The reperfusion to previously ischemic myocardium induces detrimental changes in the myocardium resulting in inflammation, oxidative damage and cardiac dysfunction known as 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 injury was assessed by measuring myocardial infarct size alongwith release of lactate dehydrogenase (LDH) and creatine kinase (CK) in the coronary effluent. Additionally, the oxidative stress parameters were analyzed in the heart which 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 Simvastatin at different concentrations (3 µMol, 10 µMol and 30 µMol) 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, the high degree of oxidative stress produced as a result of I/R injury was noted to be reduced by Simvastatin treatment. It may be concluded that reductions in myocardial infarct size and oxidative stress may be responsible for the observed cardioprotective potential of Simvastatin against I/R-induced myocardial injury.

**KEY WORDS:** Simvastatin, HMG-CoA, Ischemia-reperfusion injury, Oxidative stress

**INTRODUCTION**

The reperfusion to previously ischemic myocardium induces detrimental changes in the myocardium resulting in inflammation, oxidative damage and cardiac dysfunction known as 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 injury was assessed by measuring myocardial infarct size alongwith release of lactate dehydrogenase (LDH) and creatine kinase (CK) in the coronary effluent. Additionally, the oxidative stress parameters were analyzed in the heart which 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 Simvastatin at different concentrations (3 µMol, 10 µMol and 30 µMol) afforded cardioprotection against I/R-induced myocardial injury. It has been widely accepted that oxidative stress plays an important role in producing lethal injury associated with myocardial I/R. Surprisingly, reactive oxygen species (ROS) produced at the onset of reperfusion has been noted to enhance the oxidative stress in heart which is known to cause the detrimental changes in heart. The HMG-CoA reductase inhibitors commonly known as statins, possess manifold favorable effects above and beyond that of cholesterol lowering in affording cardioprotection. Simvastatin, a potent HMG-CoA reductase inhibitor, has been well reported to be a potent cardioprotective agent due to its antioxidant properties. Simvastatin has been noted to prevent the leukocyotic and aortic productions of reactive oxygen species (ROS) alongwith inhibition of protein and lipid oxidation products such as thiobarbituric acid reactive oxygen species (TBARS) confirming its antioxidant potential. In addition, experimental studies have shown that treatment with simvastatin attenuated the oxidative stress and produced cardioprotection by decreasing malondialdehyde (MDA) levels and increasing the superoxide dismutase (SOD) and nitric oxide (NO) levels. Further, treatment with simvastatin reduced oxidative stress and infarction volume thereby ameliorating ischemic damage in rats that confirmed the cardioprotective potential of the drug. Moreover, experimental studies in rats have shown that the treatment with simvastatin decreased oxidative stress in diabetic-hypercholesterolemic rats further confirming its antioxidant potential. In addition, Simvastatin has been well reported to alleviate myocardial contractile dysfunction and lethal ischemic injury in isolated Langendorff-perfused rat heart model. Therefore, the present study was undertaken to investigate the cardioprotective effect of Atorvastatin 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 (TTCl) staining employing 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 nitro blue tetrazolium (NBT) using the method of Wang et al. Moreover, the reduced glutathione content in each heart was estimated using the method of Beutler et al.

**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 (Sim Treated I/R-3 μMol): After 10 min of stabilization, isolated normal rat heart was infused with Simvastatin (3 μMol) for 10 min. The heart was then subjected to 30 min of global ischemia followed by 120 min of reperfusion.

Group IV (Sim Treated I/R-10 μMol): After 10 min of stabilization, isolated normal rat heart was infused with Simvastatin (10 μMol) for 10 min. The heart was then subjected to 30 min of global ischemia followed by 120 min of reperfusion.

Group V (Sim Treated I/R-30 μMol): After 10 min of stabilization, isolated normal rat heart was infused with Simvastatin (30 μMol) for 10 min. The heart was then subjected to 30 min of global ischemia followed by 120 min of reperfusion.

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.

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. Simvastatin, 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 Simvastatin on I/R-Induced Infarct size and Oxidative Stress

Treatments with Simvastatin in different concentrations (3 μMol, 10 μMol and 30 μMol) 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 10 μMol.

In addition, Simvastatin treatments (3 μMol, 10 μMol and 30 μMol) 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 10 μMol.

