

Distinct Effects of Acute Pretreatment With Lipophilic and Hydrophilic Statins on Myocardial Stunning, Arrhythmias and Lethal Injury in the Rat Heart Subjected to Ischemia/Reperfusion

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Summary

Although both lipophilic and more hydrophilic statins share the same pathway of the inhibition of HMG-CoA reductase, their pleiotropic cardioprotective effects associated with the ability to cross cellular membranes, including membranes of heart cells, may differ. To test this hypothesis, isolated rat hearts were Langendorff-perfused either with simvastatin (S, 10 µmol/l) or pravastatin (P, 30 µmol/l), 15 min prior to ischemia. Control untreated hearts (C) were perfused with perfusion medium only. Postischemic contractile dysfunction, reperfusion-induced ventricular arrhythmias and infarct size were investigated after exposure of the hearts to 30-min global ischemia and 2-h reperfusion. Both lipophilic S and hydrophilic P reduced the severity of ventricular arrhythmias (arrhythmia score) from 4.3±0.2 in C to 3.0±0 and 2.7±0.2 in S and P, respectively, (both P<0.05), decreased the duration of ventricular tachycardia and suppressed ventricular fibrillation. Likewise, the extent of lethal injury (infarct size) determined by tetrazolium staining and expressed in percentage of risk area, was significantly lower in both treated groups, moreover, the effect of P was more pronounced (27±2 % and 10±2 % in S and P groups, respectively, vs. 42±1 % in C; P<0.05). In contrast, only S, but not P, was able to improve postischemic recovery of left ventricular developed pressure (LVDP; 48±12 % of preischemic values vs. 25±4 % in C and 21±7 % in P groups; P<0.05). Our results suggest that differences in water solubility of statins indicating a different ability to cross cardiac membranes may underlie their distinct cardioprotective effects on myocardial stunning and lethal injury induced by ischemia/reperfusion.

Key words

Statins • Water solubility • Pleiotropic effects • Rat heart • Ischemia

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Statins have been initially developed to reduce the synthesis of endogenous cholesterol by the inhibition of the 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) in the liver. Now it is generally accepted that they have a wide variety of pleiotropic actions, besides the remarkable hypolipidemic effects, which significantly contribute to the reduction of the incidence of ventricular arrhythmias-associated sudden cardiac death in subjects with and without coronary artery disease (for review see Ramasubbu *et al.* 2008, Abuissa *et al.* 2009). These beneficial non-lipid effects of statins are mainly mediated by blocking the synthesis of isoprenoids through a common pathway as cholesterol that results in the inhibition of small GTP-binding proteins (Ras, Rac and Rho) regulating endothelial dysfunction, oxidative stress, inflammation, thrombogenesis and metabolism (McFarlane *et al.* 2002, Takemoto and Liao 2001). In accordance, experimental studies have clearly shown that statins reduce myocardial electrical disturbances irrespective of their lipid-lowering

activity and are able to attenuate postischemic cardiac contractile dysfunction in subjects without lipid disorders (Adameová *et al.* 2009, Ravingerová *et al.* 2009, Ikeda *et al.* 2003, Chen *et al.* 2003).

Statins, a diverse group of natural and synthetic drugs, differ in chemical structure and pharmacokinetic profile, in particular, water solubility, which may determine their lipid-modifying efficacy. In fact, lipophilic statins (simvastatin, atorvastatin, lovastatin) enter hepatocytes non-selectively by passive diffusion, while hydrophilic statins (rosuvastatin and pravastatin) are more hepatoselective due to active carrier-mediated process (Schachter 2005, Hamelin and Turgeon 1998). Data from comparative trials have confirmed that on a milligram basis, rosuvastatin is the most efficient statin for lowering LDL-cholesterol, followed by atorvastatin, simvastatin and pravastatin (Jones *et al.* 2003). In addition, it is apparent that although both types of statins, lipophilic and hydrophilic, share the same pathway of the inhibition of HMG-CoA reductase, such different pharmacokinetic characteristic may underlie their ability to cross cellular membranes including membranes of cardiomyocytes. To investigate the efficacy of two different types of statins on myocardial stunning, arrhythmias and lethal injury induced by ischemia/reperfusion (I/R) and to explore a potential relationship between the differences in the cardioprotective effects of statins and their chemical structure, in the present study we used two structurally distinct drugs, lipophilic simvastatin (S) and hydrophilic pravastatin (P).

