Protective role of fibrates in cardiac ischemia/reperfusion

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INTRODUCTION

The process of restoring blood flow to the ischemic myocardium induces injury to the myocardium, the phenomenon termed as myocardial ischemia-reperfusion (I/R) injury. Various factors have been involved in the pathogenesis of I/R-induced myocardial injury that include oxidative stress, intracellular calcium overload, apoptotic and necrotic myocytes death. Fibrates, the synthetic agonists of the peroxisome proliferator-activated receptor-a (PPAR-a), are known to be conventional, effective, and well-tolerated agents in the management of dyslipidemias. At the molecular level, fibrates attach to PPAR-a and increase the expression of genes that are involved in fatty acid uptake, β-oxidation, and ω-oxidation. 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 superoxide dismutase and glutathione peroxidase levels accounting for its antioxidant effects. Furthermore, PPAR-a activation by clofibrate afforded cardioprotection by a mechanism involving increased eNOS (endothelial nitric oxide synthase) expression and thereby increased NO production. Fenofibrate, another activator of PPAR-a, has been well reported to possess potent antioxidant and cardioprotective potential as evidenced by an increase in the content of reduced glutathione and decrease in superoxide dismutase and glutathione peroxidase levels accounting for its antioxidant effects. In addition, fenofibrate exerted cardioprotective effect against ischemia and improved NO-mediated response by enhancing antioxidant capacity of the vessel wall. Furthermore, administration of fenofibrate markedly attenuated the development oxidative stress and vascular inflammation evidencing its potent antioxidant potential in affording cardioprotection. Therefore, the present study has been conducted to investigate the

Abstract

Prevention of myocardial injury has been considered as the most important therapeutic challenge of today. Fibrates, the agonists of the peroxisome proliferator-activated receptor (PPAR)-a receptor, have been regarded as potent therapeutic agents in this context. Hence, the present study has been designed to investigate the effect of fibrates, i.e., Clofibrate and Fenofibrate, the potent agonists PPAR-a, on ischemia-reperfusion (I/R)-induced myocardial injury. The isolated Langendorff-perfused rat hearts were subjected to global ischemia for 30 minutes followed by reperfusion for 120 minutes. Myocardial infarct size and the release of lactate dehydrogenase (LDH) and creatine kinase (CK) in coronary effluent have been conducted to assess the degree of cardiac injury. Moreover, the oxidative stress in the heart was assessed by measuring lipid peroxidation, superoxide anion generation, and reduced glutathione. Clofibrate and Fenofibrate 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 along with reduction in I/R-induced oxidative stress. It may be concluded that the observed cardioprotective potential of Clofibrate and Fenofibrate against I/R-induced myocardial injury was due to the reductions in infarct size and oxidative stress.

Key words: Clofibrate, fenofibrate, I/R injury, PPAR-a, oxidative stress

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cardioprotective effect of Clofibrate and Fenofibrate against I/R-induced myocardial injury in rat hearts.

MATERIALS AND METHODS

Drugs and Chemicals
The LDH and CK enzymatic estimation kits were purchased from Vital Diagnostics, Thane, Maharashtra, India. 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and nitro blue tetrazolium (NBT) were obtained from Loba Chem, Mumbai, India. Clofibrate, Fenofibrate, 1, 1, 3, 3-tetramethoxy propane (TMP), and reduced glutathione (GSH) were procured from Sigma-Aldrich, USA. All other reagents used in this study were of analytical grade.

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 to 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. The experimental protocol was approved by the Institutional Animal Ethics Committee of NIMS University, Jaipur (Registration No. 1302/ac/09/CPCSEA).

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 minutes by blocking the inflow of physiological solution and it was followed by perfusion for 120 minutes.

Experimental Protocol
Four groups of 8 to 10 animals each were employed in the present study. In all groups, each isolated perfused heart was allowed to stabilize for 10 minutes by perfusing with K-H solution [Figure 1].

