Cardioprotective effect of preconditioning is more efficient than postconditioning in rats submitted to cardiac ischemia and reperfusion[1]

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Abstract

Purpose: To investigate the cardioprotective effects of ischemic preconditioning (preIC) and postconditioning (postIC) in animal model of cardiac ischemia/reperfusion.

Methods: Adult rats were submitted to protocol of cardiac ischemia/reperfusion (I/R) and randomized into three experimental groups: cardiac I/R (n=33), preCI + cardiac I/R (n=7) and postCI + cardiac I/R (n=8). After this I/R protocol, the incidence of ventricular arrhythmia (VA), atrioventricular block (AVB) and lethality (LET) was evaluated using the electrocardiogram (ECG) analysis.

Results: After reestablishment of coronary blood flow, we observed variations of the ECG trace with increased incidence of ventricular arrhythmia (VA) (85%), atrioventricular block (AVB) (79%), and increase of lethality (70%) in cardiac I/R group. The comparison between I/R + preIC group with I/R group demonstrated significant reduction in VA incidence to 28%, AVB to 0% and lethality to 14%. The comparison of I/R + postIC group with I/R group was observed significance reduction in AVB incidence to 25% and lethality to 25%.

Conclusion: The preconditioning strategies produce cardioprotection more efficient that postconditioning against myocardial dysfunctions and lethality by cardiac ischemia and reperfusion.

Key words: Myocardial Infarction. Myocardial Reperfusion Injury. Ischemic Preconditioning, Myocardial. Ischemic Postconditioning. Rats.

■ Introduction

The coronary reperfusion therapy constitutes the major therapeutic strategy to salvage the myocardium from tissue injury following prolonged ischemia in acute myocardial infarction (AMI) patients[1-3]. However, the beneficial effect of this therapy is compromise by myocardial injuries caused during coronary reperfusion that subsequently lead to cardiac dysfunctions, including arrhythmias, caused by bioenergetic and electrochemical misbalance caused mainly by reduction of ATP production by mitochondria and cytosolic Ca^{2+} overload in cardiomyocytes[4,5].

Strategies to limit myocardial damage in acute myocardial infarction include, in addition to early reperfusion, the improvement of the endogenous resistance of cardiac cells to blood deprivation, which can be obtained by stimulating its survival mechanisms that function as a molecular adaptation to ischemic stress[2]. Among these phenomena, we can mention the stunned and the hibernation of the myocardium. Stunned is defined as a prolonged and reversible ischemic heart dysfunction, which persists after reperfusion, despite normalization of blood flow[6].

The hibernating myocardium represents a condition in which a prolonged sub-acute or chronic state of myocardial ischemia results in a new equilibrium in which myocardial necrosis does not settle because the metabolism and function are reduce proportionally to the concomitant reduction of coronary flow[7]. Other phenomena described are myocardial pre and postconditioning[8]. Ischemic preconditioning (preCI) describes the protection afforded by brief period (or periods) of ischemia against a later ischemic episode of longer duration. This protection is typically measure as prevention against arrhythmias, reduction in myocardial infarction size or better recovery of the electrical and contractile function of the heart[9].

The main advantages of preCI are in fact this can be inducing by ischemia or directly in heart, can be programme in case of surgery or angioplasty, situations where heart function is compromise. The preCI has cardioprotective efficacy of two periods with different characteristics: the first window of protection (preCI early) is more effective in reducing the extent of infarct size, and the second window of protection (preCI late) is most effective against myocardial stunning[10]. Ischemic postconditioning consists of alternating brief periods of reperfusion and brief reocclusions applied in the initial minutes of reperfusion[11]. In ischemic postconditioning (postCI), is necessary to make brief mechanical interruptions of reperfusion early in process. The mechanisms by which postCI confers cardioprotection in cardiac I/R resemble those of preCI, and adenosine has been implicate in the cardioprotective effect conferred by postCI, since inhibitors of the action of adenosine inhibit this effect[12]. PostCI that is more clinically applicable than preconditioning, once your application occurs before an ischemic episode, but at the time of reperfusion. In this study aims to characterize the ECG changes caused by I/R cardiac process and quantify the incidence of lethality in animals subjected to I/R and evaluate the effectiveness of preCI and postCI.

■ Methods

All experimental procedures were approved by Ethical Committee, UNIFESP (#1130/11).

