

CARDIOPROTECTION OF FIBRATES IN RAT MODEL OF MYOCARDIAL ISCHEMIA: REPERFUSION

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ABSTRACT

The present study was designed to investigate the effect of Clofibrate, an agonist of peroxisome proliferator activated receptor- α (PPAR- α), on ischemia-reperfusion (I/R)-induced myocardial injury. The isolated Langendorff-perfused rat hearts were subjected to global ischemia for 30 min followed by reperfusion for 120 min. Myocardial infarct size was assessed by volume methods using triphenyltetrazolium chloride staining. Coronary effluent was analyzed for the release of lactate dehydrogenase (LDH) and creatine kinase (CK) to assess the degree of cardiac injury. Moreover, oxidative stress in the heart was assessed by measuring lipid peroxidation, superoxide anion generation and reduced glutathione. I/R was noted to produce myocardial injury, as assessed in terms of increase in myocardial infarct size, LDH and CK in coronary effluent. Moreover, oxidative stress was noted to be increased due to I/R injury as assessed in terms of decreased TBARS (thiobarbituric acid-reactive substance) and superoxide anion generation levels alongwith increase in reduced glutathione levels in the heart. Treatment with Clofibrate showed cardioprotection against I/R-induced myocardial injury in rat hearts as assessed in terms of reductions in myocardial infarct size, LDH and CK levels in coronary effluent. Moreover, I/R-induced oxidative stress was noted to be reduced by Clofibrate treatment. It may be concluded that the observed cardioprotective potential of Clofibrate against I/R-induced myocardial injury was due to the reductions in infarct size and oxidative stress.

KEYWORDS: Clofibrate, PPAR- α , Ischemia-reperfusion injury, Oxidative stress

INTRODUCTION

Coronary artery disease is a leading cause of morbidity and mortality and its prevalence is continuously increasing worldwide¹. Myocardial ischemia is a condition in which heart tissue receives inadequate blood flow, followed by inadequate oxygen and nutrient supply. The restoration of coronary blood flow to an ischemic myocardium is mandatory to avoid myocardial damage. However, reperfusion of the previously ischemic myocardium is often followed by detrimental changes in myocardial tissues, known as ischemia-reperfusion (I/R) injury^{2,3}. The pathogenesis of I/R-induced myocardial injury involves various factors including oxidative stress, intracellular calcium overload, apoptotic and necrotic myocytes death^{4,5}. Fibrates, the synthetic agonists of PPAR- α , a subfamily of the nuclear receptor superfamily, is naturally activated by ligands such as free fatty acids and eicosanoids^{6,7}. PPAR- α is expressed in the liver and in tissues with highly active fatty acid metabolism, such as the heart, kidney, endothelium, and vascular smooth muscle. Fibrates have been in clinical use as hypolipidemic agents for several decades. More recently, they have been reported to have beneficial effects on cardiovascular function^{8,9}. Clofibrate, a PPAR- α activator, has been reported to possess potent antioxidant and cardioprotective potential as evidenced by an increase in the content of reduced glutathione and decrease in the activity of glutathione-S-transferase¹⁰. Additionally, treatment with clofibrate has been shown to inhibit superoxide dismutase and glutathione peroxidase activities accounting for its antioxidant effects¹¹. Moreover, the cardioprotective effects of clofibrate-induced PPAR- α activation has been reported to include the higher production of endothelial nitric oxide (eNOS)¹². Further, PPAR- α activation by clofibrate afforded cardioprotection by a mechanism that involves NO production and inhibition of NADPH oxidase activity, accounting for its cardioprotective potential^{8,13}. Therefore, the present study was undertaken to investigate the cardioprotective effect of Clofibrate against I/R-induced myocardial injury in rat hearts.

MATERIALS AND METHODS

Experimental Animals

The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee. Wistar albino rats of either sex weighing 180-220 g were used. They were housed in Institutional animal housing and were maintained on rat feed (Kisan Feeds Ltd., Chandigarh, India) and tap water ad libitum.

