: group A, rats as controls; group B, rats received LRIP in bilateral upper limbs (BUL); group C, rats received LRIP in bilateral lower-limb (BLL); group D, rats received LRIP in bilateral upper and lower limbs (ULL).

In group A, the animals were sterilised and sheared in the operation area. Subsequently, endotracheal intubation was performed through the trachea incision and the rats were mechanically ventilated at a frequency of 60 breaths/min with a tidal volume of 20-30 ml/kg atmospheric air. Arterial pressure and heart-rate were monitored continuously using a calibrated pressure transducer (Bailey & Mackey Ltd., Birmingham, UK) that connected with an invasive pressure monitor (Spacelabs Medical, Inc., Redmond, WA). The thoracotomy was performed with a 3 cm vertical incision in the left mid-clavicular line. The thoracic cavity was exposed by careful dissection. After slitting a 2 cm incision in the intercostal muscle between the third and fourth ribs, the heart was exposed by pericardial incision. Afterwards, the ligation was conducted in the upper third of the LAD with silk. The rats were subjected to 60 min of occlusion and then reperfusion for 180 min. Body temperatures were monitored with a rectal probe and kept at $36.5 \pm 1^{\circ}C$ using a heating pad and overhead lamp during the whole experiment.

The above operations were also applied to the rats of group B, C and D. As well, six cycles of 20 min IP (5 min occlusion and 5 min reperfusion [20]) were applied on BUL, BLL and ULL respectively in group B, C and D after LAD ligation. The occlusion was performed under oxygen saturation (SpO2) < 80%. A rubber band tourniquet (1 cm width and 30 cm length) was used for occlusion at the root of the limbs. To ensure the rubber band was placed at the same site between animals and avoid inconsistent operations, one experienced surgeon was required to complete these operations with less than a one-day interval, during this study. No rats died during the experiment.

Haemodynamic Examination

The heart-rate and blood pressure were recorded continuously, especially before surgery, after 30 min of occlusion as well as after 60 min and 180 min of reperfusion. These data were analysed to show changes of haemodynamic variables.

Arrhythmia Score

During this experiment, no prophylactic antiarrhythmic drugs were applied. Ventricular arrhythmia caused by sustained haemodynamic collapse was treated with interrupted