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A comparative study on the cardioprotective potential of atorvastatin and simvastatin in hyperhomocysteinemic rat hearts

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ABSTRACT

The present study has been deliberated in order to compare the cardioprotective potential of 3-hydroxymethyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors, Atorvastatin and Simvastatin in hyperhomocysteinemic rat hearts. L-methionine (1.7 g/kg/day orally) was administered to rats for 4 weeks to produce experimental hyperhomocysteinemia (Hhcy). Isolated Langendorff-perfused normal and hyperhomocysteinemic rat hearts were subjected to global ischemia for 30 min followed by reperfusion for 120 min. The extent of myocardial damage was assessed in terms of myocardial infarct size using triphenyltetrazolium chloride (TTC) staining, and release of creatine kinase (CK) and lactate dehydrogenase (LDH) in the coronary effluent; whereas the oxidative stress in the heart was assessed by measuring lipid peroxidation, superoxide anion generation and reduced glutathione. Ischemia-reperfusion (I/R) was noted to produce myocardial injury in normal and hyperhomocysteinemic rat hearts, assessed in terms of increase in myocardial infarct size, LDH and CK in coronary effluent and oxidative stress. Treatment with Atorvatstain (50 μM) and Simvastatin (10 μM) 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. It may be concluded that reductions in the high degree of oxidative stress may be responsible for the observed cardioprotective potential of Atorva-and Simvastatin, and both statins can be used interchangeably to afford cardioprotection against I/R-induced myocardial injury in normal and hyperhomocysteinemic rat hearts.

1. Introduction

The initial ischemic damage followed by further damage induced by reperfusion is referred to as myocardial I/R injury (Qian et al., 2014). It is believed that I/R-induced myocardial injury is related to the increased reactive oxygen species generation, calcium overloading, apoptotic and necrotic myocytes death, and the loss of membrane phospholipids, especially during reperfusion (Ferdinandy et al., 2007; Balakumar et al., 2008; Rohilla et al., 2011). Hhcy, a pathological condition characterized by abnormally high levels of homocysteine in the blood, is considered as an independent risk factor for various cardiovascular diseases like atherosclerosis, endothelial dysfunction, hypertension, myocardial infarction, chronic heart failure and obesity (Thiengburanatham, 2009; Ciaccio and Bellia, 2010; Baszczuk et al., 2014; Zhou et al., 2014). Additionally, Hhcy has been well reported to increase the generation of reactive oxygen species, downregulate endothelial nitric oxide synthase (eNOS), reduce the generation of nitric oxide (NO), and increase the production of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), which may affect the function of coronary endothelium (Suematsu et al., 2007; Bogdanski et al., 2008). Statins, the HMG-CoA reductase inhibitors, have been extensively used for the treatment of hyperlipidemia (Law et al., 2003). Atorvastatin and Simvastatin, the potent inhibitors of HMG-CoA reductase, have been well reported to possess various beneficial effects in the treatment of various cardiovascular diseases such as myocardial infarction, stroke, unstable angina and revascularization due to their potent antioxidant properties (Law et al., 2003; DiNicolantonio et al., 2013; Owens et al., 2014). Atorvastatin treatment reduced the infarct size in isolated Langendorff-perfused heart model by activating pro-survival kinases and NO levels (Bell and Yellon, 2003; Efthymiou and Yellon, 2005). In addition, Simvastatin has been reported to minimize the myocardial contractile dysfunction and lethal ischemic injury in isolated Langendorff-perfused rat heart model (Zheng and Hu, 2006; Yin et al., 2007). Moreover, treatment with

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Atorvastatin reduced the thiobarbituric acid reactive oxygen substances (TBARS) levels and lipid peroxidation levels, the oxidative stress markers, which further evidenced the antioxidant potential of it in affording cardioprotection (Ozacmak et al., 2007). Also, 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 antioxidant and cardioprotective potential (Delbosc et al., 2002). Furthermore, Atorvastatin treatment reduced the vascular and cardiac free radical formation, and normalized the NADPH oxidase expression (Bolayirli et al., 2007). Treatment with Simvastatin prevented the aortic production of reactive oxygen species and inhibited the lipid peroxidation products like TBARS, confirming its antioxidant potential in affording cardioprotection (Naiyra et al., 2010). Therefore, the present study has been undertaken in order to compare and investigate the cardioprotective effect of Statins, i.e. Atorvastatin and Simvastatin against I/R-induced myocardial injury in normal and hyperhomocysteinemic rat hearts.