DISCUSSION

Ischemic heart disease (IHD) represents the leading cause of morbidity and mortality worldwide whose prevalence is continuously increasing worldwide21. 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 heart17. 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 injury2. The increase in infarct size and the release of LDH and CK are documented to be an index of I/R-induced myocardial injury22,23. 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 studies24,25.

Increases in lipid peroxidation and superoxide anion generation have been suggested as indicators of oxidative stress26,27. 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. When oxygen is reintroduced during reperfusion, conversion of excess hypoxanthine to xanthine by xanthine oxidase results in the formation of ROS, including superoxide anions (O2·−), hydroxyl radicals (OH·), hydrogen peroxide (H2O2) and peroxinitrite (ONOO−)28,29. 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.

Statins, commonly known as HMG-CoA reductase inhibitors, have been widely accepted to possess various pleiotropic effects in a way to afford cardioprotection7. Simvastatin, a member of the statins, is a synthetic derivate of a fermentation product of Aspergillus terreus, initially marketed by Merck and Company under the trade name Zocor, that has been well reported to inhibit HMG-CoA reductase enzyme found in liver and show cardioprotection2. In addition, numerous studies have demonstrated Simvastatin to possess cardioprotective effects due to its potent antioxidative properties2.

Simvastatin reduced the activity of NADPH-CoQ reductase, an enzyme required in generation of free radicals that evidenced its potent role as an antioxidant3. Moreover, treatment with Simvastatin prevented the leukocytic and aortic productions of ROS along with inhibition of lipid oxidation products such as TBARS confirming its antioxidative potential4,5,6. Experimental studies have shown that treatment with simvastatin attenuated the oxidative and produced cardioprotection by decreasing MDA levels and increasing the SOD activity7,8. Moreover, experimental studies in rats have shown that the treatment with simvastatin decreased oxidative stress in diabetic-hypercholesterolemic rats further confirming its antioxidative potential9. This contention is supported by the results obtained in the present study that treatment with Simvastatin in different concentrations (3 μMol, 10 μMol and 30 μMol), has markedly reduced the oxidative stress in rat hearts subjected to I/R, as assessed in terms of reductions in TBARS and superoxide anion generation, and consequent increase in reduced glutathione levels, with maximum reductions at a concentration of 10 μMol.

In addition, a number of studies have demonstrated Simvastatin to reduce myocardial injury parameters in order to mimic cardioprotection. Treatment with simvastatin has been noted to significantly improve the endothelial function in mice9. Further, treatment with simvastatin reduced the infarction volume thereby ameliorating ischemic damage in rats that confirmed the cardioprotective potential of the drug. In addition, Simvastatin has been well reported to alleviate myocardial contractile dysfunction and lethal ischemic injury in isolated Langendorff-perfused rat heart model10,11,12,13,14,15. The present study investigated the cardioprotective potential of Simvastatin against I/R injury in rat hearts when administered at the onset of reperfusion. The data demonstrates that
administration of Simvastatin at the onset of reperfusion results in significant, dose-dependent cardioprotection, with optimal concentration ranges of 3 µMol, 10 µMol and 30 µMol with maximal protection at 30 µMol, which is in accordance with the earlier reports. Moreover, treatments with Simvastatin 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 10 µMol.

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. Simvastatin, due to its potent antioxidative effects, showed cardioprotection in rat hearts. Further studies are under way in our laboratory to elucidate the mechanisms involved in the attenuation of myocardial injury by statins.

REFERENCES


Figure 2. Effect of Simvastatin 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. Sim Treated I/R-I= 3 µMol; Sim Treated I/R-II= 10 µMol; Sim Treated I/R-III= 30 µMol.

Figure 3. Effect of Simvastatin 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. Sim Treated I/R-I= 3 µMol; Sim Treated I/R-II= 10 µMol; Sim Treated I/R-III= 30 µMol.

Figure 4. Effect of Simvastatin 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. Sim Treated I/R-I= 3 µMol; Sim Treated I/R-II= 10 µMol; Sim Treated I/R-III= 30 µMol.

Figure 5. Effect of Simvastatin 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. Sim Treated I/R-I= 3 µMol; Sim Treated I/R-II= 10 µMol; Sim Treated I/R-III= 30 µMol.
Figure 6. Effect of Simvastatin 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. Sim Treated I/R-I= 3 µMol; Sim Treated I/R-II= 10 µMol; Sim Treated I/R-III= 30 µMol.

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