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ORIGINAL RESEARCH ARTICLE

Atorvastatin Ameliorates Ischemia Reperfusion Injury in Rat Heart

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ABSTRACT

The present study was designed to investigate the effect of Atorvastatin, a 3-hydroxymethyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitor, 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 Atorvatstain (25 µM, 50 µM and 100 µM) 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 Atorvastatin treatment. It may be concluded that reductions in infarct size and oxidative stress may be responsible for the observed cardioprotective potential of Atorvastatin against I/R-induced myocardial injury.

Key words: Atorvastatin, HMG-C0A, Ischemia-reperfusion injury, Oxidative stress

INTRODUCTION

Ischemic heart disease (IHD) has been considered to be associated with high morbidity and mortality worldwide [1]. Ischemia-reperfusion (I/R) injury can be defined as the damage to cardiac tissues when blood supply is restored after a period of ischemia, resulting in oxidative damage, inflammation and cardiac dysfunction [2,3]. Oxidative stress, intracellular calcium overload, apoptotic and necrotic myocytes death have been implicated in the pathogenesis of I/R-induced myocardial injury [3,4]. It has been reported that reactive oxygen species (ROS) play an important role in producing lethal cell injury associated with myocardial I/R [5]. The production of ROS 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 [6]. The HMG-CoA reductase inhibitors commonly known as statins, possess multiple beneficial effects above and beyond that of cholesterol lowering in affording cardioprotection [7]. Atorvastatin, a potent HMG-CoA reductase inhibitor, has been reported to possess beneficial effects in the treatment of various cardiovascular diseases and multiple risk factors associated with myocardial infarction, stroke, unstable angina and revascularization due to its pleiotropic effects [8,9,10,11]. Various studies have reported Atorvastatin to reduce the infarct size in isolated Langendorff-perfused heart model by activating pro-survival kinases and niric oxide (NO) levels [12,13]. Moreover, studies have demonstrated atorvastatin to be a potent antioxidant that has a vital role in affording cardioprotection. Atorvastatin treatment has been noted to reduce thiobarbituric acid reactive oxygen substances (TBARS) levels and lipid peroxidation levels, the oxidative stress markers that further evidenced its antioxidant action in affording cardioprotection

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[14]. In addition, treatment with atorvastatin has been reported to reduce vascular and cardiac free radical formation, normalize the expression of the NADPH oxidase and thus show anti-oxidative properties [11,15]. 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 [16]. 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; $CaCl_2$ 2.5 mM; $MgSO_4.7H_2O$ 1.2 mM; $NaHCO_3$ 25 mM; KH_2PO_4 1.2 mM; $C_6H_{12}O_6$ 1 mM) pH 7.4, maintained at 37 °C and bubbled with 95% O_2 and 5% CO_2. 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 [17]. 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. [18]. The superoxide anion generation was assessed by estimating the reduced nitro blue tetrazolium (NBT) using the method of Wang et al. [19]. Moreover, the reduced glutathione content in each heart was estimated using the method of Beutler et al. [20].

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 (Ator Treated I/R-25 µM): After 10 min of stabilization, isolated normal rat heart was infused with Atorvastatin (25 µM) for 10 min. The heart was then subjected to 30 min of global ischemia followed by 120 min of reperfusion.

Group IV (Ator Treated I/R-50 µM): After 10 min of stabilization, isolated normal rat heart was infused with Atorvastatin (50 µM) for 10 min. The heart was then subjected to 30 min of global ischemia followed by 120 min of reperfusion.

Group V (Ator Treated I/R-100 µM): After 10 min of stabilization, isolated normal rat heart was infused with Atorvastatin (100 µM) 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, Maharastra, India. DTNB and NBT were obtained from Loba Chem, Mumbai, India. Atorvastatin, 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).