Forty eight adult (14 - 16 week-old, 300 to 340 g) male Wistar rats were maintained under standard conditions of nutrition, hydration, temperature, humidity and light/ dark cycle. Rats were submitted to cardiac I/R and randomized into three groups: cardiac I/R (n=33), preCI + cardiac I/R (n=7) and postCI + cardiac I/R (n=8).

Protocol of cardiac ischemia and reperfusion (I/R)

All experimental procedures used to induce cardiac I/R in this study were previously described in other studies published by our lab[13,14]. After anesthesia with urethane (1.25 g/kg), rats were submitted to orotracheal intubation and adapted in mechanical ventilator apparatus (Insight, model EFF 312, Brazil) with room air (volume of 10 mL/kg and 70 breaths/min). The body temperature was maintained at 37.5ºC with a heated operating platform and appropriate heating lamps, and was evaluated routinely via a rectal thermometer. After this procedure, the chest was opened with a left thoracotomy between fourth and fifth intercostal space to exteriorization of heart using mechanical pressure on the abdomen. To induce ischemia, surgical tourniquet (Mononylon Ethilon 5.0, Atramac, Mexico) attached to a 10-mm micro point reverse-cutting needle was placed around the left anterior descending coronary artery (about 2 mm from its origin). The two ends of nylon silk yarn were passed inside a cylindrical tube of polypropylene to perform a surgical tourniquet. After stabilization for 15 min, this tourniquet was tied for mechanical occlusion of coronary (ischemia). After 10 min of occlusion, the nylon tourniquet was removed to allow coronary recirculation (reperfusion) during 120 min.

ECG analysis

To evaluate cardiac electrical activity, the ECG system was adapted in rats submitted to cardiac I/R. Cardiac electrical activity were measured during 10 min ischemia and 120 min reperfusion[13,14]. The ECG was recorded from the beginning of the stabilization period using a data acquisition system (AqDados 7.02; Lynx Tecnologia Ltda, Brazil). The recorded raw data was analysed using the computer program (AqDAnalysis 7, Lynx Tecnologia Ltda, Brazil). Incidence of VA, AVB, and LET in response to cardiac I/R were evaluated by ECG analysis. To simplify the presentation of the results, ventricular fibrillation, *torsades de pointes*, and ventricular tachycardia parameters were considered only as VA. ECG measurements were evaluated according to Lambeth conventions[13,14].

Protocol of preconditioning and posconditioning

The cardiac I/R group (n = 33) was proposed to analyze and establish the amendments ECG and mortality of animals submitted to I/R. In the preCI + I/R was studied the effect of 3 short cycles of ischemia lasting 3 min and 5 min intervals between I/R cycles, applied before performing longer duration of ischemia (10 min). In the cardiac I/R group treated with postCI + I/R, ischemia held 3 cycles of 1 min interspersed with 3 cycles of 3 min of reperfusion.

Statistical analysis

The incidence of VA, AVB, and LET was compared by the Fisher's exact test. Values were considered significant to $p<0.05$. Statistical analyses were performed by Prism 5.0 software (GraphPad, USA), and data were expressed as means ± SE.

■ Results

Cardiac I/R group showed changes in the ECG tracing from the 5 minutes of first ischemic arrhythmias corresponded to VA (ventricular extra systoles, ventricular tachycardia and *torsade de pointes*) and AVB in most animals tested occurred, and the rest of animals tested showed no arrhythmias during ischemia. Very few animals died because of arrhythmias during cardiac ischemia (data not showed).

After reestablishment of coronary blood flow by removing wire Nylon variations

of ECG trace, with increased incidence of VA (85%), AVB (79%) and mortality (70%) were observed (Figure 1). In animals that had no arrhythmias during ischemia, the arrhythmic events began to occur during reperfusion; no deaths were observed during first four minutes of reperfusion, but between 5th and 20th minutes of reperfusion.

The Figure 1 also shows that the preCI significantly reduced the incidence of cardiac arrhythmias (VA and AVB) and mortality. The comparison of group I/R + preCI with I/R group showed significant reduction in VA incidence to 28%, AVB to 0% and mortality to 14%.

Figure 2 shows that the postCI significantly reduced incidence of AVB and mortality but not VA. The comparison of group I/R + postCI with I/R group showed significant reduction in incidence of AVB and mortality for 25%.

