Lovastatin interferes with the infarct size-limiting effect of ischemic preconditioning and postconditioning in rat hearts

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Kocsis GF, Pipis J, Fekete V, Kovács-Simon A, Odendaal L, Molnár É, Gírz Z, Janáky T, van Rooyen J, Csont T, Ferdinandy P. Lovastatin interferes with the infarct size-limiting effect of ischemic preconditioning and postconditioning in rat hearts. Am J Physiol Heart Circ Physiol 294: H2406–H2409, 2008. First published March 21, 2008; doi:10.1152/ajpheart.00862.2007.—Statins have potent cholesterol-lowering effects and reduce cardiovascular morbidity and mortality (16, 17). Since cardioprotection by preconditioning is impaired in experimental hypercholesterolemia in both animal models and patients (5, 7, 8). We have previously reported that ischemic stress adaptation by preconditioning is impaired in experimental hypercholesterolemia, possibly due to inhibition of the mevalonate pathway (6). However, it is not known if pharmacological inhibition of the mevalonate pathway by statins interferes with the cardioprotection conferred by endogenous cardiac stress adaptation.

Therefore, in the present study, we determine if acute or chronic treatment with lovastatin affects the infarct size-limiting effect of ischemic preconditioning and postconditioning and explored if alterations in coenzyme Q9 (a mevalonate pathway product downstream of HMG-CoA reductase) as well as the activation of prosurvival kinases Akt and p42/p44 MAPK/ERK are involved in this phenomenon.

METHODS

This investigation conformed with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996) and was approved by the local ethics committee.

Experimental protocol and isolated heart perfusions. Male Wistar rats (n = 128, 350–400 g) were randomly assigned to the following three groups (Fig. 1): 1) the vehicle-treated group was treated with 1% methylcellulose per os for 12 days followed by ex vivo drug-free perfusion protocols, 2) the chronic lovastatin-treated group was given 15 mg·kg⁻¹·day⁻¹ lovastatin per os dispersed in 1% methylcellulose for 12 days followed by ex vivo drug-free perfusion protocols, and 3) the acute lovastatin-treated group was treated with 1% methylcellulose per os for 12 days followed by ex vivo perfusion protocol in the presence of 50 μmol/l lovastatin. The doses of lovastatin were selected according to our and other’s previous literature data (4, 6, 15).

After the 12-day per os treatments, rats were anesthetized with diethyl ether, heparin (500 U/kg iv) was given, and hearts were harvested. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
isolated and perfused at 37°C in the Langendorff mode with oxygenated Krebs-Henseleit buffer as previously described (6).

Hearts from the three groups were further subdivided to three subgroups (n = 8–12) and subjected to either a nonconditioning, preconditioning, or postconditioning perfusion protocol, respectively (Fig. 1). Nonconditioned hearts were subjected to time-matched aerobic perfusion followed by test ischemia-reperfusion induced by a 30-min occlusion of the left coronary artery followed by 2-h reperfusion. Preconditioned hearts were subjected to three intermittent periods of ischemia-reperfusion of 5 min in duration, followed by test ischemia-reperfusion. Postconditioning was achieved by six cycles of 10-s ischemia-reperfusion periods after the 30-min test coronary occlusion. At the end of the 2-h reperfusion, infarct size was measured in all groups.

Separate series of hearts from vehicle-, acute lovastatin-, and chronic lovastatin-treated groups were perfused for 45 min, freeze clamped, and used for biochemical measurements (Fig. 1).

Heart rate and coronary flow were monitored throughout the perfusion. The area at risk and infarct size were determined at the end of the protocol. Nine hearts were excluded from the study due to technical problems.

Induction of ischemia-reperfusion by coronary occlusion. A 3/0 silk suture was placed around the left main coronary artery and passed through a plastic tube to form a snare. Following stabilization of the heart, coronary occlusion was initiated by pulling the ends of the suture taut and clamping the snare onto the epicardial surface, and reperfusion was induced by releasing the snare as previously described (3).

