

Research Article

Study of Ethyl-2-methylquinoline-3-carboxylate in Ischaemia and Reperfusion Induced Myocardial Injury as specific inhibitor of GCN-5.

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ABSTRACT

Myocardial Infarction is the leading cause of death and disability worldwide. It has been reported that GCN-5 inhibitors inhibit GCN-5 mediated acetylation of PGC-1 alpha, which in turns enhances PGC-1alpha activity, mitochondrial function and oxidative metabolism. PGC-1 α activity is regulated by Histone deacetylases and Histone acetyl transferases. In particularly PGC-1 α is directly acetylated by HAT enzyme- general control non derepressible 5 (GCN-5). Ethyl-2-methylquinoline-3-carboxylate is reported to be a specific inhibitor of GCN-5. PGC-1 α levels are reported to be reduced in heart following myocardial infarction and up regulation of PGC-1 α confers protection against ischaemia and reperfusion in cardiomyoblast cells. Thus, in present study we investigated the protective effect of Ethyl-2-methyl quinoline-3-carboxylate as GCN-5 inhibitor in modulation of PGC-1 alpha activity during ischemia and reperfusion induced myocardial injury.

KEYWORDS

PGC-1 α , GCN-5 inhibitor, HAT enzyme, cardiomyoblast.

1. INTRODUCTION

Myocardial Infarction is the leading cause of death and disability worldwide. Early reperfusion with thrombolysis or percutaneous trans-luminal coronary angioplasty is necessary to restore blood supply and salvage ischemic myocardium. However, Reperfusion paradoxically results in oxidative stress, the loss of membrane phospholipids and Ca^{2+} overload leading to further cardiomyocyte death- termed as 'Reperfusion injury'. A short and less severe ischaemia followed by reperfusion produces reversible injury manifested as contractile dysfunction (Kim et al., 2003), endothelial and vascular dysfunction leading to 'impaired blood flow' and arrhythmias, whereas prolonged severe ischaemia followed by reperfusion produce irreversible cell death termed as lethal reperfusion injury (Yellon and Hausenloy, 2007). In the adult heart, fatty acid oxidation and expression of mitochondrial genes for oxidative phosphorylation is regulated by the transcriptional coactivator, peroxisome proliferator-activated receptor-coactivator-1 α (PGC-1 α), which is abundantly expressed in heart (Witt H et al., 2008). However, in response to pathological stressors such as hemodynamic load or ischemia, cardiac myocytes down-regulate PGC-1 alpha level and fatty acid oxidation genes in preference for glucose metabolism pathways (Rosano et al., 2008). The genetic deletion of PGC-1 α results in diminished cardiac mitochondrial enzyme activities, diminished ATP production, blunted cardiac postnatal growth, diminished chronotropic capacity and an inability to appropriately augment cardiac workload in response to exercise or to β -adrenergic stimulation (Arany et al., 2005 and Leone et al., 2005). In failing heart, synthesis of ATP is compromised as a result of mitochondrial dysfunction (Mudd and Kass, 2008). Chronic administration of low dose of metformin affords cardio protection against ischemia-induced heart failure by improving mitochondrial function via activation of AMPK and its downstream signaling pathway involving PGC-1 α and eNOS (Gundewar et al., 2009). In delayed cardiac ischemic preconditioning PGC-1 α is temporarily induced during the transient ischemic stress which is then associated with subsequent enhanced myocardial ischemia-reperfusion tolerance (McLeod et al., 2004). Transient induction of PGC-1 α alleviates neuronal cell oxidative stress and enhances skeletal myotube antioxidant defenses (St-Pierre et al., 2006). The expression of PGC-1 α and integrity of gap junctions were suppressed and the number of apoptotic bodies were increased in remote viable areas of left ventricle following acute myocardial infarction (AMI) and losartan therapy can abrogate the adverse effects of AMI in a remote area of the LV myocardium and preserves LV function (Sun et al., 2007). PGC-1 α knockout (KO) animals increase mitochondrial apoptotic susceptibility to exogenous ROS (Adhihetty et al., 2009) and enhance apoptotic neurons treated with MPTP (St- Pierre et al., 2006), whereas over expression of PGC-1 α can inhibit apoptosis induced by high glucose in human umbilical vein endothelial cells and prevent cardiomyocytes from apoptosis induced by cyclin T1/Cdk9 (Sano et al., 2004).

PGC-1 α activity is regulated by Histone deacetylases and Histone acetyl transferases. In particularly PGC-1 α is directly acetylated by HAT enzyme- general control non derepressible 5 (GCN-5) resulting in a transcriptionally inactive protein, whereas it is deacetylated by SIRT1 at lysine sites, with subsequent increase in its activity leading to induction of liver gluconeogenesis (Carradori et al., 2011). Moreover lysine acetylation of PGC-1 α by GCN-5 down-regulate catalase expression in response to angiotensin II induced VSMC hypertrophy (Xiong et al.,

2010). GCN-5 over expression reduces the activity of PGC-1 α and expression of its downstream target genes in hepatoma (Lerin et al., 2006) and muscle (Gerhart et al., 2007).