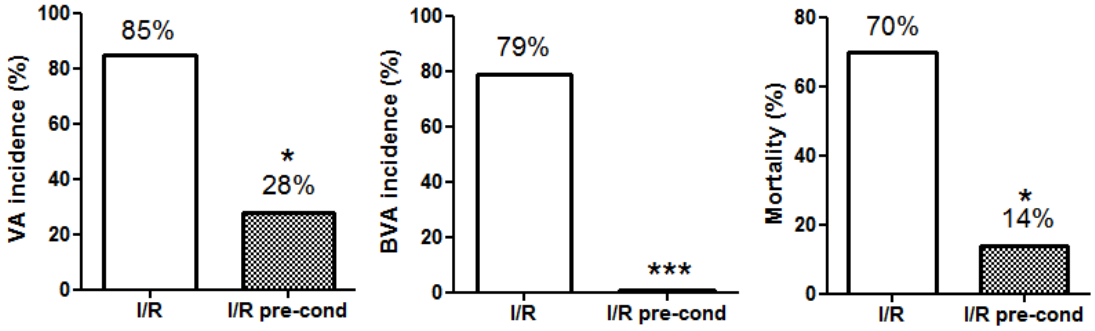

Figure 1 - Histograms representing percentages of VA, BVA and mortality incidence in groups I/R and I/R + preCI. Statistical analysis were performed from Fisher's exact test (*p<0.05, ***p<0.0005).

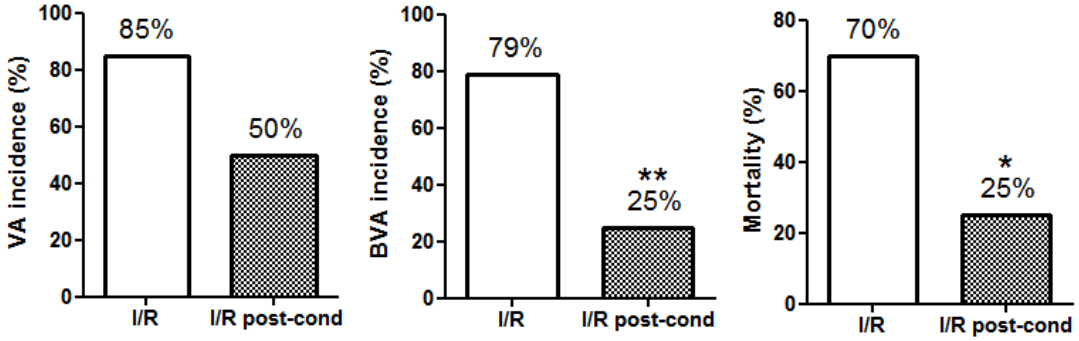

Figure 2 - Histograms representing percentages of incidence of VA and BVA and mortality in I/R and I/R + postCI groups. Statistical analysis were performed from Fisher's exact test (*p<0.05, **p<0.005).

■ Discussion

The results obtained in this study showed that the incidence of VA and AVB and mortality in animals submitted to cardiac I/R was reduce when animals were subjected to preCI, and postCI. The preCI significantly reducing the incidence of AVB and mortality in animals submitted to cardiac I/R when compared with postCI.

Ischemic preconditioning (preCI) describes the protection afforded by brief periods (or periods) of ischemia against a subsequent longer lasting ischemic episode. This protection is typically measured as prevention against arrhythmias, reduction in the size of myocardial infarction or better recovery of the electrical and contractile function of the heart.

The preCI stimulates increased

production of NO by myocardial cells, which in response activates intracellular signaling cascade that inhibits opening of mitochondrial permeability transition pore (MPTP), resulting in production of ATP and homeostasis of Ca^{2+} and Na^{+}[15]. The K^{+}ATP-dependent mitochondrial (mKATP) channels present in the inner mitochondrial membrane are activate during preCI, increasing volume of mitochondrial matrix and oxidation of fatty acids[16] with production of oxigen-reactive species (ROS)[17].

The opening these channels involves the epsilon isoform of protein kinase C (PKCε)[18], preventing hyperpolarization of electrical potential of mitochondrial membrane[19] avoiding accumulation of mitochondrial Ca^{2+}, prolonged opening of MPTP, release of cytochrome c and apoptosis[20,21].

The cardioprotection afforded by preCI is mediate by combination of multiple endogenous mechanisms involving adrenergic receptors (α1 e β_3), opioid receptor, bradykinin B2 receptor and adenosine receptor[22]. Found in our study that ischemic preCI reduce the incidence of cardiac arrhythmias and mortality by I/R. The preCI confers cardioprotection reducing the incidence of arrhythmias induced by I/R[23] in cardiomyocytes cultured and isolated hearts in different animal species[24]. Prior exposure for 1 to 2 cycles of ischemia (5 min) and reperfusion (10 min) reduced the incidence of ventricular fibrillation from 42% to 17% with 2 cycles and completely abolished this type of arrhythmia with 1 cycle in isolated hearts rabbits subjected to ischemia for 30 min followed by reperfusion for 45 min[25].

