# Cardioprotective potential of simvastatin in the hyperhomocysteinemic rat heart

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## Abstract

The present study investigated the probable role of simvastatin, 3-hydroxymethyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitor, in abrogated cardioprotection in hyperhomocysteinemic (Hhcy) rat hearts. Isolated Langendorff's perfused normal and Hhcy rat hearts were subjected to 30-min global ischemia (I) followed by 120-min reperfusion (R). Assessment of myocardial damage was done by measuring infarct size and analyzing the release of lactate dehydrogenase (LDH) and creatine kinase (CK-MB) in coronary effluent. In addition, the oxidative stress in the heart was assessed by measuring lipid peroxidation and superoxide anion generation. I/R produced myocardial injury in normal and Hhcy rat hearts by increasing myocardial infarct size, LDH and CK in coronary effluent and oxidative stress. Hhcy rat hearts showed enhanced myocardial injury and high oxidative stress as compared to normal hearts. Treatment with Simvastatin (10 $\mu$Mol) afforded cardioprotection against I/R-induced myocardial injury in normal and hyperhomocysteinemic rat hearts as assessed in terms of reductions in myocardial infarct size, LDH and CK levels in coronary effluent and oxidative stress. The reductions in the high degree of oxidative stress may be responsible for the observed cardioprotection afforded by simvastatin against I/R-induced myocardial injury in normal and hyperhomocysteinemic rat hearts.

**Key words:** Hyperhomocysteinemia, oxidative stress, simvastatin

## INTRODUCTION

I/R injury may be defined as the damage to myocardial tissue when blood supply is restored after a period of ischemia, resulting in oxidative damage, inflammation, intracellular calcium overload, apoptotic and necrotic myocytes death and cardiac dysfunction.[1-4] Hhcy, a condition of elevated serum homocysteine concentration, is considered as an independent risk factor for various cardiovascular disorders such as atherosclerosis, endothelial dysfunction, hypertension, myocardial infarction and chronic heart failure.[5,6] Hhcy has been well reported to enhance the generation of reactive oxygen species (ROS), decrease endothelail nitric oxide synthase (eNOS) expression and consequently reduces the generation of NO to produce cardiac dysfunction.[7-9] Statins, the HMG-CoA reductase inhibitors, have been regarded as potent hypolipidemic agents that accounts for their role in reducing cardiovascular mortality and morbidity.[10,11] Simvastatin, a potent inhibitor of HMG-CoA reductase, has been well reported to be a potent cardioprotective agent due to its antioxidant properties.[12] Simvastatin has been noted to prevent aortic production of ROS. Moreover, simvastatin showed inhibitions of protein and lipid oxidation products such as thiobarbituric acid reactive oxygen species (TBARS) confirming its antioxidant potential.[13] In addition, experimental studies have shown that treatment with simvastatin resulted in reductions of malondialdehyde (MDA) levels and increases in the superoxide dysmutase (SOD) and NO levels accounting for its cardioprotective potential.[14] In addition, simvastatin has been reported to lessen myocardial contractile dysfunction and lethal ischemic injury in isolated Langendorff-perfused rat heart model.[15,16] Therefore, the present study was undertaken to investigate the cardioprotective effect of

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simvastatin against I/R-induced myocardial injury in hyperhomocysteinemic rat hearts.

## MATERIALS AND METHODS

### 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. Simvastatin and 1,1,3,3-tetramethoxy propane 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 175-225 gm 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.[17] 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,[18] 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.

### Experimental Protocol

Eight groups each consisting of eight to ten animals 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 [Figure 1].

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-Control): 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 per se Normal Control): After 10-min stabilization, the isolated normal rat heart was infused with Simvastatin (10 μMol) for 10 min. Then the heart was perfused for 150 min using K-H solution.

Group IV (Sim-treated I/R-Control): 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 (Hhcy control): The isolated Hhcy rat heart was perfused for 150 min using K-H solution after 10-min stabilization.

Group VI (Hhcy-I/R control): Isolated Hhcy rat heart after 10 min of stabilization was subjected to 30 min of global ischemia followed by 120 min of reperfusion.