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

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

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

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

Laboratory Assays
Myocardial infarct size was measured macroscopically using triphenyl tetrazolium chloride (TTC) staining using volume method. 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. The superoxide anion generation was assessed by estimating the reduced NBT. Moreover, the reduced glutathione content in each heart was estimated using the method of Beutler et al.

Statistical Analysis
The results were expressed as mean ± SD (standard deviation). The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey’s multiple-comparison test (Graph Pad prism software). 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 [Table 1]. Moreover, the global ischemia for 30 minutes followed by reperfusion for 120 minutes significantly increased LDH and CK release in the coronary effluent in rat hearts. Maximum release of
LDH was noted immediately after reperfusion [Table 1], while maximum release of CK was noted at 5 minutes of reperfusion [Table 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 [Table 2].

**Effect of Clofibrate and Fenofibrate on I/R-Induced Infarct Size and Oxidative Stress**

Treatments with Clofibrate (300 mg/kg i.p.) and Fenofibrate (100 mg/kg i.p.) 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 [Table 1]. In addition, Clofibrate (300 mg/kg i.p.) and Fenofibrate (100 mg/kg i.p.) 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 [Table 2].

**DISCUSSION**

Fibrates, commonly referred to as PPAR-α agonists, are a subfamily of the nuclear receptor superfamily that have been noted to be naturally activated by ligands such as free fatty acids and eicosanoids.\[^{17-19}\] PPAR-α expression is present in liver, heart, kidney, endothelium, and vascular smooth muscle. Fibrates have been in wide clinical use due to their potent hypolipidemic effect for several decades.

More recently, they have been reported to have beneficial effects on cardiovascular function.\[^{20}\] Myocardial ischemia is a restriction in blood supply with resultant damage or dysfunction of myocardial tissue. Myocardial reperfusion is the restoration of blood flow to an ischemic heart. I/R injury are the tissue damage caused when blood supply returns to the tissue after a period of ischemia. Lack of oxygen and nutrients from blood during ischemia creates a condition in which the restoration of circulation results in inflammation and oxidative damage leading to I/R-induced myocardial injury. The increase in infarct size and the release of LDH and CK are documented to be an index of I/R-induced myocardial injury.\[^{21}\] In the present study, 30 minutes of ischemia followed by 120 minutes 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 minutes of reperfusion—both findings in accordance with our earlier studies.\[^{22}\] Moreover, increases in lipid peroxidation and superoxide anion generation have been suggested as indicators of oxidative stress.\[^{23}\] 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. Additionally, GSH levels were decreased in rat hearts subjected to I/R which suggest the development of I/R-induced oxidative stress, which may be responsible for the noted I/R-induced myocardial injury. 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.

The present study investigated the cardioprotective potential of Clofibrate and Fenofibrate against I/R injury
in rat hearts. Treatments with Clofibrate (300 mg/kg) and Fenofibrate (100 mg/kg) 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. In addition, many studies have demonstrated protective effects of Clofibrate and Fenofibrate against oxidative stress in order to mimic cardioprotection. Treatment with Clofibrate has been reported to increase the reduced glutathione levels and decrease the activity of glutathione-S-transferase. Moreover, Clofibrate has been shown to inhibit superoxide dismutase and glutathione peroxidase activities accounting for its antioxidant effect. On the other hand, Fenofibrate showed protective effects against oxidative stress by decreasing plasma malondialdehyde and C-reactive protein levels. Administration of Fenofibrate attenuated the development of oxidative stress and vascular inflammation evidencing its potent antioxidant potential. Furthermore, Fenofibrate has been shown to reduce oxidative stress and improve the integrity of vascular endothelium and enhance the generation and bioavailability of NO, confirming its cardioprotective potential. These contentions are supported by the results obtained in the present study that treatment with Clofibrate (300 mg/kg) and Fenofibrate (100 mg/Kg) 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.

CONCLUSION

I/R injury leads to increased infarct size and enhanced oxidative stress. Clofibrate and Fenofibrate showed cardioprotection which may be attributed to their potent antioxidant potentials. Further studies in this direction are undergoing in our laboratory to explicate the mechanisms involved in the attenuation of myocardial injury by fibrates.

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