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RAPID COMMUNICATION

Simvastatin Alleviates Myocardial Contractile Dysfunction and Lethal Ischemic Injury in Rat Heart Independent of Cholesterol-Lowering Effects

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Summary

Statins, the inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, are most frequently used drugs in the prevention of coronary artery disease due to their cholesterol-lowering activity. However, it is not exactly known whether these effects of statins or those independent of cholesterol decrease account for the protection against myocardial ischemia-reperfusion (I/R) injury. In this study, we investigated the effect of 5-day treatment with simvastatin (10 mg/kg) in Langendorff-perfused hearts of healthy control (C) and diabetic-hypercholesterolemic (D-H; streptozotocin + high fat-cholesterol diet, 5 days) rats subjected to 30-min global ischemia followed by 40-min reperfusion for the examination of postischemic contractile dysfunction and reperfusion-induced ventricular arrhythmias or to 30-min (left anterior descending) coronary artery occlusion and 2-h reperfusion for the infarct size determination (IS; tetrazolium staining). Postischemic recovery of left ventricular developed pressure (LVDP) in animals with D-H was improved by simvastatin therapy (62.7±18.2 % of preischemic values vs. 30.3±5.7 % in the untreated D-H; $P<0.05$), similar to the values in the simvastatin-treated C group, which were 2.5-fold higher than those in the untreated C group. No ventricular fibrillation occurred in the simvastatin-treated C and D-H animals during reperfusion. Likewise, simvastatin shortened the duration of ventricular tachycardia (10.2±8.1 s and 57.8±29.3 s in C and D-H *vs.* 143.6±28.6 s and 159.3±44.3 s in untreated C and D-H, respectively, both $P<0.05$). The decreased arrhythmogenesis in the simvastatin-treated groups correlated with the limitation of IS (in % of risk area) by 66 % and 62 % in C and D-H groups, respectively. However, simvastatin treatment decreased plasma cholesterol levels neither in the D-H animals nor in C. The results indicate that other effects of statins (independent of cholesterol lowering) are involved in the improvement of contractile recovery and attenuation of lethal I/R injury in both, healthy and diseased individuals.

Key words

Statins • Cholesterol • Pleiotropic effects • Heart • Ischemia

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It is generally known that statins, inhibitors of 3-hydroxy-3-methylgluratyl CoA reductase, reduce cardiovascular morbidity and mortality due to cholesterol-lowering effects (Gould *et al.* 2007). However, other lipid-independent pleiotropic actions may contribute to cardioprotection induced by statins (Takemoto and Liao 2001, McFarlane *et al.* 2002) that has been documented in experimental studies demonstrating a decrease in oxidative stress (Zhou *et al.* 2008, Kuželová *et al.* 2008), inflammation (van Linthout *et al.* 2007, Endres 2006), inhibition of thrombogenic response (Rossoni *et al.* 2008, Schafer *et al.* 2006) and atherosclerotic plaque formation (Shimizu *et al.* 2003,

Table 1. The effects of 5-day simvastatin (S) treatment on plasma levels of glucose (Glu) and total cholesterol (Chol) in normal control (C) and diabetic-hypercholesterolemic (D-H) rats.

	C ***n* = 9**	**C+S** ***n* = 7**	**D-H** ***n* = 8**	**D-H+S** ***n* = 7**
Glu (mmol/l)	5.52 ± 0.63	5.28 ± 0.50	17.85 ± 2.50†	13.83 ± 1.11†
Chol (mmol/l)	1.51 ± 0.11	1.62 ± 0.21	2.50 ± 0.19†	2.18 ± 0.16†

Values are means ± S.E.M. of 7-9 animals per group. † P<0.05 vs. respective normocholesterolemic controls.

Monettie *et al.* 2007) resulting in attenuation of vascular endothelial dysfunction. The mechanisms responsible for pleiotropic effects of statins involve inhibition of important intermediates, small GTP-binding proteins, such as Ras, Rho, and Rac, which modulate a wide variety of cellular processes (Takemoto and Liao 2001) including myocardial response to ischemia-reperfusion (I/R) injury. In our previous study performed in the open-chest diabetic-hypercholesterolemic rats (D-H), simvastatin pretreatment significantly reduced reperfusion arrhythmias occurring after regional ischemia (Adameová *et al*. 2006). In this study in the isolated Langendorff-perfused rat hearts we focused on the effects of simvastatin on the myocardial infarct size (IS), as well as postischemic recovery of contractile function and reperfusion arrhythmias. To elucidate whether effects of simvastatin on I/R injury are dependent on cholesterol lowering, we compared its effects in both, D-H rats and normocholesterolemic animals.