Group VII (Sim per se Hhcy-control): After 10-min stabilization, the isolated Hhcy rat heart was infused with simvastatin (10 μMol) for 10 min. Then the heart was perfused for 150 min using K-H solution.

Group VIII (Sim-treated Hhcy-I/R Control): After 10 min of stabilization, isolated Hhcy 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.

### Laboratory Assays

Myocardial infarct size was measured macroscopically using triphenyl tetrazolium chloride (TTC) staining employing volume method.[19] The myocardial injury was assessed by measuring the release of LDH and CK-MB in the coronary effluent using the commercially available enzymatic kits (Vital Diagnostics, Thane, Maharashtra, India). The level of TBARS, an index of lipid peroxidation in

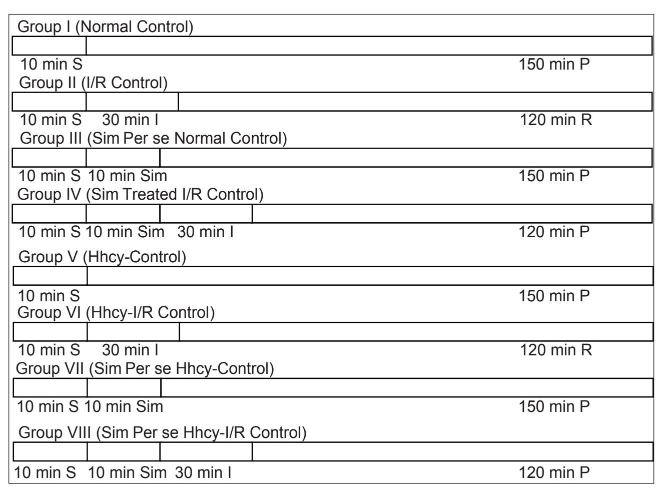


**Figure 1:** Diagram showing schematic representation of experimental protocol

the heart was estimated according to the method of Ohkawa *et al*.[20] The superoxide anion generation was assessed by estimating the reduced nitroblue tetrazolium (NBT) using the method of Wang *et al*.[21]

### Statistical Analysis

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

## RESULTS

Rats fed with L-methionine (1.7 g/kg/day, p.o.) for 4 weeks via oral gavage produced hyperhomocysteinemia (22.15±1.85 μM/L) when compared with normal rats (4.31±0.56 μM/L). In addition, L-methionine administration did not produce mortality in rats.

### Effect of Simvastatin in I/R-induced Myocardial Injury in normal and Hyperhomocysteinemic Rat Hearts

Global ischemia followed by reperfusion significantly increased LDH and CK release in the coronary effluent in normal and hyperhomocysteinemic rat hearts. Maximum release of LDH was noted immediately after reperfusion, while maximum release of CK was noted at 5 min of reperfusion [Figures 2 and 3]. In addition, I/R was noted to increase the infarct size in normal and hyperhomocysteinemic rat hearts [Figure 4]. Hyperhomocysteinemic rat hearts showed enhanced myocardial injury when compared with normal rat hearts subjected to I/R. Treatment with simvastatin (10 μMol) significantly attenuated I/R-induced myocardial injury in normal and hyperhomocysteinemic rat hearts, as assessed in terms of reduction in myocardial infarct size and decreased release of LDH and CK in coronary effluent [Figures 2-4].

### Effect of simvastatin in I/R-induced oxidative stress in normal and hyperhomocysteinemic rat hearts

Lipid peroxidations, measured in terms of TBARS, and superoxide anion generation, assessed in terms of reduced NBT, were significantly increased in normal and hyperhomocysteinemic rat hearts subjected to I/R [Figures 5 and 6]. In addition, hyperhomocysteinemic rat hearts showed high oxidative stress when compared with normal rat hearts subjected to I/R. Simvastatin treatment (10 μMol) attenuated I/R-induced oxidative stress in normal and hyperhomocysteinemic rat hearts, as assessed in terms of reduction in TBARS and superoxide anion generation [Figures 5 and 6].