Isolated Rat Heart Preparation

Rats were heparinized (500 IU i.p.) and sacrificed by stunning. The heart was rapidly excised and immediately mounted on a Langendorff apparatus¹⁴. The heart was enclosed in a double walled jacket, the temperature of which was maintained at 37°C by circulating hot water. The preparation was perfused with Krebs-Henseleit (K-H) solution (NaCl 118 mM; KCl 4.7 mM; CaCl₂ 2.5 mM; MgSO₄.7H₂O 1.2 mM; NaHCO₃ 25 mM; KH₂PO₄ 1.2 mM; C₆H₁₂O₆ 1 mM) pH 7.4, maintained at 37 °C and bubbled with 95% O₂ and 5% CO₂. The coronary flow rate was maintained at around 7 mL/min, and the perfusion pressure was kept at 80 mmHg. Global ischemia was produced for 30 min by blocking the inflow of physiological solution and it was followed by perfusion for 120 min.

Laboratory Assays

Myocardial infarct size was measured macroscopically using triphenyl tetrazolium chloride (TTC) staining employing volume method¹⁵. The myocardial injury was assessed by measuring the release of lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) in the coronary effluent using the commercially available enzymatic kits (Vital Diagnostics, Thane, Maharashtra, India). The level of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation in the heart was estimated according to the method of Ohkawa et al.¹⁶. The superoxide anion generation was assessed by estimating the reduced nitro blue tetrazolium (NBT) using the method of Wang et al.¹⁷. Moreover, the reduced glutathione content in each heart was estimated using the method of Beutler et al.¹⁸.

Experimental Protocol

Five groups of 8-10 animals each were employed in the present study. In all groups, each isolated perfused heart was allowed to stabilize for 10 min by perfusing with K-H solution.

Group I (Normal Control): Isolated normal rat heart was perfused for 150 min using K-H solution after 10 min of stabilization.

Group II (I/R): Isolated normal rat heart after 10 min of stabilization was subjected to 30 min of global ischemia followed by 120 min of reperfusion

Group III (Clo Treated I/R- I): The rat was given Clofibrate (100mg/kg/day, i.p.) for 2 weeks. After 2 weeks, the heart was then subjected to 30 min of global ischemia followed by 120 min of reperfusion, after 10 min of stabilization.

Group IV (Clo Treated I/R- II): The rat was given Clofibrate (300mg/kg/day, i.p.) for 2 weeks. After 2 weeks, the heart was then subjected to 30 min of global ischemia followed by 120 min of reperfusion, after 10 min of stabilization.

Group V (Clo Treated I/R- III): The rat was given Clofibrate (500mg/kg/day, i.p.) for 2 weeks. After 2 weeks, the heart was then subjected to 30 min of global ischemia followed by 120 min of reperfusion, after 10 min of stabilization.

Statistical Analysis

The results were expressed as mean \pm SD. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's multiple-comparison test. A P value < 0.05 was considered to be statistically significant.

Drugs and Chemicals

The LDH and CK enzymatic estimation kits were purchased from Vital Diagnostics, Thane, Maharashtra, India. DTNB and NBT were obtained from Loba Chem, Mumbai, India. Clofibrate, 1,1,3,3-tetramethoxy propane and reduced glutathione were procured from Sigma-Aldrich, USA. All other reagents used in this study were of analytical grade.

RESULTS

Effect of I/R on Myocardial Infarct size and Oxidative Stress

I/R was noted to increase the infarct size in rat hearts as assessed macroscopically using TTC (Fig. 1). Moreover, the global ischemia for 30 min followed by reperfusion for 120 min significantly increased LDH and CK release in the coronary effluent in rat hearts. Maximum release of LDH was noted immediately after reperfusion (Fig. 3), while maximum release of CK was noted at 5 min of reperfusion (Fig. 2).