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).

The hearts of anesthetized (sodium pentobarbitone, 60 mg/kg, *i.p.*) animals were rapidly excised and perfused at 37 °C in the Langendorff mode at a constant perfusion pressure of 73 mm Hg. The perfusion solution was a modified Krebs-Henseleit buffer gassed with 95 % O₂ and 5 % CO₂ (pH 7.4) containing (in mM): NaCl 118.0; KCl 3.2; MgSO₄ 1.2; NaHCO₃ 25.0; KH₂PO₄ 1.18; CaCl₂ 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 systolic pressure, diastolic pressure (LVDiP), developed pressure (LVDP; systolic minus diastolic pressure), maximal rates of pressure development and fall, heart rate and coronary flow were measured during stabilization pre-ischemic period and continuously recorded until the end of experiment. Heart function was analyzed using PowerLab/8SP Chart 7 software (ADInstruments).

Global ischemia was induced by clamping of aortic inflow for 30 min and followed by reperfusion for the evaluation of postischemic recovery of contractile function after 40-min reperfusion. Susceptibility to reperfusion-induced ventricular tachyarrhythmias, such as ventricular tachycardia (VT) and fibrillation (VF), as well as arrhythmia severity (assessed by a 5-point scoring system according to the most severe form of arrhythmia that occurred in each individual heart; Curtis and Walker 1988) was evaluated during 10-min reperfusion. The size of infarction (IS) was determined after 2-h reperfusion by staining with 2,3,5-triphenyltetrazolium chloride and a computerized planimetric method as described earlier (Ravingerová *et al.* 2009). The IS was expressed as percentage of the LV area.

In drug-treated groups, simvastatin (10 μmol/l) and pravastatin (30 μmol/l) were given as components of the perfusion medium 15 min prior to ischemia. The rationale for the used dose of statins was based on the evidence that simvastatin is more potent than pravastatin, thereby allowing its administration at the lower dose to achieve the effects on the HMG-CoA reductase that are comparable with the effects of another statin (Schachter 2005). The hearts of the untreated group (C) were perfused for additional 15 min with perfusion medium only.

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 and Fisher's exact test were used where appropriate. Differences were considered as significant at P<0.05.

No significant differences in the values of coronary flow and hemodynamic parameters between the control non-treated and treated groups were observed at baseline and after treatment (data not shown). Acute simvastatin administration prior to ischemia significantly increased postischemic recovery of LVDP (48±12 % of preischemic values vs. 25±4 % in C groups; P<0.05) (Fig. 1A). Likewise, significantly lower postischemic values of LVDiP in the S-treated group (Fig. 1B)