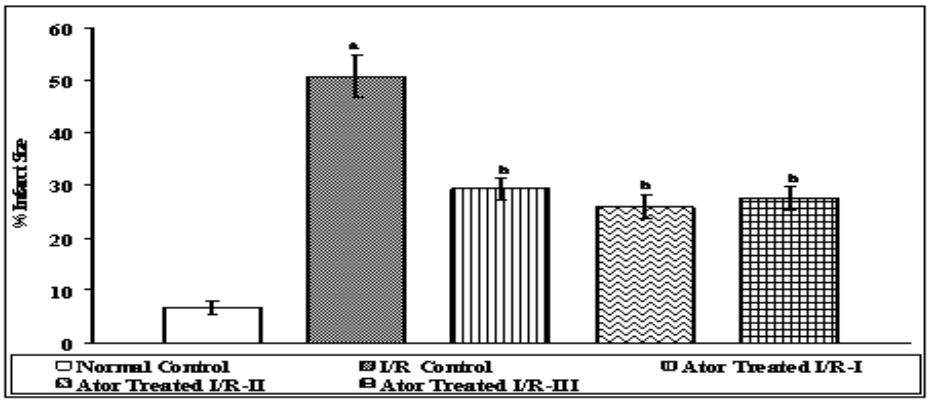
Lipid peroxidations, measured in terms of TBARS, and superoxide anion generation, assessed in terms of reduced NBT, were significantly increased in rat hearts subjected to I/R. Moreover, the levels of reduced GSH were found to be decreased in the rat hearts subjected to I/R that may be attributed to the enhanced oxidative stress in I/R-induced myocardial injury (Fig 4,5&6).

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

Treatments with Atorvastatin in different concenterations (25 µM, 50 µM and 100 µM) 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 1,2&3). However, maximum cardioprotection was noted at a concentration of 50 µM.

In addition, Atorvastatin treatments (25 µM, 50 µM and 100 µM) 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 4,5&6). However, maximum reduction of I/R-induced oxidative stress was noted at a concentration of 50 µM.

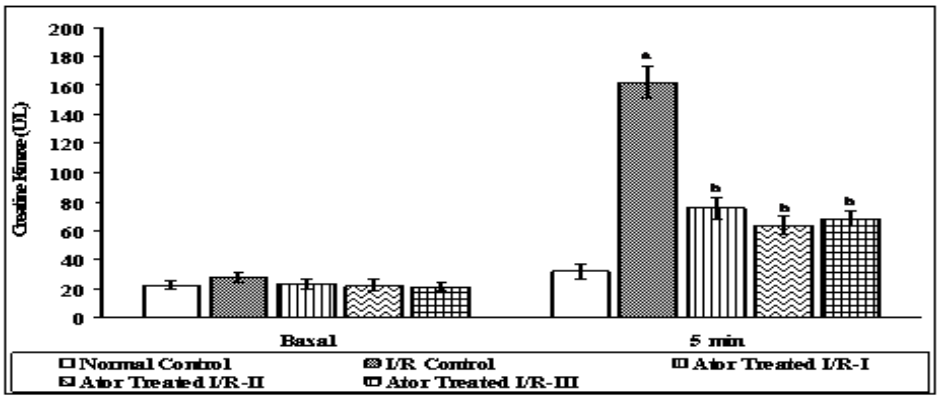
Fig 1: Effect of Atorvastatin on increases in infarct size induced by ischemia–reperfusion (I/R).



Values are expressed as mean ± SD.

a= P < 0.05 vs. normal control; b= P < 0.05 vs. I/R control. Ator Treated I/R-I= 25µM; Ator Treated I/R-II= 50 µM; Ator Treated I/R-III= 100 µM.

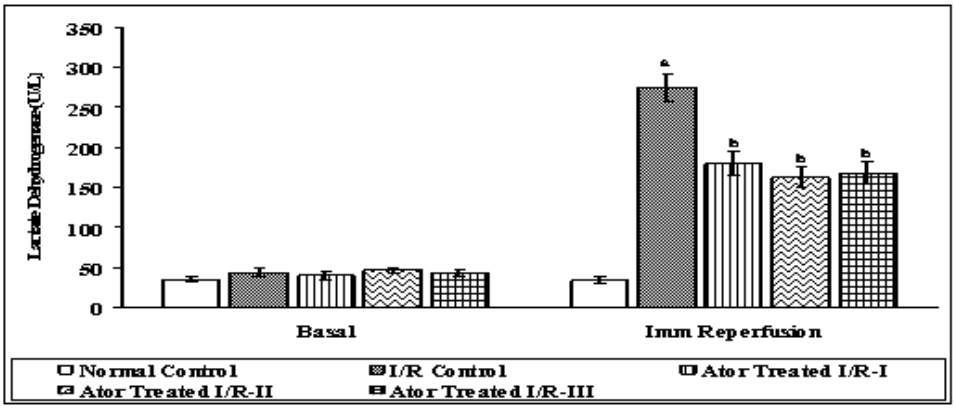
Fig 2: Effect of Atorvastatin on increases in creatine kinase (CK) levels induced by ischemia–reperfusion (I/R).