Lipid peroxidations, measured in terms of TBARS, and superoxide anion generation, assessed in terms of reduced NBT, were significantly increased in rat hearts subjected to I/R. Moreover, the levels of reduced GSH were found to be decreased in the rat hearts subjected to I/R that may be attributed to the enhanced oxidative stress in I/R-induced myocardial injury (Figs. 4-6).

Effect of Clofibrate on I/R-Induced Infarct size and Oxidative Stress

Treatments with Clofibrate in different concentrations afforded cardioprotection by significantly attenuating I/R-induced myocardial injury in rat hearts as assessed in terms of reductions in myocardial infarct size and decreased release of LDH and CK in coronary effluent (Fig. 1-3). However, maximum cardioprotection was noted at a concentration of 300 mg/kg.

In addition, Clofibrate treatments markedly attenuated the I/R-induced oxidative stress in normal rat hearts, as assessed in terms of reduction in TBARS and superoxide anion generation, and the consequent increase in GSH (Fig. 4-6). However, maximum reduction of I/R-induced oxidative stress was noted at a concentration of 300 mg/kg.

DISCUSSION

CAD has been regarded as the leading cause of morbidity and mortality worldwide whose prevalence is continuously increasing worldwide¹. Myocardial ischemia is a condition in which the coronary blood flow to the heart is reduced, which results in deficient oxygen and nutrients supply to the heart^{4,5}. Myocardial reperfusion is the restoration of blood flow to an ischemic heart. Reperfusion to an ischemic myocardium often results in lethal myocardial injury known as I/R injury^{2,3}. The increase in infarct size and the release of LDH and CK are documented to be an index of I/R-induced myocardial injury^{19,20}. In the present study, 30 min of ischemia followed by 120 min of reperfusion was noted to produce myocardial injury, as assessed in terms of increased infarct size in the heart and elevated release of LDH and CK in the coronary effluent. The maximal release of LDH was noted immediately after reperfusion, whereas peak release of CK was observed after 5 min of reperfusion - both findings in accordance with our earlier studies²¹. Lipid peroxidation refers to the oxidative degradation of lipids. It is the process whereby free radicals steal electrons from the lipids in cell membranes, resulting in cell damage. Increases in lipid peroxidation and superoxide anion generation have been suggested as indicators of oxidative stress^{22,23}. The lipid peroxidations, measured in terms of TBARS, and superoxide anion generation, assessed in terms of reduced NBT, were noted to be increased as a result of I/R. In addition, the GSH level was decreased in rat hearts subjected to I/R. These indicators suggest the development of I/R-induced oxidative stress, which may be responsible for the noted I/R-induced myocardial injury in the present study. Thus, the observed marked increase in myocardial injury in the rat heart may be due to the high degree of oxidative stress as a result of I/R.

Fibrates, commonly known as the agonists of PPAR- α , a subfamily of the nuclear receptor superfamily, are noted to be naturally activated by ligands such as free fatty acids and eicosanoids^{6,7}. The expression of PPAR- α has been already reported to be present in liver and in tissues with highly active fatty acid metabolism, such as the heart, kidney, endothelium, and vascular smooth muscle. The wide clinical use of fibrates may be attributed to their potent hypolipidemic effect for several decades. More recently, they have been reported to have beneficial effects on cardiovascular function^{8,9}. The present study investigated the cardioprotective potential of clofibrate against I/R injury in rat hearts. Treatments with clofibrate afforded cardioprotection by significantly attenuating I/R-induced myocardial injury in rat hearts as assessed in terms of reductions in myocardial infarct size and decreased release of LDH and CK in coronary effluent, with maximum cardioprotection at a concentration of 300 mg/kg.