Male Wistar rats (250-300 g body weight), fed a standard diet and tap water *ad libitum*, were employed. All studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health (NIH publication No 85-23, revised 1996).

Simvastatin was given at the dose of 10 mg/kg as a component of the normal and/or a high-cholesterol diet to the healthy control (C) and double-diseased D-H rats, respectively. The choice of the dosage was based upon the results of some previous studies, which have used the same or even higher dose of statins administered orally (Birnbaum *et al*. 2003, Tavackoli *et al.* 2004). The D-H state was induced with streptozotocin (80 mg/kg, i.p.) and administration of high-cholesterol diet (1 % cholesterol, 1 % cocounut oil; 20 g/kg per day) as described earlier (Adameová *et al*. 2006, 2007a).

Following 5 days, the hearts of anesthetized (sodium pentobarbitone, 60 mg/kg, *i.p.*) C and D-H animals were rapidly excised and perfused at 37 ºC in the Langendorff mode at a constant perfusion pressure of 70 mm Hg. The perfusion solution was a modified Krebs-Henseleit buffer gassed with 95 % O_2 and 5 % CO_2 (pH 7.4) containing (in mM): NaCl 118.0; KCl 3.2; $MgSO_4$ 1.2; $NaHCO_3$ 25.0; KH_2PO_4 1.18; $CaCl_2$ 2.5; glucose 7.0. An epicardial electrogram was registered by means of two electrodes attached to the apex of the heart and the aortic cannula. Left ventricular (LV) pressure was measured by means of a non-elastic water-filled balloon inserted into the LV cavity and connected to a pressure transducer (MLP844, ADInstruments). LV developed pressure (LVDP; systolic minus diastolic pressure), maximal rates of pressure development and fall, heart rate, pressure-rate product and coronary flow were measured during stabilization pre-ischemic period and continuously recorded until the end of experiment. Heart function and arrhythmias were analyzed using PowerLab/8SP Chart 5 software (ADInstruments).

Global ischemia was induced by clamping of aortic inflow for 30 min and followed by 40-min reperfusion for the evaluation of postischemic recovery of LVDP expressed as percentage of preischemic baseline values and of susceptibility to reperfusion-induced ventricular tachyarrhythmias, such as ventricular fibrillation (VF) and tachycardia (VT).

For the evaluation of IS, in the additional subset of experiments the hearts underwent regional test ischemia induced by left anterior descending coronary artery occlusion for 30 min followed by 2-h reperfusion. The IS and area at risk (AR) size was delineated by double staining with 5 % potassium permanganate and 2,3,5-triphenyltetra-zolium chloride and determined by a computerized planimetric method as described earlier (Ravingerová *et al.* 2007). The IS was expressed as percentage of the AR area.

The plasma glucose and cholesterol levels were measured enzymatically using a commercial assay kit (Spinreact, USA) and bioanalyzer ELISA 200 (USA) (Adameová *et al.* 2006, 2007a).

The data were expressed as means ± S.E.M. One-way ANOVA and subsequent Student-Newman-Keuls test as well as Mann-Whitney U test were used where appropriate. Differences were considered as significant at $P<0.05$.

The D-H state was confirmed by significantly increased plasma glucose and total cholesterol levels. Simvastatin decreased the plasma cholesterol levels neither in D-H nor in C animals (Table 1). Furthermore, no significant changes were observed in the plasma glucose levels between the treated and respective untreated animal groups (Table 1).

Despite development of D-H, no significant differences in the values of coronary flow and ventricular hemodynamic parameters between C and D-H groups were observed at baseline before ischemia. Administration of simvastatin did not affect these parameters in any of the groups (data not shown).