Measurement of infarct size. At the end of the perfusion protocols, the coronary artery was reoccluded and 4 ml of 0.1% Evans blue dye was injected into the aorta to delineate the area at risk zone. Stained hearts were frozen, sliced, and incubated at 37°C in 1% triphenyltetrazolium chloride to delineate infarcted tissue. Slices were then fixed and quantified by planimetry using Infarctsize 1.0 software (Pharmahungary, Szeged, Hungary). Infarct size was expressed as a percentage of the total ventricular area.

Western blot analysis of survival kinases. Standard Western blot analysis was performed from cardiac tissue homogenates as previously described (10). In brief, 20 μg protein was separated by SDS-PAGE and transferred to nitrocellulose membranes (Amersham Biosciences). Membranes were incubated with rabbit polyclonal IgG antibodies (Cell Signaling) against rat Akt (1:1,000), phosphorylated Akt [1:500 (Ser473)], p42/p44 MAPK/ERK (1:1,500), and phosphorylated p42/p44 MAPK/ERK [1:1,500 (Thr202/Tyr204)]. Intensities of the appropriate bands were analyzed by densitometry.

Measurement of myocardial coenzyme Q9. The level of cardiac coenzyme Q9 was measured by a HPLC method following lipid extraction with n-hexane as previously described (18). Coenzyme Q9 was detected at 275 nm using an UV/VIS detector following separation with a Gilson HPLC system on a C18 column (50734 LiChrospher endcapped, Merck). Calibration curves were made using a coenzyme Q9 standard (Sigma, St. Louis, MO).

Statistics. Data are expressed as means ± SE and were analyzed with one-way ANOVA followed by a Tukey post hoc test. All comparisons were made versus the vehicle-treated nonconditioned group. P < 0.05 was accepted as a statistically significant difference.

Fig. 1. Experimental protocol. Open bars, aerobic perfusion; solid bars, coronary occlusion; solid arrows, times of sample collection for biochemical measurements; open arrows, times of infarct size (IS) measurement.

Fig. 2. IS expressed as a percentage of the area at risk (AAR) in isolated hearts of vehicle-, chronic lovastatin-, and acute lovastatin-treated rats subjected to nonconditioning (Non), preconditioning (Pre), or postconditioning (Post) perfusion protocols. Values are means ± SE; n = 8–12. *P < 0.05 vs. the vehicle-treated nonconditioned group.
RESULTS

Both preconditioning and postconditioning significantly decreased infarct size in the vehicle-treated groups (Fig. 2). Whereas chronic lovastatin treatment reduced infarct size in the nonconditioned group, it did not affect the infarct size-limiting effect of preconditioning. Whereas acute lovastatin treatment failed to decrease infarct size significantly in the nonconditioned group, it abolished the infarct size-limiting effect of preconditioning; however, it did not affect postconditioning. Neither chronic nor acute lovastatin treatment affected baseline coronary flow, heart rate (data not shown), and area at risk (Table 1). Both chronic and acute lovastatin treatment significantly decreased myocardial coenzyme Q9 levels (Fig. 3A) and attenuated the phosphorylation of Akt (Fig. 3, B and C) without affecting total Akt protein levels (Fig. 3, B and D). However, the phosphorylation of p42 MAPK/ERK was increased only by acute lovastatin treatment and was unaffected by chronic lovastatin treatment. Total p42 MAPK/ERK was not changed by either acute or chronic lovastatin (Fig. 3, B, E, and F).

