Journal of Indian College of Cardiology xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Indian College of Cardiology

journal homepage: www.elsevier.com/locate/jicc

Role of erythropoietin in ischemic postconditioning induced cardioprotection in hyperlipidemic rat heart

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ARTICLE INFO

Article history:
Received 27 August 2016
Accepted 8 March 2017
Available online xxx

Keywords:
Ischemic postconditioning
Hyperlipidemia
Myocardial infarction
Erythropoietin
PI-3K

ABSTRACT

Objective: Ischemic postconditioning (IPOC) is an endogenous cardioprotective phenomenon, which gets attenuated during the hyperlipidemia. It has been documented that erythropoietin (EPO), a glycoprotein, produces IPOC-like cardioprotection through common signaling pathway such as PI-3K pathway. The aim of this study has been designed to investigate the role of EPO in IPOC induced cardioprotection in hyperlipidemic rat heart.
Materials and methods: Heart was isolated from hyperlipidemic rat and mounted on Langendorff's apparatus, subjected to 30 min ischemia and 120 min reperfusion. IPOC was mediated by four cycles of 5 min reperfusion and 5 min ischemia. The infarct size was estimated using TTC stain and coronary effluent was analyzed for LDH and CK-MB release to assess the degree of myocardial injury.
Results: Four cycles of IPOC produces cardioprotection noted in terms of decrease in infarct size and decrease in the release of LDH and CK-MB in normal rat heart. However, IPOC-induced cardioprotection was completely attenuated in isolated heart obtained from hyperlipidemic rat. Perfusion of EPO (1 U/ml) significantly restored the attenuated cardioprotection in hyperlipidemic rat heart, which was completely blocked by perfusion of LY294002 (10 μM), a selective inhibitor of PI-3K.
Conclusion: Thus, it is suggested that EPO restores the attenuated cardioprotective effect of IPOC in hyperlipidemic rat heart by the activation of PI-3K signaling pathway.

1. Introduction

Ischemic heart disease is the leading cause of mortality and morbidity worldwide.[1] Reperfusion of an ischemic heart is necessary to regain the normal functioning of heart.[2] However, abrupt reperfusion of an ischemic heart elicits a cascade of adverse events which lead to injury of myocardium i.e. ischemia reperfusion injury (I/R injury).[3,4] A phenomenon termed ischemic postconditioning (IPOC), a cardioprotective maneuver that targets the reperfusion phase, which refers to the several brief, transient cycles of alternating reperfusion-ischemia immediately after the sustained ischemic episode, which resulted in a reduction in myocardial injury.[5,6] The IPOC induces cardioprotection by activation of PI-3K–Akt pathway, activation of adenosine,[7] bradykinin,[8] erythropoietin,[9] generation of nitric oxide (NO) and by opening of mitochondrial ATP-dependent potassium channel (mito K_{ATP}).[10] However, the cardioprotective effect of IPOC is attenuated in conditions such as hyperhomocystein,[11] diabetes mellitus[12] and hyperlipidemia.[13]

Erythropoietin (EPO) is a glycoprotein, originally designated for its role in promoting erythrocytes proliferation, differentiation and survival.[14] Recently it has been documented by the different studies that erythropoietin play a protective role in spinal cord,[15] skeletal muscle,[16] brain,[17] retina[18] and most recently myocardium.[19,20] EPO exerts cardioprotective effect by inducing several intracellular mechanisms,[21] one of which is PI-3K pathway, which suppress the apoptotic cell death via its downstream effector,

Abbreviations: Akt, protein kinase B; ATP, adenosine triphosphate; CK-MB, creatine kinase; EPO, erythropoietin; I/R, ischemia/reperfusion; IPC, ischemic preconditioning; IPOC, ischemic postconditioning; LDH, lactate dehydrogenase; LY294002, PI-3K inhibitor; mito K_{ATP}, mitochondrial ATP-sensitive K^+ channel; NO, nitric oxide; PI-3K, phosphoinositide-3 kinase; PKC, protein kinase C; STAT, signal transducers and activators of transcription.

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http://dx.doi.org/10.1016/j.jicc.2017.03.003
1561-8811/

protein kinase B (PKB)/Akt.[22] Activated Akt kinase interms blocks apoptosis by inhibiting BCL-2 family, protein Bad, along with caspase 9.[22] It has been documented that pretreatment of EPO reduce, I/R induce damage in cardiomyocyte, vascular smooth muscle and endothelial cells by PI-3K/Akt signaling pathway.[21]

Hyperlipidemia is one of the major risk factor for ischemic heart disease. Cardioprotective effect of ischemic postconditioning is attenuated in hyperlipidemia.[13] It has been documented that EPO produced ischemic preconditioning (IPC) mediated cardioprotection by PI-3K pathway.[21] Therefore, the present study has been designed to investigate the role of EPO in modulation of cardioprotective effect of ischemic postconditioning in hyperlipidemic rat heart.

2. Materials and methods

Male wistar rats ($n = 6$, each group) weighing about 180–250 g kept in animal house and provided 12 h light and 12 h dark cycle were employed in this study. They were fed on standard chow diet (wheat flour 22.5%, roasted Bengal gram powder 60%, skimmed milk powder 5%, casein 4%, refined oil 4%, salt mixture with starch 4% and vitamin and choline mixture 0.5%) and provided water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (GLAIPR/CPCSEA/IAEC/2013/P.Col) in accordance with the national guidelines on the use of laboratory animals.

