World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 3.347

BIOLOGICAL EVALUATION OF ETHYL-2-METHYLQUINOLINE-3-CARBOXYLATE IN MYOCARDIAL ISCHAEMIA REPERFUSION INJURY

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Article Received on 15/02/2016 Article Revised on 08/03/2016 Article Accepted on 30/03/2016

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ABSTRACT

Isolated perfused heart preparation has been employed in the present study because it permits the use of pharmacological interventions without any interference due to changes in systemic circulation. Electrical pacing has not been used in this study because it is reported to release norepinephrine. Oxidative stress parameters in the present study have been assessed spectrophotometrically. PGC-1α interacts with GCN-5, an acetyl-transferase that has been shown to acetylate and inhibit PGC-1α activity in vitro and in vivo. PGC-1α levels are reduced in heart following MI by coronary artery ligation in rats while treatment with angiotensin II receptor blockers. Moreover, impairment of the PGC-1α-mediated mitochondrial biogenesis increased heart vulnerability to IRI and up-regulation of PGC-1α pathway confers protection against simulated I/R in cardio-myoblast cells.

KEYWORDS: GCN-5, PGC-1α, MI, cardiomyoblast.

INTRODUCTION

GCN-5 belongs to GNAT (GCN-5 related N-acetyl-transferase) family of histone acetyl-transferases (HAT). Other HATs are p300/CBP accessory factor (PCAF), TIP60 and SRC-1 (Roth *et al.,* 2001; Sterner*et al.,* 2000; Marmorstein *et al.,* 2001). Dysfunction of HATs (GCN-5/PCAF) leads to several diseases including cancer, neurodegenaration, asthma, diabetes, AIDS, and cardiac hypertrophy (Mantelingu *et al.,* 2007). By this it has been supposed that selective inhibitors of HATs (GCN-5/PCAF) may have therapeutic applications (Lehrmann *et al.,* 2002). It has been shown that natural products like garcinol

(Balasubramanyam *et al.*, 2004), curcumin (Balasubramanyam *et al.*, 2004),and anacardic acid (Balasubramanyam *et al.*, 2003) are potent p300 and PCAF inhibitors, While synthetic compounds like trifluoromethyl phenyl benzamides have been found to modulate p300 (Mantelingu *et al.*, 2007) and isothiazolones (Stimson *et al.*, 2005) as novel inhibitors of PCAF and p300 in cell cultures. The reported compounds with specific GCN-5 inhibitory activity are mentioned below:

CPTH2, cyclopentylidene-[4- (4′-chlorophenyl) thiazol-2-yl) hydrazone: CPTH2 has been reported to inhibit GCN-5 dependent functional network in *Saccharomyces cerevisiae*. CPTH2 inhibited an in vitro HAT reaction, which was reverted by increasing concentration of histone H3. In vivo, it decreased acetylation of bulk histone H3 at the specific H3-AcK14 site (Chimenti *et al.*, 2009). GCN-5 plays a role in diabetes induced endothelial oxidative stress by upregulating Nox2 through histone acetylation. GCN-5 inhibition by CPTH2 or siRNA mediated knockdown prevented hyperglycemia-induced Nox2 overexpression and subsequent O2− · increase (Costantino *et al.*, 2013).

MB3: The γ-butyrolactone MB3 is an inhibitor of histone acetyltransferase, GCN-5 (KAT2A) with an affinity comparable to the natural substrate H3 lysine (Biel *et al.*, 2004).

Quinoline derivatives: Quinoline also called as 1-aza-napthalene or benzo-pyridine is nitrogenous heterocyclic aromatic weak tertiary base with alkaloidal nature and is derived from quinine, coal tar, etc having molecular formula of C_9H_7N and molecular weight 129.16. Quinoline possesses similar reactions of pyridine and benzene and shows electrophilic and nucleophilic substitution reactions. It has pungent odour, oily texture and is nontoxic to humans on oral absorption and inhalation.

Ethyl-2-methylquinoline-3-carboxylateand Ethyl-3-quinoline carboxylate: Ethyl-2-methylquinoline -3-carboxylate and Ethyl quinoline-3-carboxylate were found as *specific* GCN-5 *inhibitors* in yeast. In *Saccharomyces cerevisiae,* the inhibitory effects of both compounds mimic the effect of GCN-5loss-of-function mutation (Ornaghi *et al.*, 2005; Mai *et al.*, 2006).Yeast strains with disrupted *gcn5*gene wereless sensitive to both inhibitors and showed persistent growth, while in wild type strain with normal GCN-5p both inhibitors impaired cell growth (Ornaghi *et al.*, 2005; Mai *et al.*, 2006). Further it was demonstrated that the inhibitory activity of Ethyl-2-methyl-quinoline-3-carboxylate is correlated to HAT catalytic activity of GCN-5.