It has been reported that GCN-5 inhibitors inhibit GCN-5 mediated acetylation of PGC-1 alpha, which in turns enhances PGC-1alpha activity, mitochondrial function and oxidative metabolism. Ethyl-2-methylquinoline-3-carboxylate is reported to be a specific inhibitor of GCN-5 (Mai et al., 2005). It exerted inhibition on catalytic activity of GCN-5 and showed inhibition of mutant yeast strains. PGC-1 α levels are reported to be reduced in heart following myocardial infarction (Sun et al., 2007) and upregulation of PGC-1 α confers protection against ischaemia and reperfusion in cardiomyoblast cells (Sun et al., 2013). Thus, in present study we investigated the protective effect of Ethyl-2-methyl quinoline-3-carboxylate as GCN-5 inhibitor in modulation of PGC 1 alpha activity during ischemia and reperfusion induced myocardial injury.

2. MATERIALS AND METHODS

Wistar rats of either sex, weighing 200-250 gm, were used in the present study. They were housed in the Animal House in group of three in polypropylene cages with husk bedding under standard conditions of light and dark cycle with food and water ad libitum. Animals were acclimatized to laboratory conditions before the test.

2.1. Isolated Rat Heart Preparation

Rats were heparinised (500 I.U, i.p.) and sacrificed by cervical dislocation. Hearts were rapidly excised and immediately mounted on Langendorffs apparatus (Langendorffs, 1895). The heart was enclosed in a double walled jacket and the temperature of which was maintained at 37⁰ C by circulating hot water. The preparation was perfused with Krebs Heinsleit (K-H) solution (NaCl 118mM; KCl 4.7mM; CaCl₂ 2.5mM; MgSO₄ .7H₂O 1.2mM; NaHCO₃ 25mM; KH₂PO₄ 1.2mM and C₆H₁₂O₆ 11mM) pH 7.4, maintained at 37⁰ C and bubbled with 95% O₂ and 5% CO₂. The coronary flow rate was maintained 6-9ml/ min and perfusion pressure was kept constant at 70 mm Hg. Global ischaemia was produced for 30 min by closing the inflow of physiological solution and it was followed by reperfusion for 120 min. Four ECG electrodes fixed at the ventricles and auricles were employed to record ECG (Physiograph, INCO, India) for monitoring heart rate.

2.2. Infarct Size Measurement

Heart was removed from Langendorff's apparatus. Both the auricles, the root of aorta and right ventricle were excised and left ventricle was kept overnight at 4°C. Frozen ventricle was sliced into uniform sections of 2-3 mm thickness. The slices were incubated in 1% tripheny 1 tetrazolium (TTC) solution in 0.1M Tris buffer pH 7.8, for 20 min at 37°C. Dehydrogenase enzyme and cofactor NADH present in the viable myocardium react with tetrazolium salt to form a formazon pigment, which is intensely coloured brick red. The enzyme and cofactor were lost from the infarcted cardiac cells. Thus infarcted portion remains unstained while the viable myocardium was stained brick red with TTC. Infarct size was measured by macroscopic methods i.e. volume method.

2.3. Volume Method

Ventricular slices were placed between two glass slides and a transparent plastic grid with 100 squares in 1cm² was placed over it. Average area of each ventricular slice was calculated by counting the number of squares on either side. Similarly numbers of squares falling on stained and unstained area were counted on both sides. Non-stained dull yellow area represented the infarcted portion and red area represented the viable portion. Infarct size was expressed as percentage of total left ventricular volume (%LVV).

2.4. Estimation of Lactate Dehydrogenase (LDH)

LDH was estimated in coronary effluent by 2, 4-DNPH method (King. 1959).

2.4.1. Principal

LDH catalyses the following reaction:



The pyruvate so formed is coupled with 2, 4-dinitrophenylhydrazine (2, 4-DNPH) to give correspondence hydrazone which gives a brown colour in alkaline medium. The intensity of this colour is proportional to the amount of LDH activity and is measured spectrophotometrically at 440nm.

2.4.2. Estimation of Creatine Phosphokinase (CK)

CK was measured in the coronary effluent by modified method of Hughes, 1961.

Principal

CK catalyzes the following reaction:



At pH 7.4, CK catalyses the forward reaction. The creatine so formed, reacts with diacetyl and α -naphthol in alkaline medium to give pink coloured complex. The intensity of this colour is proportional to enzyme activity and is measured spectrophotometrically at 520 nm. Mg²⁺ and cysteine were added as a activators. P-chloromercuribenzoate stops the reaction by inactivating the enzyme.