## DISCUSSION

Cardiovascular diseases correspond to the leading cause of morbidity and mortality whose incidence is continuously increasing worldwide.[22,23] The myocardial, vascular or electrophysiological dysfunction induced by the restoration of blood flow to previously ischemic tissue refers to I/R injury, the manifestations of which include reperfusion arrhythmias, endothelial cell damage leading to microvascular dysfunction, myocardial stunning, myocyte death and infarction.[24,25] The increase in infarct size and the release of LDH and CK have been well reported to be an index of I/R-induced myocardial injury.[26,27] In the present study, 30 min of ischemia followed by 120 min of reperfusion produced 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 peak release of CK was observed after 5 min of reperfusion whereas the maximal release of LDH was noted immediately after reperfusion, which are in accordance with the earlier reports.[28,29] Moreover, increase in lipid peroxidation and superoxide anion generation have been reported to be

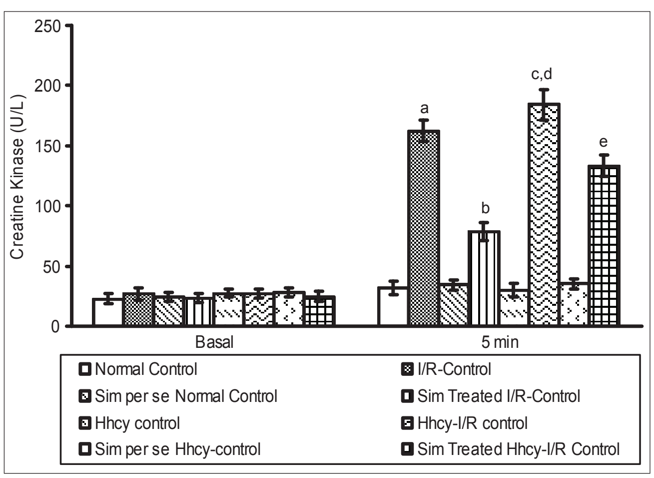


**Figure 2:** Effect of simvastatin in I/R-induced increase in CK levels Values are expressed as mean ± S.D. a = *P*< 0.05 vs Normal Control; b = *P*< 0.05 vs I/R Control; c = *P*<0.05 vs Hhcy-Control; d = *P*< 0.05 vs I/R control; e = *P*<0.05 vs Hhcy-IR Control.

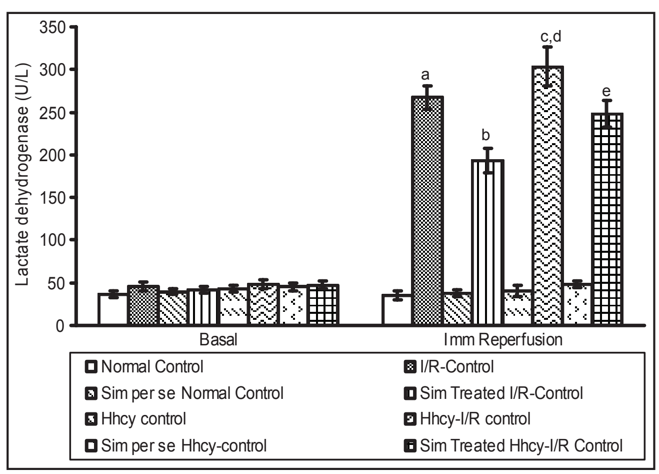


**Figure 3:** Effect of simvastatin in I/R-induced increase in LDH levels Values are expressed as mean ± S.D. a = *P*< 0.05 vs Normal Control; b = *P*< 0.05 vs I/R Control; c = *P*<0.05 vs Hhcy-Control; d = *P*< 0.05 vs I/R control; e = *P*<0.05 vs Hhcy-IR Control.

