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Investigations on the role of leukotrienes in remote hind limb preconditioning-induced cardioprotection in rats

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ABSTRACT

The cardioprotective effects of remote hind limb preconditioning (RIPC) are well established, but its mechanisms still remain to be explored. Therefore, the present study was aimed to explore the possible involvement of 5-lipoxygenase-derived leukotrienes in RIPC. The hind limb was tied by a pressure cuff and was subjected to four episodes of inflation and deflation (5 min each) to induce remote hind-limb preconditioning. Thereafter, the hearts were isolated and were subjected to global ischemia (30 min) followed by reperfusion (120 min) on the Langendorff apparatus. The extent of myocardial injury was assessed by measuring lactate dehydrogenase (LDH) and creatine kinase (CK) levels in the coronary effluent; the infarct size using TTC staining, and the hemodynamic parameters including LVDP, dp/dt_{max} and dp/dt_{min}. RIPC significantly decreased ischemia and reperfusion-induced increase in LDH, CK release, infarct size and improved LVDP, dp/dt_{max} and dp/dt_{min}. Administration of montelukast, leukotriene receptor antagonist (10 and 20 mg/kg) and zileuton, 5-lipoxygenase inhibitor, (2.5 and 5 mg/kg) abolished RIPC-induced cardioprotection. It may be concluded that hind limb ischemia stimulates 5-lipoxygenase to release leukotrienes which may elicit cardioprotection by humoral or neurogenic pathway.

1. Introduction

Remote preconditioning is a novel phenomenon in which short ischemia-reperfusion episodes are delivered to tissues (other than heart) to induce cardioprotection from sustained ischemia-reperfusion injury [43,46]. Preclinical studies from our as well as from other laboratories have documented that short episodes of ischemia-reperfusion to remote tissues by occluding the arteries such as cerebral, mesenteric, intestinal, femoral, renal arteries and abdominal aorta produce cardioprotection [12,21,22,40,53,56,60,63]. Furthermore, the preclinical cardioprotective effects of remote preconditioning have also been translated in human beings [29]. RIPC has been reported to alleviate ischemia-reperfusion injury in patients undergoing coronary angioplasty, coronary artery bypass surgery, percutaneous coronary intervention, elective abdominal aortic aneurysm repair and coronary artery bypass graft surgery [5,7,45,46].

Preconditioning ischemia to different organs including kidneys, liver, intestine or limbs produces cardioprotection with a comparable efficacy [60,63], suggesting that the cardioprotective factors released during remote preconditioning are not unique to the organs being subjected to preconditioning ischemia. Rather, it suggests that a common tissue present in kidneys, skeletal muscle and intestine responds to preconditioning ischemia and releases some factors, which subsequently confer cardioprotection during index ischemia. Endothelium may be proposed as a common tissue present in different organs which responds quickly to changes in the blood flow and hypoxia by releasing different humoral factors including leukotrienes [20,33].

Leukotrienes including LTC_4, LTD_4 and LTE_4 are synthesized by 5-lipoxygenase [42] and their receptors are widely distributed on different parts of the body including heart, bronchial airways, spinal nerves and submucosal plexus of intestine [3,37,38]. Studies have shown the release of cysteinyl leukotrienes in the coronary effluent after being subjected to global ischemia using Langendorff preparation [15]. Leukotrienes primarily produce deleterious effects on heart and promote myocardial injury [27]. Interestingly, the agents/mediators such as ischemia, CGRP [41] bradykinin [28], free radicals [52], angiotensin [65] and calcium [35] induce myocardial injury; however, these also tend to precondition the myocardium against sustained ischemic injury.

Montelukast is a cysteinyl leukotriene receptor antagonist and blocks the actions of LTC_4, LTD_4 and LTE_4 [32]. It has been used clinically for the treatment of bronchial asthma. Montelukast has been found to profoundly reduce intestinal, ovarian, renal, hepatic, spinal and cerebral ischemia-reperfusion-induced injury [48,64]. Zileuton is a specific 5-lipoxygenase inhibitor that inhibits the synthesis of cysteinyl leukotrienes i.e. LTC_4, LTD_4 and LTE_4 [16] and has also been used for the maintenance therapy of asthma. Zileuton has been found to reduce cerebral infarct size and the release of inflammatory cytokines in murine model of ischemia-reperfusion injury [51]. Furthermore, it has also been found to reduce myocardial ischemia-reperfusion-induced injury

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in rats [26]. In addition, both montelukast and zileuton have been found to reduce ischemia-reperfusion-induced arrhythmias in murine model of ischemia-reperfusion injury [16]. Therefore, in the current study montelukast and zileuton were employed to investigate the involvement of leukotrienes in RIPC-induced cardioprotection.

2. Material and methods

2.1. Animals, drugs and chemicals

Wistar albino rats (150–220 g) were fed on the standard laboratory chow (Ashirwad industries, Kharar, Mohali, India). The experimental protocol was approved by the Institutional Animal Ethics Committee and care of the animals was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg. No. - 107/99/CPCSEA/2014-07). Montelukast sodium (Jubilant, Noida) and Zileuton (Biophore, India) were employed as pharmacological agents in this study. Montelukast sodium (10 and 20 mg/kg) [9] and Zileuton (2.5 and 5 mg/kg) [16] were administered intraperitoneally. Creatine phosphokinase (CK) estimation kit was procured from Agappe Diagnostics Ltd. Kerala, India. All other chemicals were of analytical grade and were obtained from S.D. Fine chemicals, Mumbai, India. All the drugs were dissolved in distilled water.