Several analogues of 3-carbethoxy-2-methyl quinoline, with or without substitutions on 2nd and 3rd position of the quinoline ring such as insertion of a hydroxyl group at the C4 position as well as the replacement of the quinoline ring with the pyridine as in quinoline-3- carboxylic acid; ethyl-4-hydroxy-2 methyl quinoline-3- carboxylate; 2-methyl quinoline, quinoline-3-Carboxamide; ethyl-3-naphthalene carboxylate and ethyl-2-methyl pyridine carboxylate (Mai *et al.,* 2006). 3-carbethoxy quinoline was able to inhibit the growth of yW303 cells, it being less effective in inhibiting the gcn5Δ strain cell growth. The compounds with deletion of the C3-carbethoxy function abolished the GCN5 inhibiting activity. Differently, ethyl-3-quinoline carboxylate lacking the C2-methyl substituent retained the GCN5 inhibitory activity, but the replacement of its quinoline with naphthalene or the introduction of a carboxyl or carboxamide function at the quinoline C3-position instead of the 3-carbethoxy moiety gave inactive products a compound to show GCN-5 inhibition activity (Mai *et al.,* 2006).

EXPERIMENTAL WORK

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.

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.

ASSESSMENT OF OXIDATIVE STRESS PARAMETERS

4.8.1) Homogenization of Heart

After perfusion protocol, the hearts were weighed and homogenized in 1.15% w/v KCl. The suspension was centrifuged at 1000 rpm for 10min at 0–4°C to remove the nuclei and cell debris (Llesuy *et al.*, 1985) and supernatants were stored at -20 ◦ were used for measuring antioxidant enzymatic activity.

4.8.2) Estimation of Thiobarbituric Acid Reactive Substances (TBARS)

The Malondialdehyde (MDA), a measure of Lipid peroxidation was assayed in the form of Thiobarbituric acid Reacting Substances (TBARS) (Catala, 2006).

Briefly, 1 ml of supernatatnt and 1 ml of Tris HCL were incubated at 37ºC for 2 hours. After incubation, 1 ml of 10% Trichloroacetic acid (TCA) was added and centrifuged at 10, 000× g for 10 min. To 2 ml of supernatant 2 ml of 0.375%w/v thiobarbituric acid was added and the tubes were kept in boiling water for ten minutes. After cooling 1 ml of distilled water was added and absorbance was measured at 532 nm using a spectrophotometer (Shimadzu, Japan). Extent of lipid peroxidation was expressed as nanomoles of MDA consumed per minute at 25ºC.

$$\text{nmoles MDA/ml} = \frac{6.41 \times \text{Absorbance sample} \times \text{Total volume}}{\text{volume of sample}}$$

4.8.3) Estimation of Total Protein Content

The total protein content will be determined by Lowry's method (Lowry *et al.*, 1951) using bovine albumin as a standard. 0.3 mL of supernatant of tissue homogenate was diluted to 1 mL. The 100 µL of diluted supernatant was made up to 1 ml using distilled water. To this, 5 mL of Lowry's reagent was added. The contents were mixed thoroughly and the mixture was allowed to stand for 15 min at room temperature. Then 0.5 mL of Folin-Ciocalteu reagent was added and the contents were vortexed vigorously and incubated at room temperature for 30 min. The standard curve was plotted using 50-500 µg of BSA. The protein content will be determined using spectrophotometer at 750 nm and expressed as milligram per gram of tissue weight.

4.8.4) Estimation of GSH

The assay of GSH with DTNB was performed by following a standard Ellman's method, 1959.

3 ml of potassium phosphate [0.2 M, pH 7.6] buffer was taken in the test tube, followed by the addition of 1 ml supernatant and 0.5 ml Ellman's reagent (19.8 mg of DTNB (0.001 M) in

100ml of 0.1% sodium citrate) was added. An absorbance of reaction product in the cuvette was read after 5 min at 412 nm using Shimadzu 1601 UV/Visible double bean spectrophotometer.

4.8.5) Estimation of Catalase

Catalase activity was measured by method of Sinha, 1972.

0.1 ml of the homogenate was taken to which 1 ml of phosphate buffer and 0.5 ml of hydrogen peroxide was added and a timer started. The reaction was arrested by addition of 2 ml of dichromate acetic acid reagent. The tubes were heated in a boiling water bath for 10 minutes. The green colour developed was read at 570 nm in a spectrophotometer (Shimadzu, Japan). Catalase activity was then calculated as follows: % decrease = (A570 Blank – A570 Treatment / [A570 Blank]) x 100.Catalase activity was expressed as µmoles of hydrogen peroxide utilized /mg / protein / min.

4.9) Statistical Analysis

Data analysis was performed using Graph Pad instat 3 software. Values were expressed as mean ± S.D. Statistical significance was calculated by repeated way analysis of variance (ANOVA). Student–Newman–Keul's test and Tukey Kramer test were used as post hoc tests for multiple comparison between groups and for comparison with control group, respectively. A value of $p<0.05$ was considered to be statistically significant.

