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Ischemic postconditioning of the isolated human myocardium: Role of the applied protocol

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ABSTRACT

Background: Ischemic postconditioning (IPostC), has been proposed as a useful approach to reduce infarct size in all species, but its clinical utility remains unclear.
Objective: To investigate the role played by the protocol used on the efficacy of IPostC in protecting the diseased human myocardium.
Methods: Myocardial atrial samples from patients were subjected to a 90 min ischemia/120 min reoxygenation followed by different IPostC protocols to investigate the role of the time of ischemia (30, 60, 90 and 120 s) and the number of cycles (1, 2, 3 and 4) with 60 and 120 s of total ischemic time. Muscles were also subjected to ischemic preconditioning (IPreC). The release of lactate dehydrogenase (LDH) and the measurement of tetrazolium bromide (MTT) were determined.
Results: IPostC increased the LDH and decreased the MTT values from those of control, independently of the duration of the conditioning ischemia. LDH and MTT values also worsened by augmenting the number of IPostC cycles whereas they were significantly improved by IPreC. However, analysis of individual results indicated that in approximately 1/3 of the cases IPostC exhibited some degree of protection especially in the presence of increased ischemic injury.
Conclusions: The present findings show that IPostC of the human myocardium may be influenced by the protocol used and also by the degree of the preceding ischemic injury. IPostC was beneficial in approximately 1/3 of the cases; however in the remaining cases it increased ischemic damage and, therefore, these results raise a word of caution on its broad clinical use.

1. Introduction

Infarct size is recognized as a major determinant of myocardial functional recovery and mortality after an acute myocardial infarction [1]. Hence limitation of infarct size is critical to improve survival and to prevent the development of heart failure. The most effective treatment to reduce infarct size is the re-opening of the culprit occluded coronary artery by coronary angioplasty or thrombolysis. Adjunctive treatments at reperfusion, such as β-blockers and angiotensin converting enzyme inhibitors, can ameliorate morbidity and mortality, although not via a reduction in infarct size [2]. Despite these improvements in treatment, mortality remains elevated in high risk patients [3] and the prevalence of heart failure is also increasing [4], which justifies the search for therapies that would effectively reduce infarct size.

It has been suggested that reperfusion injury accounts for 50% of the final size of a myocardial infarction [5] and that ischemic postconditioning (IPostC), a sequence of short reperfusion/ischemia episodes after a prolonged ischemic period, can be a useful approach to reduce infarct size. The first evidence of infarct size reduction associated with IPostC was reported by Zhao et al. [6] in a canine model. Afterwards, experimental studies in various models and species (dog, rabbit, mouse, rat and pig) have confirmed the beneficial action of IPostC [7], although other studies have reported no protection [8–10] or even a detrimental effect [11]. The reason for this discrepancy could be due to the use of different IPostC protocols, variations on the duration of the ischemic insult, and even the anesthetic regimen, and the animal species and type of strain utilized [12].

Staat et al. [13] performed the first prospective clinical trial on the efficacy of IPostC in patients with ST-segment elevation myocardial infarction (STEMI). In this study involving a small number of patients, they showed a reduction in myocardial infarct size, as estimated by the blood levels of creatine kinase. However, afterwards, small size randomized trials [14] and, more recently, larger randomized studies [15–17] have reported a lack of reproducibility of the IPostC response. The source of disagreement is not clear but it is possible that the lack of uniformity on the IPostC protocol between studies may play a role.

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Therefore, in order to determine the most effective IPostC protocol to protect the ischemic and reoxygenated human myocardium, we used a well characterized in vitro experimental model [18]. This model provides us a matchless opportunity to test cardioprotective strategies in the human myocardium and to investigate the underlying mechanisms in a safe, rapid and inexpensive manner.

2. Materials and methods

2.1. Patients

The study was approved by the local Ethics Committee and informed consent was obtained from all the participating patients. The right atrial appendage was obtained from patients undergoing elective cardiac surgery prior to cannulation of the heart and the establishment of cardiopulmonary bypass. Demographic data, presence of vascular risk factors and medical treatment received for each participant patient were recorded. A total of 150 patients (50 in each study) were sequentially recruited without the exclusion criteria.

2.2. Study groups

2.2.1. Study 1

2.2.1.1. Duration of the IPostC ischemic time. To study the role of the ischemia time in IPostC, the muscles obtained from the right atrial appendage were postconditioned with 30, 60, 90 and 120 s of total ischemia and divided into 3 cycles of 10, 20, 30 and 40 s, of ischemia/reoxygenation (I/R) respectively (n = 50), following 90 min of ischemia (Fig. 1).

2.2.2. Study 2

2.2.2.1. Number of IPostC cycles. To investigate the role of the number of cycles in IPostC, the muscles obtained from the right atrial appendage of another 100 patients were postconditioned by 1, 2, 3 and 4 cycles of I/R for a total of 60 (Study 2A; n = 50) and 120 s (Study 2B; n = 50) of ischemia after 90 min of ischemia (Fig. 2a & b).