On the other hand, 5-day acute simvastatin administration to the D-H rats increased post-ischemic recovery of LVDP (62.7±18.2 % of preischemic values *vs*. 30.3±5.7 % in untreated D-H; $P<0.05$; Fig. 1). Likewise, in C group, simvastatin improved LVDP recovery from 24.1±2.9 % to 76.1±9.8 %; $P<0.05$; Fig. 1). Interestingly, no worsening of the left ventricle function was documented in the D-H rats as compared with the C animals. Similarly, the size of myocardial infarction in these animals did not differ from that in the normocholesterolemic ones (IS/AR 37.3±3.1 % in D-H *vs*. 33.7±4.0 % in C; $P>0.05$), but was higher in comparison with IS observed in normocholesterolemic rats in the acute phase of diabetes showing lower susceptibility to I/R injury (Adameová *et al*. 2007a). Simvastatin therapy significantly reduced infarct size in both groups (IS/AR 14.2±1.3 % and 11.5±0.4 % in D-H and C, respectively; both $P<0.05$; Fig. 1). A lower infarct size in the simvastatin-treated animals correlated with the severity of arrhythmogenesis; a shorter duration of VT was observed in both treated groups of D-H and C animals (10.2±8.1 s and 57.8±29.3 s *vs*. 143.6±28.6 s and 159.3±44.3 s in the untreated C and D-H, respectively, both $P<0.05$; Fig. 1).

The present study demonstrated that both electrical and mechanical myocardial function may be influenced by statins independently of cholesterol-lowering not only in the healthy but also in the diseased D-H animals. Acute simvastatin therapy for 5 days showed hypolipidemic effects neither in the normal nor in the double-diseased rats. However, when measured in the liver, simvastatin normalized the higher cholesterol content induced by the fat-cholesterol diet and diabetes (Adameová *et al.* 2006).

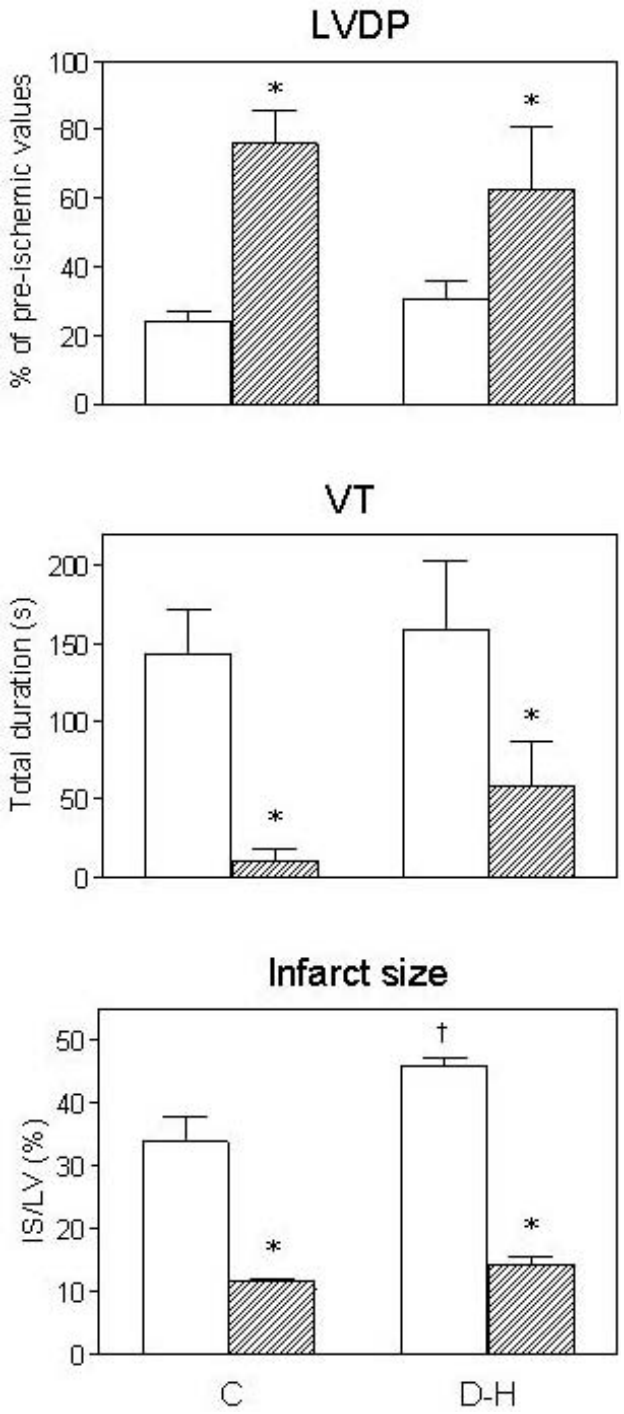

Fig. 1. Effect of 5-day simvastatin treatment on postischemic recovery of left ventricular developed pressure (LVDP), duration of ventricular tachycardia (VT) and size of infarction (IS) expressed as percentage of area at risk (AR) size in the hearts of normal (C) and diabetic-hypercholesterolemic (D-H) rats. Empty bars, untreated rats; filled bars, treated rats. Values are means ± S.E.M. from 8-10 hearts per group. * $P<0.05$ vs. untreated group.

