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Study of Ethyl-2-methylquinoline-3-carboxylate in Ischemia and Reperfusion Induced Myocardial Injury as Specific Inhibitor of GCN-5

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Keywords PGC-1 α; GCN-5 Inhibitor; HAT Enzyme; Cardiomyoblast.

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-1α activity is regulated by Histone deacetylases and Histone acetyl transferases. In particularly PGC-1α 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 upregulation 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.

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-1α (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-1α* 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 cardioprotection against ischemia-induced heart failure by improving mitochondrial function via activation of AMPK and its downstream signaling pathway involving PGC-1α and eNOS (Gundewar *et al*., 2009).

In delayed cardiac ischemic preconditioning PGC-1α 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-1α alleviates neuronal cell oxidative stress and enhances skeletal myotube antioxidant defenses (St-Pierre *et al.,* 2006).The expression of PGC-1α 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-1α 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-1α 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-1α activity is regulated by Histone deacetylases and Histone acetyl transferases. In particularly PGC-1α is directly acetylated by HAT enzyme- general control nonderepressible 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-1alpha by GCN-5 down-regulate catalase expression in response to angiotensin II induced VSMC hypertrophy (Xiong *et al*., 2010). GCN-5 overexpression reduces the activity of PGC-1α 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.

MATERIALS & 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 libitium. Animals were acclimatized to laboratory conditions before the test.

Experimental protocol

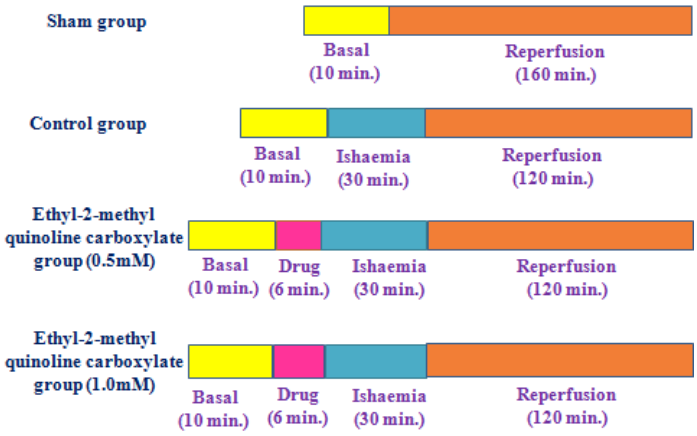


Fig. 1

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 37^0 C by circulating hot water. The preparation was perfused with Krebs Heinseleit (K-H) solution (NaCl 118mM; KCl 4.7mM; $CaCl_2$ 2.5mM; $MgSO_4$.$7H_2O$ 1.2mM; $NaHCO_3$ 25mM; KH_2PO_4 1.2mM and $C_6H_{12}O_6$ 11mM) pH 7.4, maintained at 37^0 C and bubbled with 95% O_2 and 5% CO_2. 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.

Infarct Size Measurement

Heart was removed from Langendroff's appartus. 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.

Volume Method

Ventricular slices were placed between two glass slides and a transparent plastic grid with 100 squares in $1cm^2$ was placed over it. Average area of each ventricular slice was calculated by counting the number of squares on either side. Similarly number of squares falling on stained and unstained area were counted on both sides. Non-stained dull yellow area represented the infracted portion and red area represented the viable portion. Infarct size was expressed as percentage of total left ventricular volume (%LVV).

Estimation Of Lactate Dehydrogenase (LDH)

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

Principle

LDH catalyses the following reaction

$$\text{Lactate} + \text{NAD} \rightleftharpoons \text{Pyruvate} + \text{NADH}$$

The pyruvate so formed is coupled with 2, 4-dinitrophenylhydrazine (2, 4-DNPH) to give correspondance 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.

Estimation Of Creatine Phosphokinase (CK)

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

Principle

CK catalyzes the following reaction :

$$\text{Creatine phosphate} + \text{ADP} \rightleftharpoons \text{Creatine} + \text{ATP}$$

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. Mg^{2+} and cysteine were added asa activators. P-choloromercuribenzoate stops the reaction by inactivating the enzyme.

RESULTS AND DISCUSSION

Effect Of Ethyl-2-Methylquinoline-3-Carboxylateon 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 1 and 2).

Effect Of Ethyl-2-Methylquinoline-3-Carboxylateon 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 2a and 2b.

Effect Of Ethyl-2-Methylquinoline-3-Carboxylateon 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 3 and 4).

Table 1: Effect of Ethyl-2-Methyl Quinoline-3-Carboxylate on Heart Rate

Groups	Basal	0 min RP	30min RP	120 min RP
Sham	220± 10.354	220± 10.354	220± 10..354	204 ± 3.878
Control	217.2± 6.87	69.2± 6.723*	98± 7.874*	62± 11.747*
2 methyl ethyl 3 Quinoline carboxylate (0.5 mM)	206.8± 17.527	128.8± 8.672*	177.02± 14.464*	157±9.798*
2 methyl ethyl 3 Quinoline carboxylate (1 mM)	212 ± 15.65	125.4 ± 11.44*	186.4± 9.029*	167.2±12.80*

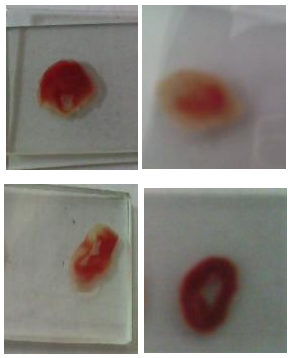
Values are expressed as mean ± S.D. (n=5). Basal denotes heart rate measured during stabilization before ischaemia. O min RP, 30 min RP and 120 min RP denotes heart rate measured after 0 min, 30 min and 120 min reperfusion following sustained ischaemia. *p < 0.001 vs basal.