2.1. Drugs and chemicals

Erythropoietin (1 U/ml) (Gennova Biopharmaceuticals Ltd., Pune) was dissolved in Krebs-Henseleit (K-H) buffer and then perfused to the isolated heart in the four cycle of reperfusion. LY294002 (10 μM) (Sigma Aldrich, Ltd., Bangalore, India) was dissolved in dimethyl sulfoxide (DMSO) (Merck Pvt. Ltd., Mumbai, India) and finally 0.02% concentration of DMSO in Krebs-Henseleit buffer solution was taken. All the other reagents used in this study were of analytical grade and always freshly prepared before use.

2.2. Induction of experimental hyperlipidemia

Male wistar rats (180–250 g) were maintained on high cholesterol diet (corn starch 44.74 g, casein 14 g, sucrose 10 g, butter 20 g, mineral mix 3.5 g, vitamin mix 1 g, choline 0.25 g, terbutylhydroquinone 0.0008 g, cholesterol 1 g, cholic acid 0.5 g) for 6 weeks for the induction of hyperlipidemia.[23] Estimation of the total cholesterol (TC) and triglycerides (TG) level was used as a marker of hyperlipidemia using commercial available kits (Span Diagnostics Ltd., Surat, India).

2.3. Isolated rat heart preparation

Rats were administered heparin (500 IU/L, i.p.) (Gland Pharma Ltd., Hyderabad, India) 20 min prior to sacrificing the animal by cervical dislocation. Heart was rapidly excised and immediately mounted on Langendorff's apparatus. Isolated heart was retrogradely perfused at constant pressure of 80 mmHg with Krebs-Henseleit (K-H) buffer (NaCl 118 mM; KCl 4.7 mM; $CaCl_2$ 2.5 mM; $MgSO_4{\cdot}7H_2O$ 1.2 mM; KH_2PO_4 1.2 mM; $C_6H_{12}O_6$ 11 mM), pH 7.4, maintained at 37 °C bubbled with 95% O_2 and 5% CO_2. Flow rate was maintained at 7–9 ml/min using Hoffman's screw. Temperature surrounding of heart was maintained by enclosing the heart in a double-wall jacket, by circulating water heated at 37 °C. Global ischemia was produced for 30 min by blocking the inflow of K-H buffer solution which was followed by 160 min of reperfusion. Ischemic postconditioning was produced by 5 min of reperfusion followed by 5 min of ischemia by closing the inflow of K-H buffer solution. Four such episodes were employed. Coronary effluent was collected before 30 min of global ischemia and further effluent was collected immediately and 5 min after completion of four cycles of ischemic postconditioing.[11]

2.4. Assessment of myocardial injury

Myocardial injury was assessed by estimation of LDH and CK-MB in coronary effluents,[24,25] using commercial available kits (Span Diagnostics Ltd., Surat, India). Values were expressed in international units per liter (IU/L).

2.5. Assessment of myocardial infarct size

Heart was removed from Langendorff's apparatus. Both auricles and root of aorta were excised and ventricles were kept overnight at −4 °C temperature. Slices of uniform sections 1–2 mm thickness were prepared from frozen ventricles and then slices were incubated in 1% (w/v) triphenyltetrazolium chloride (TTC) at 37 °C in 0.2 M Tris buffer for (pH 7.4) 30 min.[23,26] Infarcted portion remained unstained while normal myocardium has brick red color due to conversion of TTC to red formazone pigment by NADH and dehydrogenase enzyme. Infarct size was measured by the volume method.[23,27]

2.6. Experimental protocol

The present study was carried out on seven groups and each group comprises of six rats. The diagrammatic representation of experimental protocol is shown in Fig. 1.

Group 1: (Sham control; n = 6): Isolated rat heart preparation was stabilized for 10 min and then perfused with K-H buffer solution for 190 min without subjecting the global ischemia and reperfusion.

Group 2: (Ischemia reperfusion control; n = 6): Isolated rat heart preparation was allowed to stabilize for 10 min and then subjected to 30 min global ischemia followed by 160 min of reperfusion.

Group 3: (Ischemic postconditioning control; n = 6): Isolated rat heart preparation was allowed to stabilize for 10 min and subjected to 30 min of global ischemia on Langendorff's apparatus. Then the preparation was subjected to four cycles of ischemic postconditioning, each cycle comprised of 5 min of reperfusion followed by 5 min of ischemia which is further followed by 120 min of reperfusion with K-H buffer solution.

Group 4: (Ischemic postconditioning in hyperlipidemic rat heart; n = 6): Isolated rat heart preparation from hyperlipidemic rat was allowed to stabilize for 10 min and subjected to 30 min of global ischemia followed by four cycles of ischemic postconditioning as described earlier in group III.

Group 5: (Ischemic postconditioning in DMSO (vehicle) treated hyperlipidemic rat heart; n = 6): Isolated rat heart preparation was perfused with DMSO (0.2 ml/L) during stabilization of 10 min and subjected to 30 min of global ischemia. Then the preparation was perfused with DMSO (0.2 ml/L) in each cycle of 5 min of reperfusion of postconditioning which is further followed by 120 min of reperfusion with K-H buffer solution.

Group 6: (Ischemic postconditioning in erythropoietin (EPO) (1 U/ml) perfused hyperlipidemic rat heart; n = 6): Isolated rat heart preparation was allowed to stabilize for 10 min and subjected to 30 min of global ischemia. Then the preparation was perfused with EPO (1 U/ml) in each cycle of 5 min of reperfusion of postconditioning which is further followed by 120 min of reperfusion with K-H buffer solution.

Please cite this article in press as: Varshney V, et al. Role of erythropoietin in ischemic postconditioning induced cardioprotection in hyperlipidemic rat heart, *J Indian Coll Cardiol.* (2017), http://dx.doi.org/10.1016/j.jicc.2017.03.003