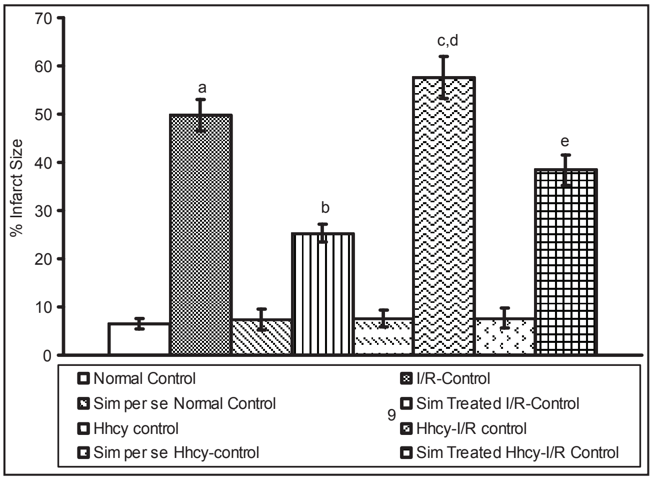


**Figure 4:** Effect of simvastatin in I/R-induced increase in infarct size Values are expressed as mean ± S.D. a = p< 0.05 vs normal control; b = *P*< 0.05 vs I/R Control; c = *P*<0.05 vs Hhcy-Control; d = *P*< 0.05 vs I/R control; e = *P*<0.05 vs Hhcy-IR Control.

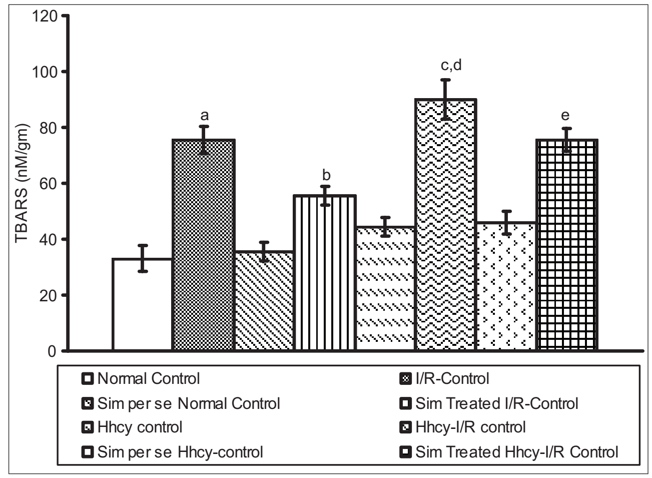


**Figure 5:** Effect of simvastatin on I/R-induced increase in TBARS levels. Values are expressed as mean ± S.D. a = *P*< 0.05 vs Normal Control; b = *P*< 0.05 vs I/R Control; c = *P*<0.05 vs Hhcy-Control; d = *P*< 0.05 vs I/R control; e = *P*<0.05 vs Hhcy-IR Control.

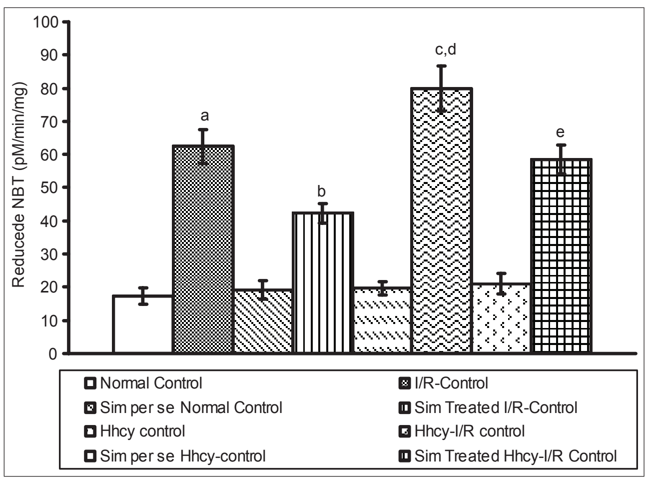


**Figure 6:** Effect of simvastatin on I/R-induced increase in superoxide anion generation Values are expressed as mean ± S.D. a = *P*< 0.05 vs Normal Control; b = *P*< 0.05 vs I/R Control; c = *P*<0.05 vs Hhcy-Control; d = *P*< 0.05 vs I/R control; e = *P*<0.05 vs Hhcy-IR Control.

the indicators of oxidative stress.[30,31] 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. These indicators suggest the development of I/R-induced oxidative stress, which may be responsible for the noted I/R-induced myocardial injury.