Group I (Sham group, n=5) : Isolated rat heart was perfused with K-H solution for 160 min after stabilization for 10 min.

Group II (Control group, n=5) : Isolated rat heart was perfused with K-H solution for 10 min stabilization and then subjected to 30 min global ischaemia followed by reperfusion for 120 min.

Group III (Ethyl-2-methyl quinoline-3-carboxylate (0.5 mM) treated group, n=5) : After stabilization, isolated rat heart was perfused with K-H solution containing ethyl-2-methyl-quinoline-3-carboxylate (0.5mM) for 10 min and then subjected to 30 min global ischaemia followed by reperfusion for 120 min.

Group IV (Ethyl-2-methyl quinoline-3-carboxylate (1 mM) treated group, n=5) : After stabilization, isolated rat heart was perfused with K-H solution containing ethyl-2-methyl-quinoline-3-carboxylate (1mM) for 10 min and then subjected to 30 min global ischaemia followed by reperfusion for 120 min.

RESULTS AND DISCUSSION

Effect of ethyl-2-methyl quinoline-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-methyl quinoline-3-carboxylate (0.5mM and 1.0 mM) treatment before global ischaemia significantly improves the heart rate and coronary flow rate (Table 7,8).

Effect of ethyl-2-methyl quinoline-3-carboxylate on gsh activity

Global ischaemia followed by reperfusion for 120 min. significantly decreased GSH level in tissue supernatant. Ethyl-2-methyl quinoline-3-carboxylate (0.5mM and 1.0 mM) treatment before global ischaemia significantly increased the GSH level (Fig 51).

Effect of 2-methyl ethyl-3 quinoline carboxylate on catalase activity

Global ischaemia followed by reperfusion for 20 min. significantly decreased catalase level in tissue supernatant. Ethyl-2-methyl quinoline-3-carboxylate (0.5mM and 1.0 mM) treatment before global ischaemia significantly increase the catalase level (Fig 52).

Effect of 2-methyl ethyl-3 quinoline carboxylate on tbars

Global ischaemia followed by reperfusion significantly increased MDA level in tissue supernatant. Ethyl-2-methyl quinoline-3-carboxylate (0.5mM and 1.0 mM) treatment before global ischeamia significantly decrease the MDA level (Fig 53).

Table: Effect of Ethyl-2-MethylQuinoline-3-Carboxylateon 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 : Effect of Ethyl-2-MethylQuinoline-3-Carboxylateon 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.

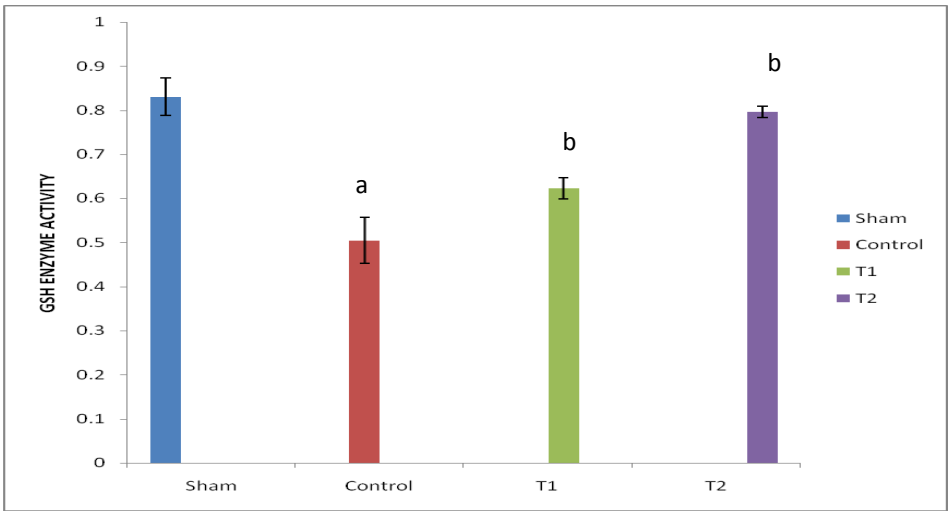

Fig: Effect of Ethyl-2-MethylQuinoline-3-Carboxylateon GSH Activity.

