Fenofibrate Prevents Myocardial Ischemia-Reperfusion Injury in Rat Hearts

Singh Gurfateh*, Rohilla Ankur1, Khan M.U.2, Khanam Razia3

1Department of Pharmacy, NIMS University, Jaipur - Rajasthan, India
2Sri Sai College of Pharmacy, Badhani, Pathankot-Punjab, India
3Department of Pharmacy, Jamia Hamdard University, Delhi-India

ABSTRACT

The present study was designed to investigate the effect of Fenofibrate, a activator 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 were 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 along with increase in reduced glutathione levels in the heart. Fenofibrate afforded 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 Fenofibrate treatment. It may be concluded that the observed cardioprotective potential of Fenofibrate against I/R-induced myocardial injury was due to the reductions in infarct size and oxidative stress.

Key Words: Fenofibrate, PPAR-α, Ischemia-reperfusion injury, Oxidative stress

INTRODUCTION

The condition in which the heart tissue receives inadequate blood flow, followed by inadequate oxygen and nutrient supply is termed as myocardial ischemia [1]. The restoration of coronary blood flow to an ischemic myocardium prevents myocardial damage. However, reperfusion of the previously ischemic myocardium is often

Corresponding Author
Gurfateh Singh
Email- dr_sugg@yahoo.co.in
followed by detrimental changes in myocardial tissues, known as ischemia-reperfusion (I/R) injury. Various factors such as oxidative stress, intracellular calcium overload, apoptotic and necrotic myocytes death have been noted to be involved in the pathogenesis of I/R-induced myocardial injury. Peroxisome proliferator-activated receptors (PPARs) are the ligand-activated transcription factors belonging to the large superfamily of nuclear receptors. Fibrates are the synthetic agonists of PPAR-α, which is naturally activated by ligands such as free fatty acids and eicosanoids. PPAR-α is expressed at a high level in tissues exhibiting high rates of fatty acid beta-oxidation including liver, heart and kidney. Fibrates have been in clinical use in the prevention of hyperlipidemia for several decades but they have been recently reported to have beneficial effects on cardiovascular function. Fenofibrate, an activator of PPAR-α, is a well known hypolipidemic agent and is generally used to treat hypertriglyceridemia, hypercholesterolemia and mixed dyslipidemia. Moreover, fenofibrate have been well reported to upregulate the expression of eNOS (endothelial nitric oxide synthase) and thereby increase the NO production. The reduction in NO production and increase in oxidative stress in the vessel wall often lead to vascular endothelial dysfunction and fenofibrate has been noted to improve the endothelium-and NO-mediated vasodilatation in the aorta evidencing its cardioprotective potential. In addition, fenofibrate exerted cardioprotective effect against ischemia and improved NO-mediated response probably by enhancing antioxidant capacity of the vessel wall. Fenofibrate showed a protective effect in by decreasing plasma malondialdehyde and C-reactive protein levels. Administration of fenofibrate markedly attenuated the development oxidative stress and vascular inflammation evidencing its potent antioxidant potential in affording cardioprotection. 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 apparatus. 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 method [16]. 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.[17]. The superoxide anion generation was assessed by estimating the reduced nitro blue tetrazolium (NBT) using the method of Wang et al. [18]. Moreover, the reduced glutathione content in each heart was estimated using the method of Beutler et al. [19].

**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 (Feno Treated I/R- I):** The rat was given Fenofibrate (80mg/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 (Feno Treated I/R- II):** The rat was given Fenofibrate (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 V (Feno Treated I/R- III):** The rat was given Fenofibrate (150mg/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 ± 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.

**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 Fenofibrate on I/R-Induced Infarct size and Oxidative Stress**

Treatments with Fenofibrate 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 100 mg/kg.

In addition, Fenofibrate 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 100 mg/kg.

**DISCUSSION**

Coronary artery disease is a leading cause of morbidity and mortality and its prevalence is continuously increasing worldwide. Early reperfusion to an ischemic myocardium often results in detrimental changes in the myocardium which is referred to 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 \[^20, 21\]. 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 studies \[^22\].

Lipid peroxidation refers to the oxidative degradation of lipids during which 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 \[^23, 24\]. 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 are the ligands of the PPAR-α, which is expressed in a number of tissues, including the myocardium and the vessel wall. PPAR-α has been well documented to regulate the metabolism of lipoprotein and fatty acids, as well as the transcription of several factors involved in inflammatory response and oxidative stress\[^{6,7}\]. Fibrates have been in clinical use in the prevention of hyperlipidemia for several decades but they have been recently reported to have beneficial effects on cardiovascular function \[^{8, 9}\]. It has been suggested that fenofibrate exerted cardioprotective effect against ischemia and improved NO-mediated response that evidenced its cardioprotective potential. The present study investigated the cardioprotective potential of fenofibrate against I/R injury in rat hearts. Treatments with fenofibrate 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 100 mg/kg.

In addition, a number of studies have demonstrated modulatory role of fenofibrate against oxidative stress in order to mimic cardioprotection. Fenofibrate showed a protective effect against oxidative stress by decreasing plasma malondialdehyde and C-reactive protein levels \[^{14}\]. Administration of fenofibrate markedly attenuated the development of oxidative stress and vascular inflammation evidencing its potent antioxidant potential in affording cardioprotection\[^{12}\]. In addition, Fenofibrate has been shown to reduce oxidative stress and improve the integrity of vascular endothelium and enhance the generation and bioavailability of NO\[^{13}\]. The additional property of fenofibrate such as activation of eNOS and generation of NO in the vessels and consequent reduction in oxidative stress has been noted to play a key role in cardiopretction. This contention is supported by the results obtained in the present study that treatment with Fenofibrate (80 mg/kg, 100 mg/kg and 150 mg/kg), a PPARα 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 100 mg/kg.

On the basis of the above discussion, it may be concluded that I/R-injury modulates the heart more susceptible to increased infarct size and enhanced oxidative stress. Fenofibrate 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.

REFERENCES

Figure 1- Effect of Fenofibrate on increases in infarct size 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. Feno Treated I/R-I= 80 mg/kg/day; Feno Treated I/R-II= 100 mg/kg/day; Feno Treated I/R-III= 150 mg/kg/day.

Figure 2- Effect of Fenofibrate on increases in creatine kinase (CK) 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. Feno Treated I/R-I= 80 mg/kg/day; Feno Treated I/R-II= 100 mg/kg/day; Feno Treated I/R-III= 150 mg/kg/day.
**Figure 3** - Effect of Fenofibrate 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. Feno Treated I/R-I= 80 mg/kg/day; Feno Treated I/R-II= 100 mg/kg/day; Feno Treated I/R-III= 150 mg/kg/day.

**Figure 4** - Effect of Fenofibrate 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. Feno Treated I/R-I= 80 mg/kg/day; Feno Treated I/R-II= 100 mg/kg/day; Feno Treated I/R-III= 150 mg/kg/day.
Figure 5- Effect of Fenofibrate 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. Feno Treated I/R-I= 80 mg/kg/day; Feno Treated I/R-II= 100 mg/kg/day; Feno Treated I/R-III= 150 mg/kg/day.

Figure 6- Effect of Fenofibrate 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. Feno Treated I/R-I= 80 mg/kg/day; Feno Treated I/R-II= 100 mg/kg/day; Feno Treated I/R-III= 150 mg/kg/day.