Prior exposure of isolated hearts in male Wistar rats to one cycle of ischemia (5 min) and reperfusion (5 min) reduced the number of premature ventricular beats, the incidence and duration of ventricular tachycardia in isolated hearts subjected to 30 min ischemia[26]. Kolettis *et al.*[24] showed that prior exposure to 2 cycles of ischemia (5 min), reperfusion (5 min) in Wistar rats undergoing cardiac ischemia for 30 min and reperfusion for 24 hours significantly reduced the incidence of VA, mortality and myocardial lesion size[24].

Experimental studies in different models of I/R have shown that inhibitor of NO biosynthesis L-NAME, reduce cardioprotective response stimulated by preCI, suggesting involvement of cellular signaling via NO/cGMP/PKG response[27]. The signaling via NO/cGMP/PKG pathway has a crucial role in excitation-contraction coupling and cellular Ca^{2+} homeostasis during I/R. This increase in NO/cGMP/PKG system activity is mediate by increased NOS activity induced by preCI[28].

The increase in PKG activity stimulates opening of mKATP, decreasing mitochondrial Ca^{2+} overload, ROS production and prolonged opening of MPTP to maintain ATP production[29-32]. As well as its application in the myocardium, ischemic preconditioning can also be induced by brief interruptions of blood flow to other organs, particularly skeletal muscle. Transient ischemia induced noninvasively by inflating a cuff on a limb, followed by reperfusion, helps reduce the damage caused to the myocardium by interruption of the coronary circulation. Remote ischemic preconditioning involves activation of humoral and/or neural pathways that open mitochondrial ATP-sensitive potassium channels in the myocardium and close mitochondrial permeability transition pores, making cardiomyocytes less vulnerable to ischemia-induced cell death[33].

This cardioprotective mechanism is now being translated into clinical practice, with positive results in several clinical trials in coronary artery bypass surgery, surgical repair of abdominal aortic aneurysms, valve replacement surgery and percutaneous coronary intervention[34-40].

In our study postCI reduce the incidence of cardiac arrhythmias and mortality caused by I/R. This procedure has been proposed as an

important non-pharmacological experimental strategy to reduce area of injury produced by I/R[41].

The postCI reduces incidence of arrhythmia and mortality in animal models of cardiac I/R[42]. The postCI reversed sustained ventricular tachyarrhythmia and ventricular fibrillation preventing mortality of animals subjected to I/R[43], and the application of 4 cycles of I/R (20s each) immediately after regional ischemia for 5 min decreased the incidence of ventricular arrhythmias compared to control animals[44].

Improvement of electrocardiographic parameters and myocardial perfusion through ischemic postCI (2 cycles of 90s separated by 3 and 5 min) in 17 patients who had suffered MI who underwent percutaneous coronary intervention PCI was observed for reperfusion therapy standard[45].

In a study in which 30 patients admitted for coronary angioplasty for AMI underwent post-CI (4 cycles of I/R 1min each), there was reduction in release of creatine kinase, improves microvascular circulation and reduction of infarct size[10].

In postCI interruption of reperfusion after 1 hour of coronary artery occlusion in canine model (3 cycles of 30 s reperfusion followed by 30 s ischemia) followed by reperfusion for 3h reduced the size of cardiac infracted tissue[46,47]. Reducing size of infarcted area afforded by postCI has been observe in different species, including pigs, dogs, rabbits, mice and rats[41].

The postCI performed within approximately less than 1h of serious ischemia followed by reperfusions and occlusions of the coronary artery significantly reduced the QT dispersion and ventricular fibrillation associated with acute cardiac ischemia[48].

Short periods of ischemia applied in early minutes of reperfusion reduced infarct size, endothelial dysfunction, accumulation of neutrophils, ROS generation and activated different signaling pathways for cell survival, including ERK1/2 (extracellular signal regulated protein kinase), PI3K-Akt (phosphatidylinositol 3-kinase)[41] neuronal nitric oxide synthase (nNOS)[49] or preventing prolonged opening MPTP[50].

■ Conclusion

The cardiac preconditioning is more efficient than postconditioning to reduce the incidence of cardiac arrhythmias and mortality resulting from cardiac ischemia and reperfusion, conferring cardioprotection.

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