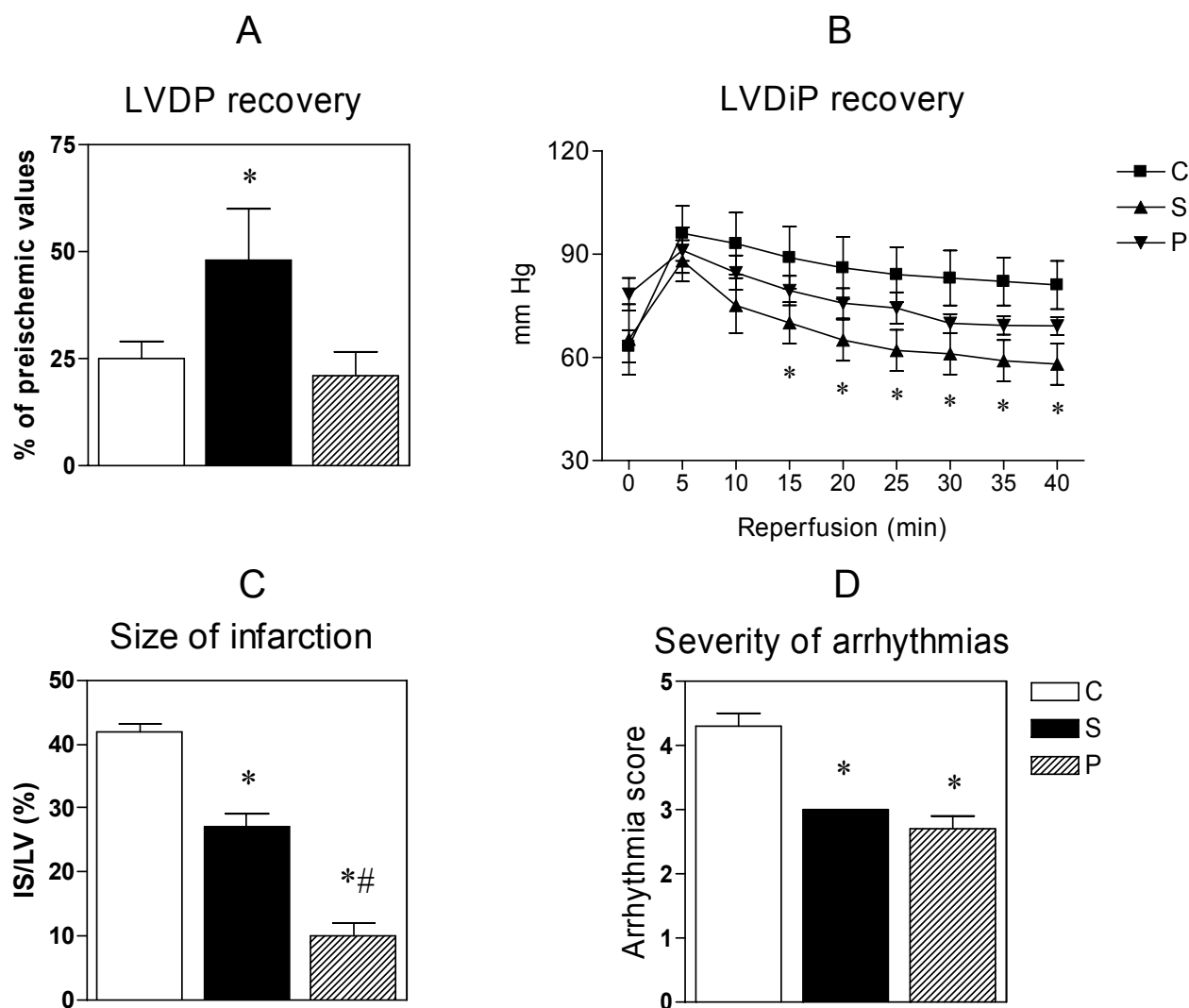


Fig. 1. Effect of acute pretreatment with simvastatin (S) and pravastatin (P) on postischemic recovery of left ventricular developed pressure (LVDP; **A**), left ventricular diastolic pressure (LVDiP; **B**), size of infarction (IS) expressed as percentage of left ventricular area (LV; **C**) and the severity of ventricular arrhythmias (**D**) in the hearts of normocholesterolemic rats. Empty bars – untreated control hearts, filled bars – S-treated hearts, hatched bars – P-treated hearts. Values are means \pm S.E.M. from 10-12 hearts per group. * $P < 0.05$ vs. untreated control group; # $P < 0.05$ vs. simvastatin-pretreated group.