Table 2: Effect of Ethyl-2-Methyl Quinoline-3-Carboxylate on Coronary Flow Rate

Groups	Basal	0 min RP	30min RP	120 min RP
Sham	8.02±0.1949	8.02±.1949	7.84±.08718	7.84±.08718
Control	8.02±0.5933	4.6±0 0.5831*	5.12± 0.9445*	2.12± 0.743*
2 methyl ethyl 3 Quinoline carboxylate (0.5 mM)	7.28± 0.251	6.52± 0.295**	8.02± 1.094**	6.78± 0.8075**
2 methyl ethyl 3 Quinoline carboxylate (1 mM)	7.78± 0.960	6.34± 0.4615**	8.38± 0.4324**	8.16± 0.5899**

Values are expressed as mean ± S.D. (n=5). Basal denotes coronary flow rate measured during stabilization before ischaemia. 0 min RP, 30 min RP and 120 min RP denotes Coronary flow rate measured after 0 min, 30 min and 120 min reperfusion following sustained ischaemia, *p < 0.001 vs basal. **p < 0.001 vs Control.

Sham Control



Treatment-1 Treatment-2

Fig 2a: 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.

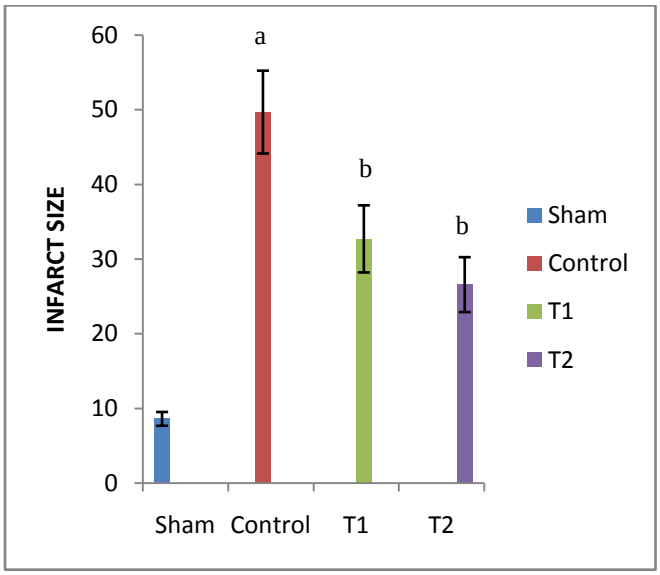


Fig 2b: Effect ofEthyl-2-MethylQuinoline-3-Carboxylateon Infarct Size.

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

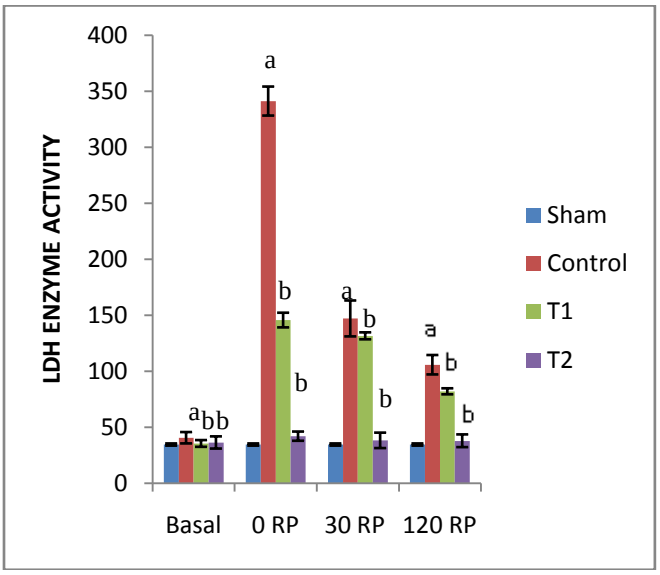


Fig 3: Effect ofEthyl-2-MethylQuinoline-3-Carboxylateon LDH Release.

LDH was estimated in coronary effluent. Basal denotes LDH measured during stabilization before ischaemia. O 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 ± S.D. (n=5). a=***p < 0.001 vs basal, b= ***p<0.001 vs control.

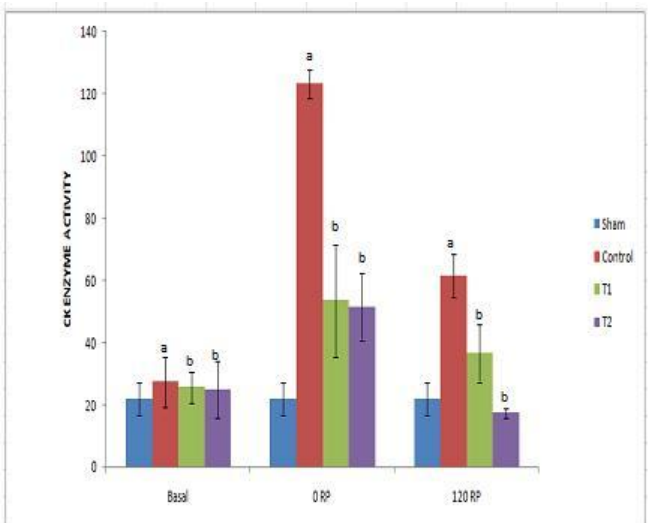


Fig 4: Effect ofEthyl-2-MethylQuinoline-3-Carboxylateon CK Release.

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 ± S.D. (n=5). a=***p < 0.001 vs basal, b= ***p<0.001 vs control.

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