DISCUSSION

We have shown here that the chronic application of lovastatin for 12 days markedly reduces infarct size in the ischemic-reperfused rat heart, but acute lovastatin treatment has no effect. This shows that acute and chronic lovastatin treatment may trigger different cellular mechanisms. As to the lack of an acute effect of lovastatin, our results are in contrast to those of Di Napoli et al. (4), who showed that the acute application of 25 μM simvastatin protected the ischemic-reperfused heart against contractile dysfunction, release of creatine kinase, and postischemic hyperpermeability. Interestingly, in that study, protection by simvastatin became less evident at 50 μM and reverted to increased damage at 100 μM. Nevertheless, as to the protective effect of chronic lovastatin treatment, our results are in line with the outcome of clinical trials showing the reduction of cardiovascular events due to statins (11).

Here, we have shown that lovastatin abrogates the cardioprotective effect of preconditioning when applied acutely but not when given chronically. The cardioprotective effect of postconditioning was attenuated when chronic lovastatin treatment was applied, and acute lovastatin treatment had no effect. As chronic lovastatin showed cardioprotection by itself, the fact that postconditioning in the chronic lovastatin-treated group did not reduce infarct size shows that there may be an antagonism between the cellular mechanisms of postconditioning and those of chronic statin treatment. These results show that acute and chronic lovastatin treatment, by interfering with different cellular mechanisms, may modulate cardioprotective mechanisms.

![Fig. 3. A: myocardial coenzyme Q9 levels in vehicle-, chronic lovastatin-, and acute lovastatin-treated rat hearts. Values are means ± SE; n = 6. *P < 0.05 vs. the vehicle-treated group. B: representative Western blots demonstrating phosphorylated and total protein levels of Akt and p42/p44 MAPK/ERK. C–F: densitometric analyses of changes in protein levels of phosphorylated Akt (C), total Akt (D), phosphorylated p42 MAPK/ERK (E), and total p42 MAPK/ERK (F) due to chronic or acute lovastatin treatment. Values are means ± SE; n = 5–8. *P < 0.05 vs. the vehicle-treated group.]

![Table 1. Areas at risk in the treatment groups](http://ajpheart.physiology.org/)
Indeed, in the present study, both acute and chronic lovastatin administration inhibited the basal cardiac level of the antioxidant enzyme Q9, which has been previously shown to be a cardioprotective molecule and an important determinant of normal cardiac function (8, 18). Furthermore, both acute and chronic lovastatin treatment significantly decreased the phosphorylation of Akt on Ser473 in the heart in the present study. As phosphorylation of Akt is a cell survival signal (2, 9), decreased Akt phosphorylation may contribute to the lovastatin-induced deterioration of cardiac adaptation. Acute and chronic lovastatin treatment showed differential effects on p42/p44 MAPK/ERK in the present study. Neither total nor phosphorylated p42/p44 MAPK/ERK protein levels were affected significantly by chronic lovastatin treatment. In contrast, acute lovastatin treatment significantly increased p42 MAPK/ERK phosphorylation; however, it did not affect the total p42 MAPK/ERK level. These effects of lovastatin might play a role in its differential action on cardioprotective mechanisms.

In contrast to our present results, Bell and Yellon (1) showed increased Akt phosphorylation in response to acute atorvastatin treatment at reperfusion in mice subjected to global ischemia-reperfusion. Other studies from the same group (12, 21) have shown that the salvage kinase cascade is activated during the early period of reperfusion. It should be noted that in our present study we measured nonischemic levels of survival kinases. Furthermore, the upstream triggers for activation of survival kinases and the sequences of their activation are far from clear, and the interpretation of different experiments using different species and experimental protocols in cardiac ischemic adaptation is complicated [see Ferdinandy et al. (7)].

In conclusion, this is the first demonstration that acute lovastatin treatment abolishes the infarct size-limiting effect of ischemic preconditioning and that chronic lovastatin treatment attenuates the protective effect of postconditioning. This may be of therapeutic relevance when preconditioning or postconditioning is applied in clinical settings of coronary interventions and cardiac surgery (22, 23). A limitation of the present study is the use of single doses of lovastatin in both acute and chronic treatment protocols; further studies are necessary to investigate the adequate use of statins in the case of acute myocardial ischemic events.

GRANTS


REFERENCES