3. RESULTS AND DISCUSSION

3.1. Effect of Ethyl-2-Methylquinoline-3-Carboxylate on Haemodynamic Responses

Global ischaemia followed by reperfusion for 120 min. significantly reduced heart rate (217.2±3.07 to 62±5.254) and coronary flow rate (8.02±.2653 to 2.12±.332). Ethyl-2-methylquinoline-3-carboxylate (0.5mM and 1.0 mM) treatment before global ischaemia significantly improves the heart rate and coronary flow rate (Table 7,8).

3.2. Effect of Ethyl-2-Methylquinoline-3-Carboxylate on Ischaemia-Reperfusion Induced Myocardial Infarct Size

Global ischaemia for 30 min followed by reperfusion for 120 min produced significant increase in myocardial infarct size calculated by volume method. Treatment with ethyl-2-

methylquinoline-3-carboxylate (0.5mM and 1.0 mM), a GCN-5 inhibitor, before global ischaemia significantly reduced myocardial infarct size. Moreover, there was greater decrease in infarct size at 1.0 mM dose as compared to 0.5 mM. (Fig. 48a, 48b).

3.3. Effect of Ethyl-2-Methylquinoline-3-Carboxylate on Ischaemia-Reperfusion Induced Ldh and Ck Release

Global ischaemia for 30 min followed by reperfusion for 120 min significantly increased the release of LDH in coronary effluent noted immediately, 30 min and 120 min after reperfusion. Similarly, there was a significant increase in release of CK noted 5 min after reperfusion. Ethyl-2-methylquinoline-3-carboxylate (0.5mM and 1.0 mM) treatment before global ischaemia significantly attenuated the ischaemia- reperfusion induced release of LDH and CK release at 120mM, dose dependently. Ethyl-2-methylquinoline-3-carboxylate (0.5mM and 1.0 mM) before global ischaemia result in similar decrease in CK level at 0 min RP.

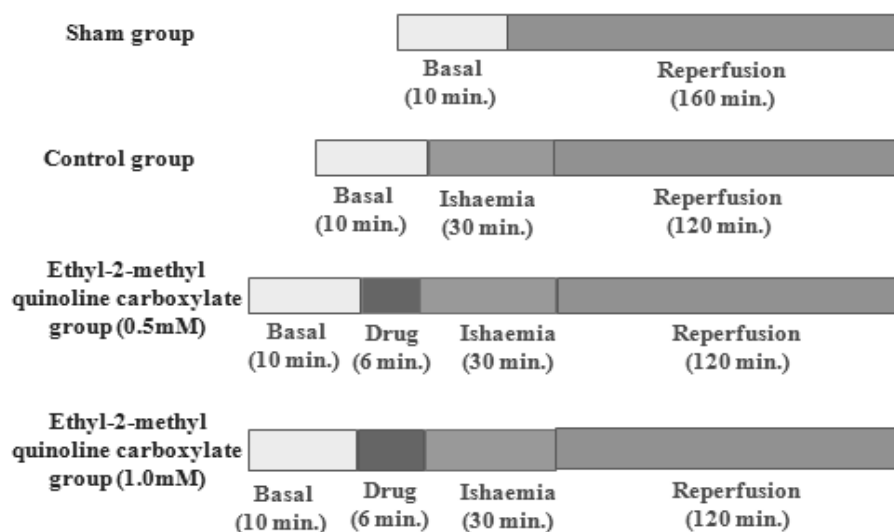
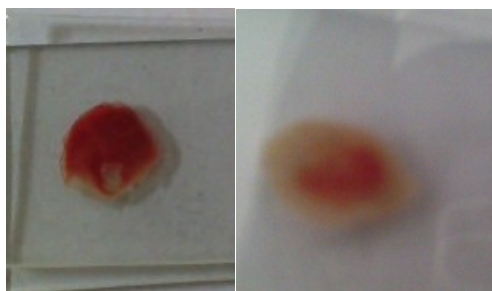
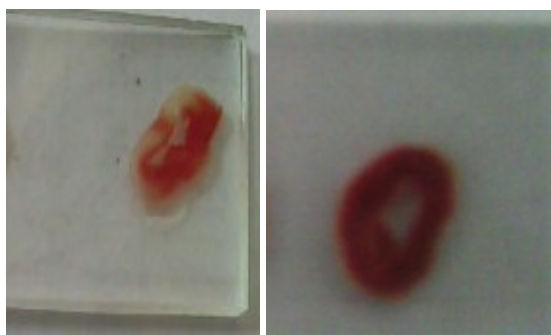


Fig.1: Experimental Protocol.





Treatment-1 Treatment-2

Fig. 2: Myocardial Infarct size.

Section of representative LV myocardium, stained with TTC after 24 hrs. Yellow area represents infarcted myocardium & red stained area represents viable myocardium.