In all the studies the IPostC stimulus was applied 30 s after the 90 min ischemic time, a period that has been shown to be necessary to induce protection [19].

2.3. Experimental preparation

The right atrial appendages were collected in buffer Krebs Henseleit Hepes (KHH) containing: 118 mM NaCl, 4.8 mM KCl, 27.2 mM $NaHCO_3$, 1.2 mM $MgCl_2$, 1.0 mM KH_2PO_4, 1.25 mM $CaCl_2$, 10 mM glucose, 20 mM HEPES and pH 7.4 at 4–5 °C. Briefly, the appendage was mounted onto a ground glass plate with the epicardial surface facing down and then sliced using surgical skin graft blades (Swann-Morton, UK) to a thickness of between 300–500 μm. The right atrial appendage and the slide were always kept moist throughout the procedure. The muscles (weight 30–50 mg) were then transferred to an Erlenmeyer (Trallero and Schlee, Barcelona, Spain) containing 10 ml of oxygenated buffer KHH (pH 7.4) and placed into a shaking water bath maintained at 37 °C. For the induction of simulated ischemia, the buffer KHH was bubbled with 95% N_2–5% CO_2 (pH 6.8–7.0) and D-glucose (Sigma, St. Louis, MO) was removed and substituted with 2-deoxy-D-glucose (Sigma, St. Louis, MO).

After sectioning the right atrial appendage, the muscles were equilibrated for 30–40 min. In all studies simulated ischemia was induced for a period of 90 min followed by 120 min of reoxygenation and then randomly allocated to the various protocols of IPostC (see below). Some muscles were aerobically incubated for an identical time period and others were subjected to ischemic preconditioning (IPreC), induced by a 5 min ischemia followed by a 5 min reoxygenation prior to the 90 min ischemia, a protocol shown to elicit optimal protection in this preparation [18].

2.4. Measurement of tissue injury and viability

Tissue injury was determined by measuring the leakage of lactate dehydrogenase (LDH) into the incubation medium during the 120 min of reoxygenation. An advanced kinetic, based on the formation of NAD + L-lactate which is directly proportional to the amount of LDH activity used. The absorbance was measured at a 340-nm wavelength with a MultiSkan FC spectrometer and the results, obtained after subtraction of the aerobic control values, were expressed as AU/g wet wt.

Tissue viability was assessed by the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO) to a blue formazan product in the muscles at the end of the 120 min reoxygenation period. The absorbance of the formazan formed was measured at a 550-nm wavelength with the MultiSkan FC spectrometer and the results, obtained after subtraction of the aerobic control values, were expressed as AU/g wet wt.

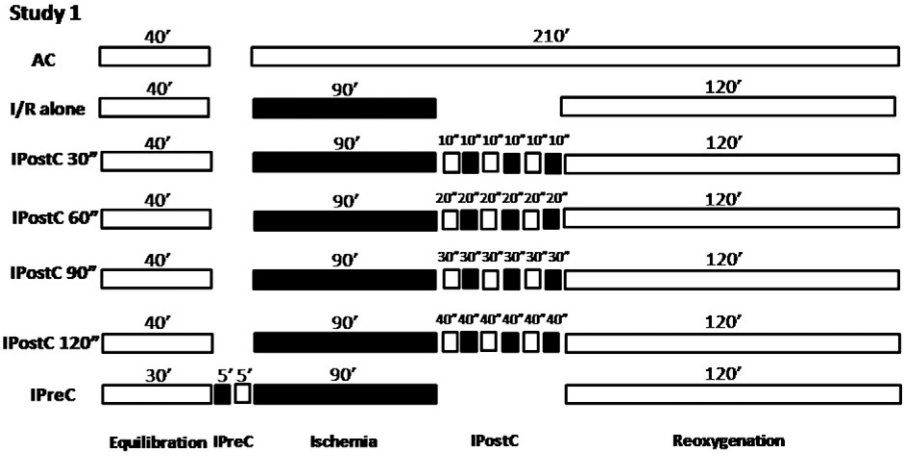

Fig. 1. Experimental protocol for Study 1. All groups were equilibrated for 30–40 min at 37 °C in aerobic conditions. Muscles (n = 50) were subjected to 90 min of ischemia followed by 120 min of ischemia/reoxygenation (I/R). Some muscles did not undergo further treatment (I/R alone),others were postconditioned (IPostC) by 30, 60, 90 and 120 s of total ischemia divided in 3 cycles of 10, 20, 30 and 40 s or preconditioned (IPreC) with 5 min of ischemia and 5 min of reoxygenation while others were maintained under aerobic conditions (AC) for the entire experimental period.