Values are expressed as mean ± SD.

a= P < 0.05 vs. normal control; b= P < 0.05 vs. I/R control. Ator Treated I/R-I= 25µM; Ator Treated I/R-II= 50 µM; Ator Treated I/R-III= 100 µM.

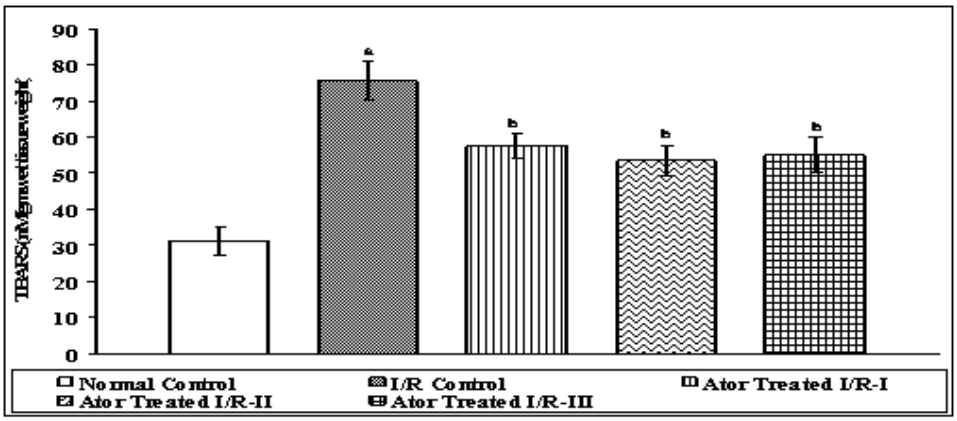
Fig 3: Effect of Atorvastatin on increases in lactate dehydrogenase (LDH) levels induced by ischemia–reperfusion (I/R).



Values are expressed as mean ± SD.

a= P < 0.05 vs. normal control; b= P < 0.05 vs. I/R control. Ator Treated I/R-I= 25µM; Ator Treated I/R-II= 50 µM; Ator Treated I/R-III= 100 µM.

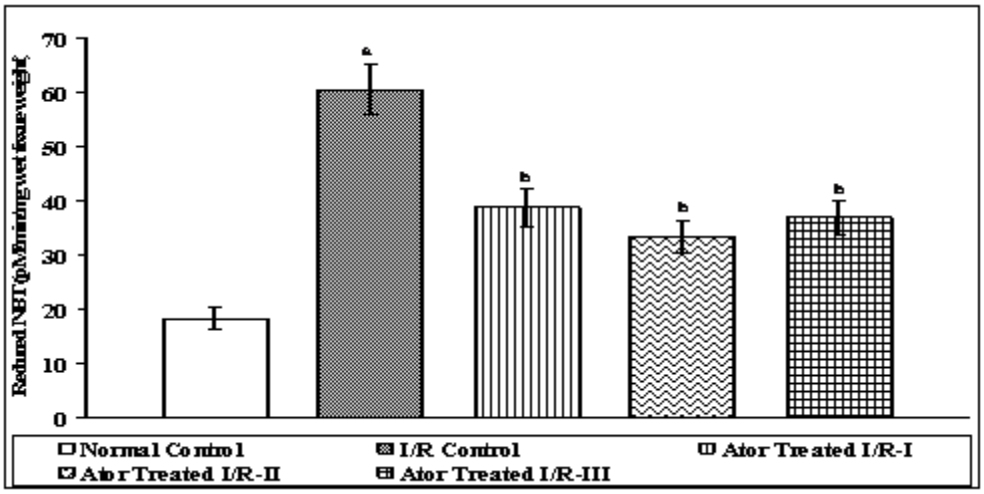
Fig 4: Effect of Atorvastatin on increases in thiobarbituric acid reactive substance (TBARS) levels induced by ischemia–reperfusion (I/R).



Values are expressed as mean ± SD.

a= P < 0.05 vs. normal control; b= P < 0.05 vs. I/R control. Ator Treated I/R-I= 25µM; Ator Treated I/R-II= 50 µM; Ator Treated I/R-III= 100 µM.

