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RAPID COMMUNICATION

Effect of Acute and Chronic Simvastatin Treatment on Post-Ischemic Contractile Dysfunction in Isolated Rat Heart

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Summary

Statins are powerful lipid-lowering drugs, widely used in patients with hyperlipidemia and coronary artery disease. It was found, however, that statins appear to have a pleiotropic effect beyond their lipid-lowering ability. They exert anti-inflammatory, antithrombotic and antioxidant effects, increase nitric oxide production and improve endothelial dysfunction. The aim of our study was to examine the effect of chronic and acute treatment with simvastatin on the contractile function of the isolated perfused rat heart after ischemia/reperfusion injury. Contractile function was measured on isolated rat hearts, perfused according to Langendorff under constant pressure. The hearts were subjected to 20 min of global ischemia, followed by 40 min of reperfusion. To investigate the acute effect, simvastatin at a concentration of 10 μmol/l was added to the perfusion solution during reperfusion. In chronic experiments the rats were fed simvastatin at a concentration of 10 mg/kg for two weeks before the measurement of the contractile function. Acute simvastatin administration significantly increased reparation of the peak of pressure development [$(+dP/dt)_{max}$] (52.9±8.2 %) after global ischemia, as compared with the control group (28.8±5.2 %). Similar differences were also observed in the time course of the recovery of [$(+dP/dt)_{max}$]. Chronic simvastatin was without any protective effect. Our results reveal that the acute administration of simvastatin during reperfusion, unlike the chronic treatment, significantly reduced contractile dysfunction induced by ischemia/reperfusion injury. This supports the idea of possible cardioprotective effect of statin administration in the first-line therapy of the acute coronary syndrome.

Key words

Simvastatin • Isolated rat heart • Ischemia/reperfusion injury • Acute and chronic treatment

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Statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) lower cholesterol level and are widely used in patients with hyperlipidemia and coronary artery disease. Statins reduce cardiovascular morbidity and mortality in a number of large clinical trials (LIPID Study Group 1998, Scandinavian Simvastatin Survival Study Group 1994, Sacks *et al.* 1996). During the past decade it was found that statins appear to have a pleiotropic effect beyond their lipid-lowering ability. It was demonstrated that they exert anti-inflammatory, antithrombotic, and antioxidant effects, increase nitric oxide production or improve endothelial dysfunction (Ošťádal 2006). Furthermore, statins can reduce experimental ischemia/reperfusion injury expressed as myocardial infarct size (Di Napoli *et al.* 2001, Lefer *et al.* 2001, Jones *et al.* 2002, Wayman *et al.* 2003, Tiefenbacher *et al.* 2003, Yamakuchi *et al.* 2005) or incidence of severe ventricular ischemic arrhythmias (Chen *et al.* 2007). To our best knowledge, there are no

Table 1. Effects of chronic and acute simvastatin treatment on baseline contractile parameters and coronary flow of the isolated perfused hearts of rats.

	n	$(+dP/dt)_{max}$ (mmHg/s)	LVDevP (mmHg)	LVDP (mmHg)	CF (ml/min/g)
Control	7	3913±262	113.0±7.3	5.8±1.5	16.8±0.5
Acute statin	7	3773±363	113.1±6.6	8.9±0.8	15.9±0.7
Chronic statin	6	3820±382	117.9±7.0	7.3±1.1	17.1±0.4

$(+dP/dt)_{max}$, peak rate of pressure development; LVDevP, left ventricular developed pressure; LVDP, left ventricular diastolic pressure; CF, coronary flow; *n*, number of animals. Values are means ± S.E.M.; * $P<0.05$ vs. corresponding controls.

studies which compare the effect of chronic and acute statin treatment on post-ichemic contractile dysfunction in the isolated heart. Therefore, the aim of our study was to examine the effect of chronic and acute administration of simvastatin on the recovery of contractile function of the isolated perfused rat hearts after ischemia/reperfusion injury.

Adult male Wistar rats weighing 280 to 350 g were used. The investigations were performed in accordance with the *Guide for Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The rats were anesthetized with intraperitoneal sodium pentobarbital (60 mg/kg). Their hearts were perfused by the Langendorff technique under constant pressure (100 cm H_2O) with non-recirculating Krebs-Henseleit solution containing (mmol/l): NaCl 118, KCl 4.7, $CaCl_2$ 1.25, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2 and glucose 7.0. The solution was saturated with 95 % O_2 and 5 % CO_2 (pH 7.4) and maintained at 37 °C. The left ventricle was vented at the apex and stimulated at 300 beats/min with platinum electrodes attached to the base of the right ventricle. Contractile function (left ventricular systolic – LVSP, diastolic – LVDP and developed pressure – LVDevP, and the peak rate of pressure development $(+dP/dt)_{max}$), was measured with a non-elastic balloon inserted into the left ventricle (Bešík *et al.* 2007). Coronary flow was measured by timed collection of coronary effluent. After 25 min of stabilization, the hearts were subjected to 20 min of global ischemia followed by 40 min of reperfusion. After restoration of flow, the functional parameters were recorded at 5-min intervals and their recovery was expressed as percentage of initial pre-ischemic values. To investigate the acute effect of statins, simvastatin at a concentration of 10 μmol/l was added to the perfusion solution at the beginning of reperfusion. Simvastatin (Sigma) was diluted in 0.5 ml of 100 % ethanol, mixed with 0.75 ml of 0.1 mol/l NaOH, heated to 50 °C for 2 h, neutralized with 0.1 mol/l HCl to pH 7.2, and adjusted with deionized water to the final concentration. Control hearts were treated in the corresponding manner with a solution without simvastatin. In the chronic experimental group the rats were fed simvastatin at a concentration of 10 mg/kg/day for two weeks before experiment. Daily dose of the laboratory diet with statin was weighed and its consumption controlled by repeated weighing.