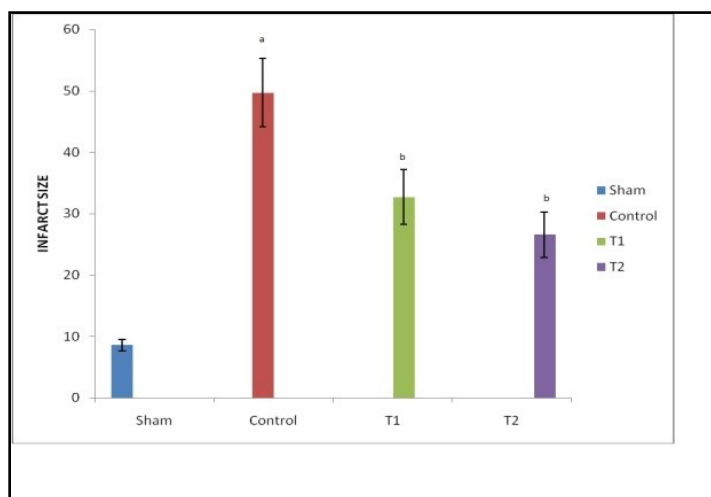


Fig. 3: Effect of Ethyl-2-Methyl Quinoline-3-Carboxylate on Infarct Size.

Values are expressed as mean \pm S.D. (n=5). a=***p < 0.001 vs basal, b= ***p < 0.001 vs control.

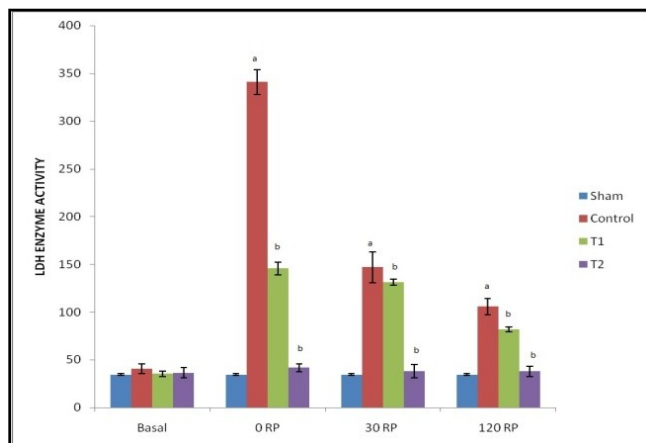


Fig. 4: Effect of Ethyl-2-Methyl Quinoline-3-Carboxylate on LDH Release.

LDH was estimated in coronary effluent. Basal denotes LDH measured during stabilization before ischaemia. 0 RP, 30 RP and 120 RP denotes LDH measured after 0 min, 30 min and 120 min reperfusion, respectively following sustained ischaemia,. Values are expressed as mean \pm S.D. (n=5). a=***p < 0.001 vs basal, b= ***p<0.001 vs control.

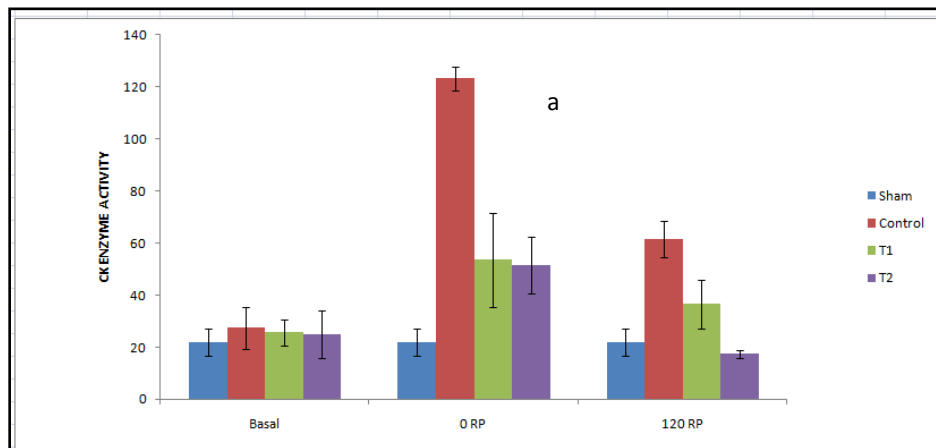


Fig. 5: Effect of Ethyl-2-Methyl Quinoline-3-Carboxylate on CK Release.

CK was estimated in coronary effluent. Basal denotes LDH measured during stabilization before ischaemia. 5 RP and 120 RP denotes CK measured after 5 min and 120 min reperfusion following sustained ischaemia. Values are expressed as mean \pm S.D. (n=5). a=***p < 0.001 vs basal, b= ***p<0.001 vs control.

4. ACKNOWLEDGEMENT

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