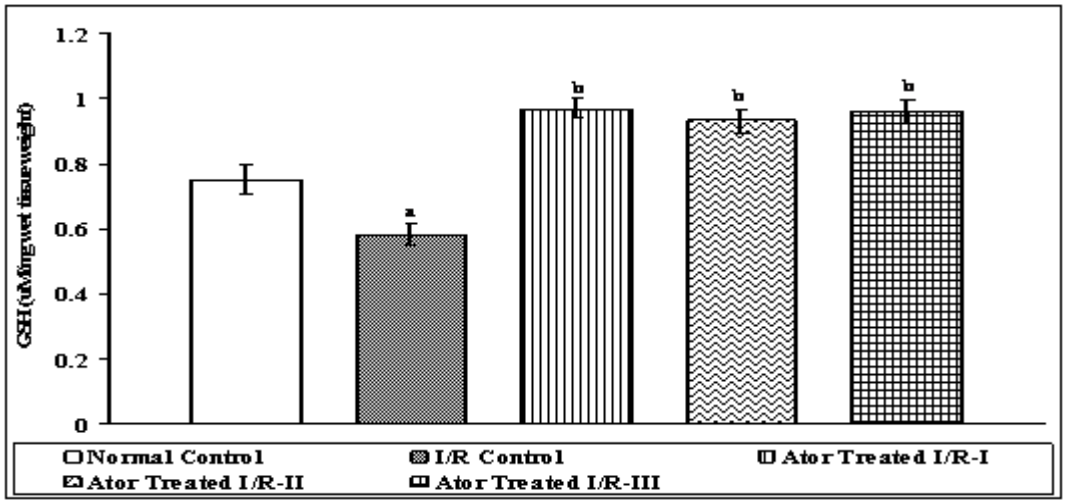
Fig 5: Effect of Atorvastatin on increases in superoxide anion levels induced by ischemia–reperfusion (I/R).



Values are expressed as mean ± SD.

a= P < 0.05 vs. normal control; b= P < 0.05 vs. I/R control. Ator Treated I/R-I= 25µM; Ator Treated I/R-II= 50 µM; Ator Treated I/R-III= 100 µM.

Fig 6: Effect of Atorvastatin 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. Ator Treated I/R-I= 25µM; Ator Treated I/R-II= 50 µM; Ator Treated I/R-III= 100 µM.

DISCUSSION

IHD represents the leading cause of morbidity and mortality worldwide whose prevalence is continuously increasing worldwide [1]. 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 [2,3]. 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 [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 [21,22]. 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 [23,24].

Increases in lipid peroxidation and superoxide anion generation have been suggested as indicators of oxidative stress [25,26]. 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 (O^{2-}), hydroxyl radicals (OH^{-}), hydrogen peroxide (H_2O_2) and peroxinitrite ($ONOO^{-}$) [3,4,6]. 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 cardioprotection [7]. Atorvastatin, sold by Pfizer under the trade name Lipitor, is a potent member of the statins class that has been well reported to inhibit HMG-CoA reductase enzyme found in liver and show cardioprotection [11]. Various studies have reported Atorvastatin to reduce the infarct size in isolated Langendorff-perfused heart model by activating pro-survival kinases such as phosphatidyl inositol 3-kinase/protein kiase B (PI3-Akt) and increasing NO levels [12,13]. The present study investigated the cardioprotective potential of atorvastatin against I/R injury in rat hearts when administered at the onset of reperfusion. The data demonstrates that atorvastatin administered as an adjunct to reperfusion results in significant, dose-dependent cardioprotection, with optimal concentration ranges of 25 μM, 50 μM and 100 μM with maximal protection at 50 μM, which is in accordance with the earlier reports[12,13]. Moreover, treatments with atorvastatin 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 50 μM.

In addition, numerous studies have demonstrated atorvastatin to possess protective effects against oxidative stress in order to mimic cardioprotection. Atorvastatin treatment has been noted to reduce the oxidative stress markers such as TBARS and lipid peroxidation levels, confirming its antioxidant action in affording cardioprotection [14]. Moreover, treatment with atorvastatin has been reported to reduce vascular and cardiac free radical formation, normalize the expression of the NADPH oxidase and thus show anti-oxidative properties [11,15]. Atorvastatin has been reported to induce a significant decrease in malondialdehyde (MDA) levels alongwith a significant increase of superoxide dysmutase (SOD) activity that accounts for its cardioprotective and antioxidant action [27]. This contention is supported by the results obtained in the present study that treatment with Atorvastatin (25 μM, 50 μM and 100 μM), a selective inhibitor of HMG-CoA 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 50 μM.

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. Atorvastatin, due to its pleiotropic effects, have shown 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 statins.

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