indicated an improvement of the diastolic function and attenuated contracture. However, a reduction of myocardial stunning was not observed in the pravastatin-treated hearts; the values of LVDP at 40 min of reperfusion were comparable with those in the control group (21 ± 7 % in P vs. 25 ± 4 % in C group; $P > 0.05$; Fig. 1A) and diastolic dysfunction was not mitigated (Fig. 1B). On the other hand, postischemic recovery of coronary flow was significantly higher in the P group than in C (80 ± 9 % and 56 ± 9 %, respectively; $P < 0.05$), while it was not modified by treatment with simvastatin ($P > 0.05$ vs. C) (Table 1). This effect of pravastatin is in line with the effects of a less hydrophilic fluvastatin that restored function of endothelium within six weeks

(Eichstädt *et al.* 1995). It could be hypothesized that in our study, specific endothelium-related effects of statins, which are mediated through an increase of the activity/expression of constitutive endothelial nitric oxide synthase (eNOS) and a reduction of caveolin-1, could attenuate decreased NO bioavailability and thus increase myocardial perfusion (Pelat *et al.* 2003, Wolfrum *et al.* 2003, Feron *et al.* 2001).

The size of myocardial infarction (Fig. 1C) in both treated groups was significantly lower in comparison to the untreated group (IS/LV; 27 ± 2 % and 10 ± 2 % in S and P groups, respectively, vs. 42 ± 1 % in C; $P < 0.05$). Moreover, the effect of pravastatin on the extent of lethal injury was more pronounced than the effect of

Table 1. The effects of treatment with simvastatin and pravastatin on postischemic recovery of left ventricular diastolic pressure (LVDiP), heart rate (HR, in % of preischemic values), coronary flow (CF, in % of preischemic values) and reperfusion-induced ventricular tachycardia (VT) and fibrillation (VF) in isolated rat hearts subjected to 30-min global ischemia and 40-min reperfusion.

Group	Recovery			Incidence (%)		Duration (s)
	LVDiP (mmHg)	HR (%)	CF (%)	VT	VF	VT
Control	81 ± 7	82 ± 4.8	56 ± 9	100	83	143 ± 28
Simvastatin	58 ± 6*	90 ± 3	72 ± 17	100	0*	57 ± 24*
Pravastatin	69 ± 3	75 ± 8	80 ± 9*	71	0*	8 ± 3*#

Data are means from 10-12 hearts per group ± S.E.M. * P<0.05 vs. control untreated group; # P<0.05 vs. simvastatin-treated group.

simvastatin (P<0.05; P vs. S). A smaller infarct size in the treated animals was associated with the reduced severity of ventricular arrhythmias (Fig. 1D, Table 1). Although arrhythmias occurred in both S and P-treated groups, they were less severe in comparison to those in the untreated C group; the incidence of ventricular fibrillation was completely suppressed, duration of ventricular tachycardia was shorter (Table 1) and arrhythmia severity score (Fig. 1D) was lower in both treated groups (3±0 and 2.7±0.2 in S and P groups, respectively, as compared with 4.3±0.2 the controls; both P<0.05). There were no substantial differences in the antiarrhythmic efficacy between both drugs except shorter duration of VT in the P group (P<0.05, vs. S; Table 1).

In the present study we investigated the role of water solubility, an important pharmacokinetic property of two statins, in pleiotropic cardioprotective effects, such as antiarrhythmic action, infarct size limitation and the ability to restore cardiac contractile function upon I/R. Although both lipophilic simvastatin and hydrophilic pravastatin reduced electrical instability, they differed in the extent of their infarct size-limiting effect. Pravastatin conferred more effective anti-infarct protection that could be partially explained by an improved restoration of myocardial perfusion. On the other hand, only simvastatin but not pravastatin attenuated myocardial contractile dysfunction. Suppression of ventricular arrhythmias upon statin treatment is in accordance with other studies which have suggested that increase of NO bioavailability, antiinflammatory and antioxidative effects as well as regulation of thrombus formation may indirectly underlie antiarrhythmic effects of statins (Takemoto and Liao 2001, McFarlane *et al.* 2002). In addition to these effects, other statins-mediated antiarrhythmic mechanisms, such as normalization of the electrophysiological disturbances associated with