2. Materials and methods

2.1. 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 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.

2.2. Isolated rat heart preparation

Rats were heparinized (500 IU i.p.) and killed by stunning. The heart was rapidly excised and immediately mounted on a Langendorff apparatus (Langendorff, 1895). 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 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.

2.3. Laboratory assays

Myocardial infarct size was measured macroscopically using the TTC staining employing volume method (Parikh and Singh, 1999). The myocardial injury was assessed by measuring the release of CK-MB and LDH 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 the heart was estimated according to the method of Ohkawa et al. (1979). The superoxide anion generation was assessed by

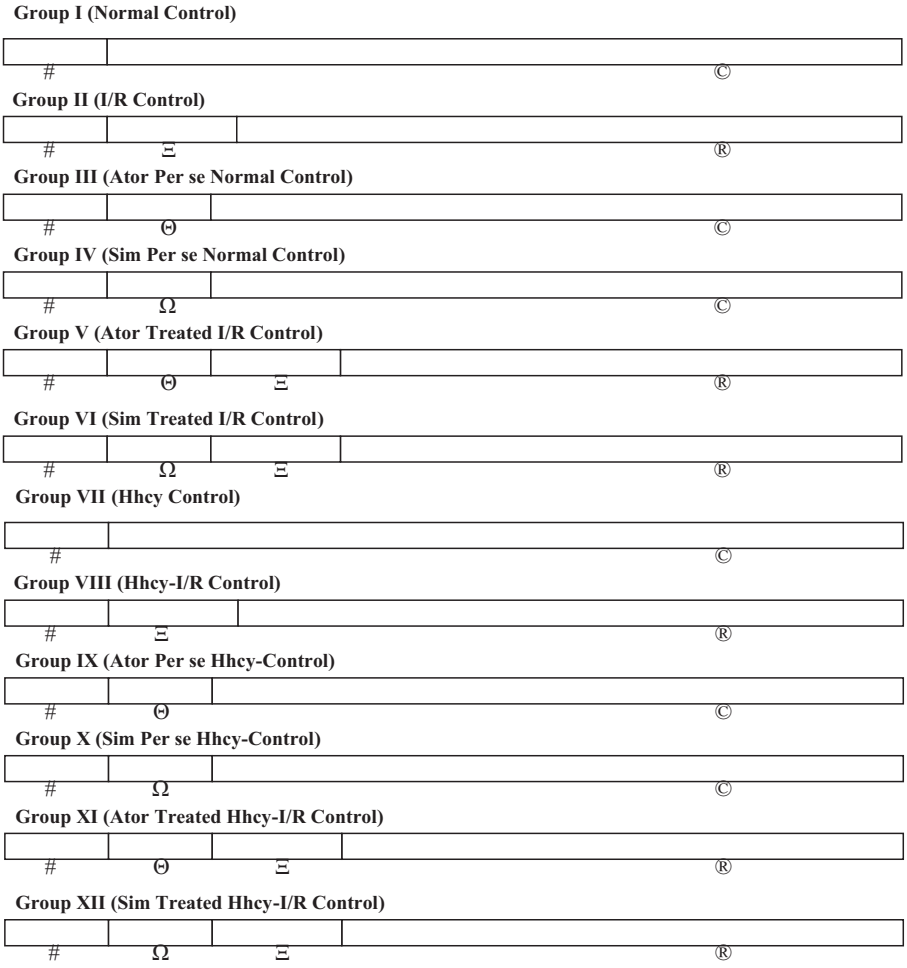

Fig. 1. Diagram showing schematic representation of experimental protocol. # represents 10 min stabilization; © represents 150 min of K-H perfusion, Ξ represents 30 min of global ischemia; ® represents 120 min of K–H reperfusion; Θ represents 10 min of Atorvastatin infusion; Ω represents 10 min of Simvastatin infusion.