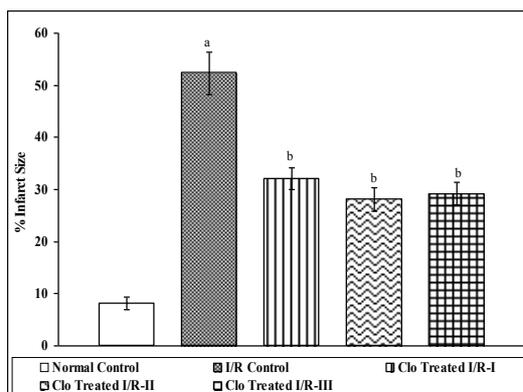
In addition, numerous studies have demonstrated clofibrate to possess protective effects against oxidative stress in order to mimic cardioprotection. Clofibrate treatment has been noted to reduce the oxidative stress markers and lipid peroxidation levels, confirming its antioxidant action in affording cardioprotection. Clofibrate, a PPAR α activator, has been reported to increase the content of reduced glutathione and decrease the activity of glutathione-S-transferase¹⁰. It was shown that the treatment with clofibrate decreased the levels of Fe/ADP-ascorbate-, as well as t-butyl hydroperoxide (Bu'OOH)-induced lipid peroxidation²⁴. Treatment with clofibrate has been shown to inhibit superoxide dismutase and glutathione peroxidase activities accounting for its antioxidant effect¹¹. This contention is supported by the results obtained in the present study that treatment with Clofibrate (100 mg/kg, 300 mg/kg and 500 mg/kg), a PPAR α activator, has markedly reduced the oxidative stress in rat hearts subjected to I/R, as assessed in terms of reduction in TBARS and superoxide anion generation, and

consequent increase in reduced glutathione levels, with maximum reductions at a concentration of 300 mg/kg.

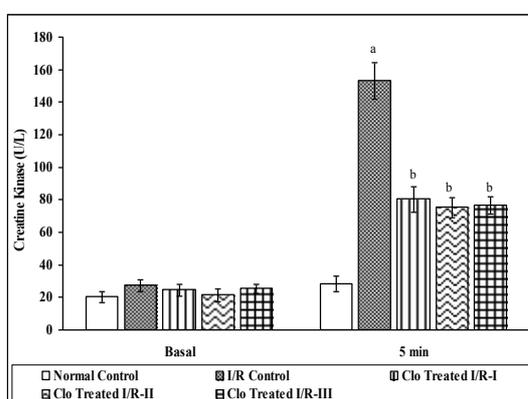
On the basis of the above discussion, it may be concluded that I/R-injury may formulate the heart susceptible to increased infarct size and enhanced oxidative stress. Clofibrate showed cardioprotection which may be attributed to its potent antioxidant effects. Further studies are under way in our laboratory to elucidate the mechanisms involved in the attenuation of myocardial injury by fibrates.

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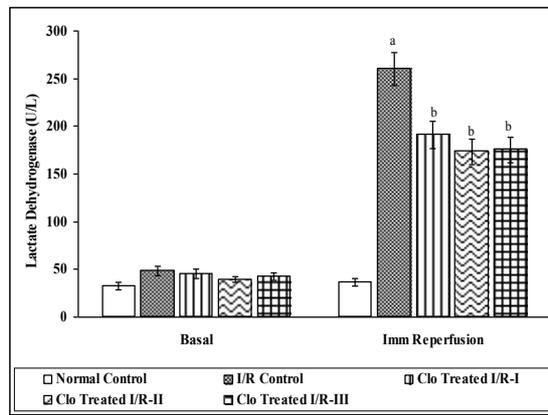
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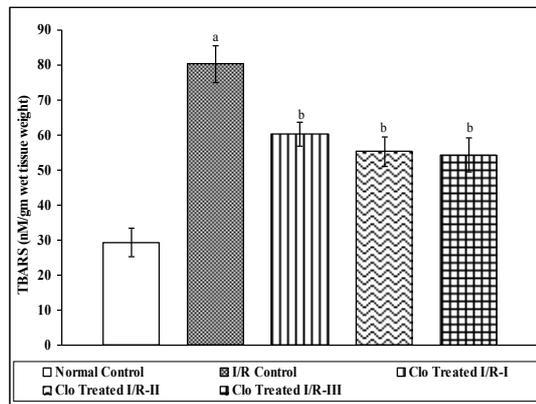
Effect of Clofibrate on increases in infarct size induced by ischemia-reperfusion (I/R). Values are expressed as mean \pm SD. a= P < 0.05 vs. normal control; b= P < 0.05 vs. I/R control. Clo Treated I/R-I= 100 mg/kg/day; Clo Treated I/R-II= 300 mg/kg/day; Clo Treated I/R-III= 500 mg/kg/day.