Administration of L-methionine (1.7 g/kg/day orally) in rats for 4 weeks produced Hhcy.[29] In the present study, a marked increase in infarct size and release of LDH and CK were noted in the hyperhomocysteinemic rat heart when compared with the normal rat heart. Hhcy has been noted to downregulate NO bioavailability by accumulating asymmetric dimethylarginine, which is an endogenous inhibitor of eNOS.[32] In addition, Hhcy has been reported to produce high oxidative stress in the heart by activating NADPH oxidase-mediated ROS generation.[33] Hhcy-induced oxidative stress may occur as a result of decreased expression and activity of key antioxidant enzymes, as well as increased enzymatic generation of superoxide anion.[34] Thus, development of high degree of oxidative stress may be responsible for the observed marked increase in myocardial injury in the hyperhomocysteinemic rat heart. This contention is supported by the fact that a marked increase in lipid peroxidation and superoxide anion generation were noted in hyperhomocysteinemic rat hearts when compared with normal rat hearts.

Statins, commonly referred to as HMG-CoA reductase inhibitors, have been widely accepted to possess various pleiotropic effects in order to afford cardioprotection.[35,36] Simvastatin, a potent member of statins, has been well reported to inhibit HMG-CoA reductase and show cardioprotection.[37] Numbers of studies have demonstrated simvastatin to reduce myocardial injury parameters in order to mimic cardioprotection. Treatment with simvastatin has been noted to improve endothelial function in mice.[38] Moreover, simvastatin reduced the infarction volume and ameliorated the ischemic damage in rats that further confirmed its cardioprotective potential. Additionally, various studies have reported that Simvastatin lessened myocardial contractile dysfunction and lethal ischemic injury in isolated Langendorff-perfused rat heart model.[15,16,39,40] The present study investigated the cardioprotective potential of simvastatin against I/R injury in normal and hyperhomocysteinemic rat hearts when administered at the onset of reperfusion. The data demonstrates that administration of Simvastatin (10 μMol) at the onset of reperfusion results in significant attenuation of I/R-induced myocardial injury in normal and hyperhomocysteinemic rat hearts as assessed in terms of reductions in myocardial infarct size and decreased release of LDH and CK in coronary effluent, which is in accordance of our earlier reports.[37]

Further, numerous studies have reported that simvastatin possesses cardioprotective effects due to its potent antioxidant properties.[12] In support, simvastatin has been noted to reduce the activity of NADPH-CoQ reductase, an enzyme required in generation of free radicals that evidenced its potent role as an antioxidant.[41] Moreover, treatment with simvastatin prevented the aortic productions of ROS along with inhibition of lipid oxidation products such as TBARS. In addition, experimental studies have shown that treatment with Simvastatin decreased MDA levels and increased the SOD activity.[14,42] Furthermore, another experimental study in rats showed that treatment with simvastatin decreased oxidative stress in diabetic-hypercholesterolemic rats that further confirmed its antioxidant potential.[43] This contention is supported by the results obtained in the present study that treatment with simvastatin (10 μMol), markedly reduced the oxidative stress in normal and hyperhomocysteinemic rat hearts subjected to I/R, as assessed in terms of reductions in TBARS and superoxide anion generation.

## CONCLUSIONS

On the basis of the above discussion, it may be concluded that I/R-injury prepares the myocardium susceptible to increased infarct size and enhanced oxidative stress. Simvastatin, due to its potent antioxidant effects, showed cardioprotection in normal and hyperhomocysteinemic rat hearts. Further studies are going on in our laboratory to reveal various mechanisms possessed by statins in attenuating myocardial injury in normal and hyperhomocysteinemic rat hearts.

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