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

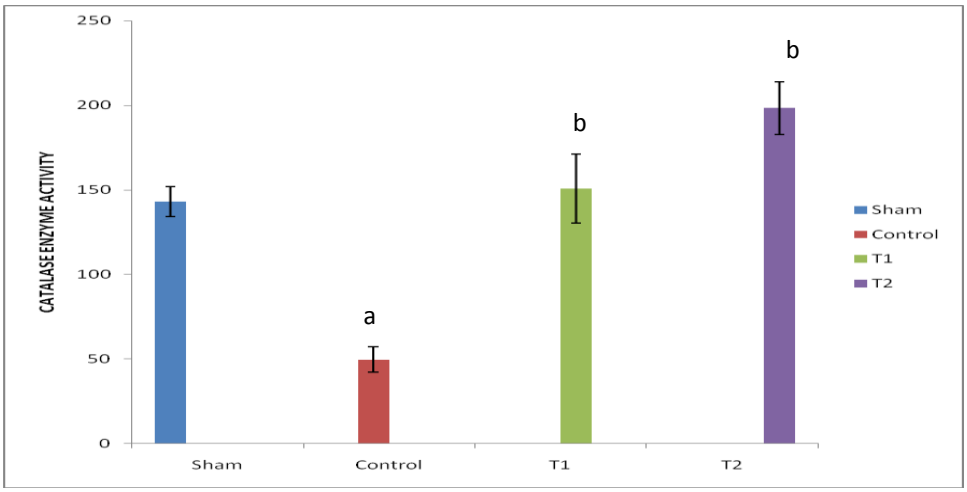

Fig: Effect of Ethyl-2-MethylQuinoline-3-Carboxylate on CATALASE Activity.

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

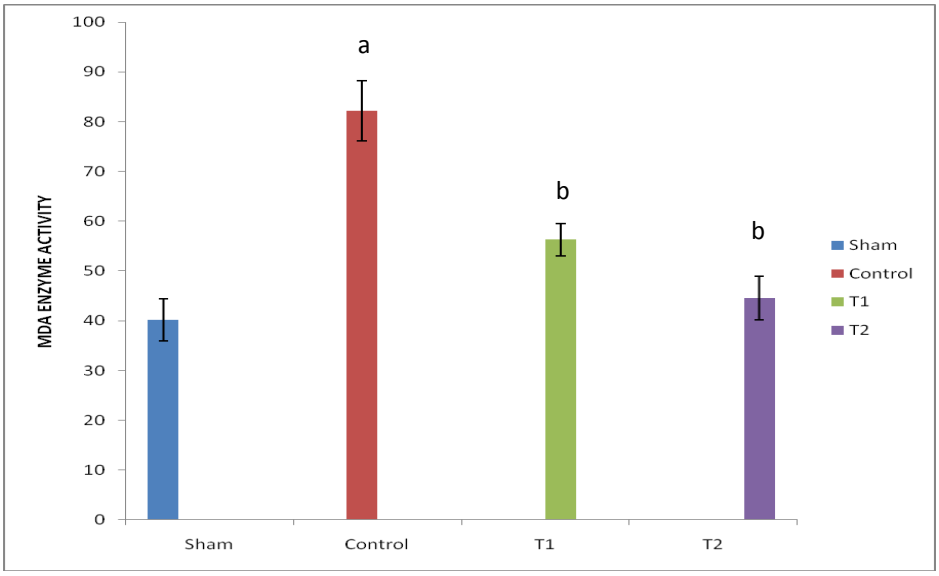

Fig: Effect of Ethyl-2-MethylQuinoline-3-Carboxylateon TBARS ACTIVITY.

MDA level was estimated in heart ventricle. Values are expressed as mean ± S.D. (n=5). a=***p < 0.001 vs basal, b= ***p<0.001 vs control.

On the basis of results obtained in present study the following salient findings may be summarised:

(1) Heamodynamic parameters such as heart rate and coronary flow rate are reduced inIsolated rat heart subjected to ischaemia for 30 min followed by reperfusion for 120 min.Ethyl-2-methyl quinoline-3-carboxylate treatment (0.5 mM and 1 mM) significantly improves reduction in heart rate and coronary flow rate.

(2) Lipid peroxidation, measured in terms of TBARS, was significantly increased and levels of reduced GSH and catalase were found to be decreased in rat hearts subjected to ischaemia and reperfusion. Ethyl-2-methyl quinoline-3-carboxylate treatment (0.5 mM and 1 mM) attenuated the ischaemia and reperfusion -induced oxidative stress as assessed in terms of reduction in TBARS and the consequent increase in GSH and catalase.

In summary, our results demonstrate that Ethyl-2-methyl quinoline-3-carboxylate, a specific GCN-5 inhibitor attenuates ischaemia and reperfusion induced Myocardialinjury. Our results suggest that cardioprotective effect of Ethyl-2-methyl quinoline-3-carboxylate may be mediated through inhibition of GCN-5. Thus, inhibition of GCN-5 may be a novel therapeutic strategy for developing cardioprotective agents in treatment of ischaemia and reperfusion induced myocardial injury

ACKNOWLEDGEMENT

Authors are thankful to Khalsa College of Pharmacy and IELTS academy, Amritsar for their providing facilities and their constant support for this work.

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