Although it is known that statins may reduce the glucose levels (McFarlane *et al.* 2002) and the administration of simvastatin for 10 days has decreased plasma glucose levels in the D-H rats (Adameová *et al.* 2007b), in the present study we did not observe the hypoglycemic effects. The results of our study indicate that pleiotropic effects of statins might account for decreased outcome of myocardial I/R. It is thus probable that statin-induced attenuation of oxidative stress (Zhou *et al.* 2008, Kuželová *et al.* 2008) and/or inflammatory response (Van Linthout *et al*. 2007, Endres 2006) involved in the genesis of I/R injury (Ravingerová *et al.* 1999) may explain the observed antiarrhythmic effects and IS limitation. In addition, activation of prosurvival cascade PI3K/Akt has been found to occur as a consequence of treatment with statins (Wang *et al.* 2007). Administration of statins prior to the onset of myocardial ischemia up-regulated the levels of phosphorylated Akt and exerted preconditioning-like infarct size-limiting effect (Sanada *et al.* 2004, Manickavasagam *et al.* 2007) that has been explained by Akt-mediated activation of eNOS and another downstream molecule 5‘-nucleotidase (Sanada *et al.* 2004, Efthymiou *et al.* 2005). It has also been suggested that the PI3K/Akt pathway and an increase of eNOS resulting in the increased cappilary recruitment and glucose disposal may account for the hypoglycemic effects of statins (McFarlane *et al.* 2002). However, hypoglycemic activity of statins seems to be specific only for some of them. In fact, pravastatin, unlike atorvastatin, has been shown to exert beneficial effects on glucose metabolism (Ishikawa *et al.* 2006). Moreover, non-lipid action of statins also seems to contribute to the alleviation of postischemic contractile dysfunction in both healthy and diseased animals. Better LVDP recovery in addition to the inhibition of enzymes characterizing myocardial cell damage (e.g. creatine kinase, lactate dehydrogenase) in the treated normocholesterolemic rats was achieved even with the lower doses of simvastatin (Rossoni *et al.* 2008). However, in that study simvastatin was given by gavage, whereas we used it as a component of the food. It should be pointed out that such myocardial protection against I/R is accomplished by a short-term but not a long-term statin therapy (Birnbaum *et al*. 2007, Tiefenbacher *et al*. 2003, Wayman *et al*. 2003). In fact, statins failed to reduce infarct size and to increase postischemic functional recovery when administered for more than one week (Mensah *et al*. 2005, Szárszoi *et al.* 2008). Here we also observed a lower infarct size in addition to a significantly shorter duration of VT in both acute simvastatin-treated groups. This is in line with our study in the normocholesterolemic rats demonstrating simvastatin-induced protection against ischemic arrhythmias (Ravingerová *et al.* 2008). Moreover, lethal arrhythmias (sustained VF) occurred neither in the simvastatin-treated normocholesterolemic nor in the D-H animals (data not shown). These findings are in accordance with the results of our previous study performed in the open-chest D-H animals; the severity of reperfusion ventricular arrhythmias occurring after regional ischemia was significantly decreased (Adameová *et al*. 2006). From the foregoing discussion it appears that short-term statin therapy might be a prospective strategy used for mitigation of the outcome of cardiac surgeries linked with reperfusion injury. Another non-lipid lowering indication of statins suggested partially also by results of this study could be their using in the pathological conditions such as myocardial ischemia, hypertrophy and heart failure (Liao 2004).

In conclusion, 5-day treatment with simvastatin improved postischemic recovery of myocardial function, suppressed the severity of reperfusion-induced tachyarrhythmias and attenuated lethal injury in the hearts of both, normal and diabetic-hypercholesterolemic rats without normalizing plasma levels of cholesterol. The latter indicates that other than cholesterol-lowering effects of simvastatin are involved in its cardioprotective action.

Conflict of Interest

There is no conflict of interest.

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