The results are expressed as means ± S.E.M. One-way ANOVA or ANOVA for repeated measures and subsequent Student-Newman-Keuls test were used for comparison of differences between groups. Differences were assumed as statistically significant when $P<0.05$.

Baseline preischemic values of contractile parameters and coronary flow did not differ between control, acute and chronic simvastatin-treated hearts (Table 1). Acute simvastatin administration during reperfusion significantly increased the maximum recovery of $(+dP/dt)_{max}$ to 52.9±8.2 % of preischemic value compared to the control group (28.8±5.2 %) (Fig. 1A). The same differences were observed also in the time course of the recovery of $(+dP/dt)_{max,}$ (Fig. 1B). Similar results were also found in LVDP (56.9±8.6 % of preischemic value vs. 29.5±6.5 %, $P<0.05$). Chronic simvastatin treatment was without any protective effect.

Our results show that acute administration of simvastatin during reperfusion significantly reduced the contractile dysfunction. However, this protective effect of statins was not present after chronic treatment. It was shown previously that acute treatment of animals with statins prior to the onset of myocardial ischemia reduced the infarct size in mice (Lefer *et al.* 2001, Jones *et al.* 2002, Yamakuchi *et al.* 2005), rats (Di Napoli *et al.* 2001, Wayman *et al.* 2003, Tiefenbacher *et al.* 2003), dogs (Sanada *et al.* 2004) or pigs (Lazar *et al.* 2003).

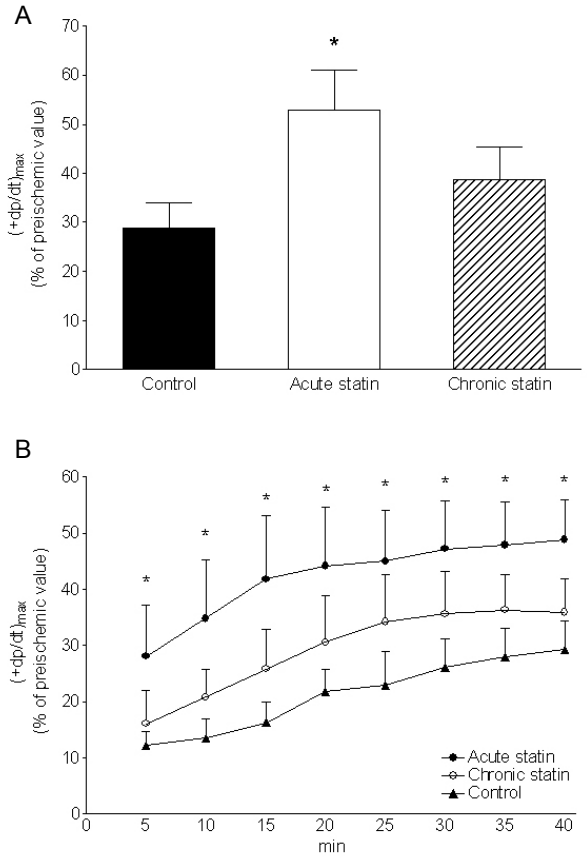

Fig. 1. The maximum recovery of the peak rate of pressure development $(+dP/dt)_{max}$ (A) and time course of $(+dP/dt)_{max}$ (B) during reperfusion in the control, acute and chronically simvastatin-treated treated hearts. Values are means ± S.E.M.

While the above experimental studies demonstrated a protective effect of prophylactic therapy, they did not simultaneously address the question whether the animals with acute myocardial infarction might also benefit from the initiation of statin therapy during reperfusion. There are only a few papers dealing with this particular issue. Zheng and Hu (2006) found on the isolated rat heart that simvastatin added to the perfusion solution during reperfusion improved post-ischemic contractile recovery after 15 min ischemia. Bell and Yellon (2003) demonstrated the beneficial effect of atorvastatin (reduction of infarct size) administered at the time of reperfusion in the isolated perfused mouse heart. Finally, Wolfrum *et al.* (2004) showed in their *in vivo* study that simvastatin administered intravenously 3 min before restoration of flow significantly decreased infarct size in rats. On the other hand, chronic treatment of rats with simvastatin for two weeks did not change the severity of the contractile dysfunction in the isolated hearts in our study. Our results are in agreement with those of Mensah *et al.* (2005) showing that the infarct size lowering effect of atorvastatin, when given for less than three days before ischemia/reperfusion, is lost, when administered chronically for one or two weeks before the ischemic insult. Nevertheless, it seems that the effect of chronic statin treatment depends not only on the duration of treatment but also on the end-point of the ischemia/reperfusion injury. Chen *et al.* (2007) found that chronic administration of pravastatin for 22 days before the ischemic insult in the anesthetized rats reduced the incidence of ventricular fibrillations.

The protective effect of acute statin treatment against ischemia/reperfusion injury is well documented but its mechanism is still under investigation. The molecules that appear to play an important role in this protective mechanism are phosphatidylinositol-3-kinase (Bell and Yellon 2003, Wolfrum *et al.* 2004), nitric oxide synthase 3 (Jones *et al.* 2002, Tiefenbacher *et al.* 2003, Wolfrum *et al.* 2004), ATP-dependent potassium channels (Tavackoli *et al.* 2004) or PTEN (phosphatase and tensin homolog deleted on chromosome 10) a protein phosphatase known to inhibit the function of phosphatidylinositol-3-kinase (Mensah *et al.* 2005).

In conclusion, statins have an unambiguous cholesterol-independent cardioprotective effect that can be lost after chronic treatment. These results support the idea of possible cardioprotective effect of statin administration in the first-line therapy of acute coronary syndrome.

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Conflict of Interest

There is no conflict of interest.

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