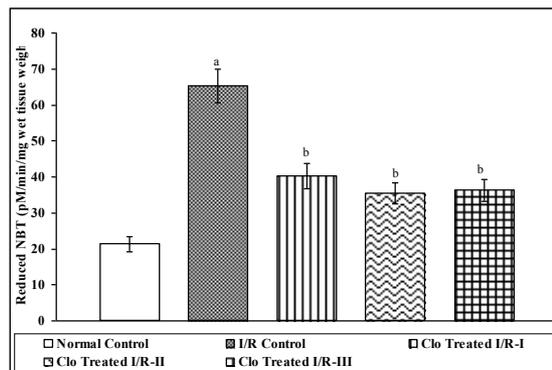
Effect of Clofibrate on increases in creatine kinase (CK) levels induced by ischemia-reperfusion (I/R). Values are expressed as mean \pm SD. a= P < 0.05 vs. normal control; b= P < 0.05 vs. I/R control. Clo Treated I/R-I= 100 mg/kg/day; Clo Treated I/R-II= 300 mg/kg/day; Clo Treated I/R-III= 500 mg/kg/day.



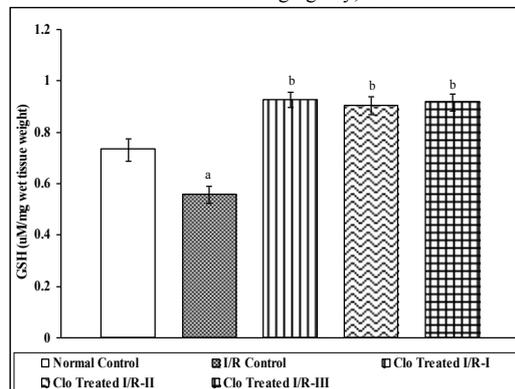
Effect of Clofibrate on increases in lactate dehydrogenase (LDH) levels induced by ischemia–reperfusion (I/R). Values are expressed as mean ± SD. a= P < 0.05 vs. normal control; b= P < 0.05 vs. I/R control. Clo Treated I/R-I= 100 mg/kg/day; Clo Treated I/R-II= 300 mg/kg/day; Clo Treated I/R-III= 500 mg/kg/day.



Effect of Clofibrate on increases in thiobarbituric acid reactive substance (TBARS) levels induced by ischemia–reperfusion (I/R). Values are expressed as mean ± SD. a= P < 0.05 vs. normal control; b= P < 0.05 vs. I/R control. Clo Treated I/R-I= 100 mg/kg/day; Clo Treated I/R-II= 300 mg/kg/day; Clo Treated I/R-III= 500 mg/kg/day.



Effect of Clofibrate on increases in superoxide anion levels induced by ischemia–reperfusion (I/R). Values are expressed as mean ± SD. a= P < 0.05 vs. normal control; b= P < 0.05 vs. I/R control. Clo Treated I/R-I= 100 mg/kg/day; Clo Treated I/R-II= 300 mg/kg/day; Clo Treated I/R-III= 500 mg/kg/day.



Effect of Clofibrate on decreases in reduced glutathione (GSH) levels induced by ischemia–reperfusion (I/R). Values are expressed as mean ± SD. a= P < 0.05 vs. normal control; b= P < 0.05 vs. I/R control. Clo Treated I/R-I= 100 mg/kg/day; Clo Treated I/R-II= 300 mg/kg/day; Clo Treated I/R-III= 500 mg/kg/day.

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