heterogeneity in ventricular repolarization as well as of the heart rate can be also considered (Vrtovec *et al.* 2005, Mark *et al.* 2000, Pehlivanidis *et al.* 2001, Pelat *et al.* 2003). Recently, we have suggested another mechanism of anti-arrhythmic action of statins; simvastatin given for one week altered the calcium channel composition indicating a reduction of calcium influx into the cardiomyocytes (Szobi *et al.* 2011). This was shown in both hypercholesterolemic and normocholesterolemic rats confirming the effects of simvastatin independent on lipid-lowering activity. It should be noted that both statins used in the present study were given acutely prior to the induction of ischemia, however, in all above mentioned studies, statin treatment lasted for several days. From the foregoing discussion it appears that octanol-water coefficient indicating the degree of lipophilicity of statin, is unlikely to determine the efficacy of arrhythmia development. On the other hand, statin lipophilicity seems to affect the regulation of energy metabolism. In fact, Satoh and Ichihara (2000) demonstrated that cerivastatin, atorvastatin and fluvastatin, but not hydrophilic pravastatin reduced myocardial ATP levels in the reperfused heart of the rats treated for 3 weeks. Of note, these three lipophilic compounds worsened the segment shortening in the reperfused myocardium suggesting that myocardial stunning is associated with the lower ATP content in subjects chronically treated with lipophilic statins. These results are not in line with the findings of the present study; simvastatin but not pravastatin improved postischemic recovery of contractile function. Likewise, only simvastatin considerably reduced postischemic diastolic contracture indicating its greater lusitropic effect. Interestingly, Szárszoi *et al.* (2008) have shown that administration of simvastatin given during reperfusion is able to restore the recovery of cardiac

contractility; however, chronic simvastatin treatment failed to exert such cardioprotection. Thus, the duration of statin treatment seems to be an important factor with respect to the restoration of mechanical function of the heart. Although a short-term 7-day treatment with simvastatin, as well as its administration before/during I/R attenuates cardiac contractile dysfunction, chronic several-week premedication of rats induces myocardial stunning (Adameová *et al.* 2009, Szárszoi *et al.* 2008). In contrast, Kocsis *et al.* (2008) have reported that lovastatin, a statin with a lower octanol-water coefficient than that of simvastatin, is cardioprotective when given chronically but not acutely. It is thus apparent that water solubility besides the duration of treatment may determine the restoration of heart function upon I/R. In addition, some discrepancies in the efficacy of statins in terms of mechanical function of the heart may be also associated with the dose of statin as all authors of the previously mentioned studies used a different dosage regiment. It should be also pointed out that this physical-chemical property, water solubility, of statins is likely to be associated with undesirable effects, such as myalgia, myopathy and rhabdomyolysis, which incidence was the highest in the deregistered most lipophilic cerivastatin,

a statin known to exert a passive widespread tissue distribution due to the highest octanol-water coefficient (Staffa *et al.* 2002, Furber and Pitt 2001).

In conclusion, acute treatment with lipophilic simvastatin and hydrophilic pravastatin suppressed the severity of reperfusion-induced tachyarrhythmias and attenuated lethal injury in the rat hearts. While infarct size-limiting effect was more pronounced in pravastatin-treated hearts, only simvastatin improved postischemic recovery of myocardial function. Consideration of the physical-chemical and pharmacokinetic properties of statins may provide a basis for a rational choice of a preferable agent to prevent/treat different manifestations of acute myocardial ischemia including arrhythmias, myocardial infarction and contractile dysfunction.

Conflict of Interest

There is no conflict of interest.

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