2.2. Remote hind limb preconditioning

In anaesthetized rats (thiopental sodium 50 mg/kg, *i.p.*), the pressure cuff was tied on the hind limb to induce hind limb ischemia. The cuff was inflated with air up to 200 mm of Hg to produce ischemia in the limb, and thereafter, the pressure was released to reperfuse the ischemic limb. Four such episodes of ischemia and reperfusion (each comprising of 5 min) were used to produce remote limb preconditioning [4,22].

2.3. Isolated rat heart preparation and measurement of haemodynamic parameters

Heparin (500 IU, *i.p.*) was injected in rats 20 min before sacrificing the animals and heart was rapidly excised and mounted immediately on the Langendorff apparatus [68]. The hearts were perfused in a retrograde manner with Kreb's Henseleit (KH) solution (pH 7.4; temperature 37 °C; bubbled with 95% O_2 and 5% CO_2; flow rate 7–8 ml/min). A fluid filled latex balloon was inserted into left ventricle and was connected to a pressure transducer (AD instruments, Australia) to record the left ventricular developed pressure (LVDP) and its first derivatives dp/dt_{max} and dp/dt_{min}. The inflow of KH solution was blocked to induce global ischemia (30 min) followed by reinstitution of flow in the form of reperfusion (120 min). The heart rate was also evaluated at various time intervals and coronary effluent was collected at different time intervals i.e., basal (immediately after stabilization), 0 min, 5 min and 30 min after reperfusion for biochemical estimations.

2.4. Assessment of infarct size

The hearts were removed from the Langendorff apparatus and was kept overnight at 0 °C. The frozen hearts was cut into uniform sections of 2–3 mm thickness. Thereafter, these slices were stained by incubating in 1% triphenyltetrazolium chloride (TTC) solution (0.2 M Tris buffer, pH 7.4) at 37 °C for 20 min [23]. The myocardial infarct size was measured by volume and weight method and data of infarct size was expressed as a percentage of total ventricular volume/weight [12,69]. Previous studies have demonstrated that TTC staining and histological evaluation of the infarct size yield quantitatively similar results and also have close correlation [14].

2.5. Estimation of lactate dehydrogenase (LDH) levels

The LDH levels in the coronary effluent were estimated using 2,4-DNPH method [70]. The levels were estimated in coronary effluents collected after stabilization, immediately and 30 min after reperfusion.

2.6. Estimation of creatine kinase (CK) levels

The levels of CK in the coronary effluent were estimated using a commercial diagnostic kit. The levels were estimated in coronary effluents collected after stabilization and 5 min after reperfusion.

2.7. Experimental protocol

Eight groups, each comprising six Wistar albino rats, were employed in the present study.

2.7.1. Group I (Control)

The rat hearts were perfused on the Langendorff apparatus and subjected to global ischemia (30 min) followed by reperfusion (120 min).

2.7.2. Group II (remote hind limb preconditioning)

A pressure cuff was tied on the hind limb of anaesthetized rat and was inflated to produce ischemia in the limb and deflated for reperfusion. Four episodes of ischemia and reperfusion, each comprising of 5 min of inflation and 5 min of deflation, were used to induce remote limb preconditioning. Immediately after the last episode of remote preconditioning, heart was excised and subjected to ischemia-reperfusion as described in group I.

2.7.3. Groups III and IV (montelukast 10 and 20 mg/kg i.p in remote limb preconditioning)

Montelukast (10 or 20 mg/kg *i.p*) was administered 30 min prior to performing RIPC. Thereafter, heart was excised after the last episode of RIPC and subjected to ischemia-reperfusion as described in group I.

2.7.4. Groups V and VI (zileuton 2.5 and 5 mg/kg i.p in remote limb preconditioning)

Zileuton (2.5 or 5 mg/kg *i.p*) was administered 30 min prior to performing RIPC. Thereafter, heart was excised after the last episode of RIPC and subjected to ischemia-reperfusion as described in group I.

2.7.5. Group VII (montelukast 20 mg/kg i.p per se)

Montelukast (20 mg/kg *i.p*) was administered in rats and after 30 min, the heart was excised and subjected to ischemia-reperfusion as described in group I.

2.7.6. Group VIII (zileuton 5 mg/kg i.p per se)

Zileuton (5 mg/kg *i.p*) was administered in rats and after 30 min, the heart was excised and subjected to ischemia-reperfusion as described in group I.

2.8. Statistical analysis

The data of the study were expressed as mean ± standard error of mean (S.E.M). Two way ANOVA followed by Bonferroni's *post hoc* test was employed to analyze the statistically significant differences between different experimental groups for LDH, CK, % change in LVDP, dp/dt_{max}, dp/dt_{min}. On the other hand, one-way ANOVA followed by Tukey's multiple range *post hoc* test was employed for infarct size. A value of $p < 0.05$ was considered to be statistically significant.