Simvastatin Attenuates Oxidant-Induced Mitochondrial Dysfunction in Cardiac Myocytes

Steven P. Jones, Yasushi Teshima, Masaharu Akao, Eduardo Marbán

3-Hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase inhibitors (statins) can exert beneficial effects independently of serum cholesterol reduction by increasing the bioavailability of nitric oxide. However, it is unclear whether statins can exert such effects directly on cardiac myocytes and whether mitochondria are potential targets. Neonatal rat cardiac myocytes were cultured and subjected to oxidant stress (1 hour of 100 μmol/L H₂O₂). Mitochondrial membrane potential, a key determinant of cardiomocyte viability, was assessed by flow cytometric analysis of tetramethylrhodamine ethyl ester (TMRE)–loaded cells. Hydrogen peroxide significantly reduced mitochondrial membrane potential. Incubation of the cardiac myocytes in simvastatin (≥1 μmol/L) 1 hour before peroxide exposure significantly attenuated the loss of TMRE fluorescence. This effect was inhibited by the nitric oxide synthase inhibitor Nω-nitro-1-arginine methyl ester (L-NAME) or the ATP-sensitive mitochondrial potassium channel (mitoKₐTP) blocker 5-hydroxydecanoate. Simvastatin attenuates mitochondrial membrane depolarization after exposure to oxidant stress. These findings provide primary evidence that myocytes can act as triggers and effectors in the cardioprotective cascade of simvastatin therapy. These results bear implications of statin therapy as a potential clinical application of pharmacological preconditioning.

Materials and Methods

Cardiac myocytes were isolated from 1- or 2-day-old Sprague-Dawley rats (Zivic Laboratories, Inc, Pittsburgh, Pa) and cultured in modified DMEM media as previously described. Cardiac myocyte cultures were incubated with indicated concentrations of simvastatin for 1 hour before 0.1 mmol/L hydrogen peroxide challenge. Simvastatin (supplied by Dr D.J. Lefer, Louisiana State University Health Sciences Center, Shreveport, La) was activated via alkaline hydrolysis as previously described.

Flow Cytometric Analysis

Mitochondrial membrane potential (ΔΨₘ) was assessed by tetramethylrhodamine ethyl ester (TMRE; Molecular Probes) using a flow cytometer as previously described. TMRE (100 μmol/L) was loaded for 20 minutes in the dark at 37°C. Cardiac myocytes were subjected to flow cytometry by activation with the 488-nm wavelength. Fluorescence was monitored in the FL-2 channel.

Reagents

All chemicals were purchased from Sigma unless otherwise indicated.

Results and Discussion

Maintenance of mitochondrial membrane potential (ΔΨₘ) is necessary for production of energy (ATP) and preservation of cellular homeostasis. We have previously demonstrated that maintenance of ΔΨₘ is a critical primary determinant of myocyte survival. Figure 1A shows results of flow cytometric analysis in cardiomyocytes loaded with the ΔΨₘ indicator TMRE. Consistent with previous work, oxidative stress by H₂O₂ exposure led to a loss of ΔΨₘ as indicated by the decrease in fluorescence of the higher peak (>10²). In addition, a significant increase in the number of depolarized/dying cells is shown by the enhanced size of the lowest peak (10¹). One hour of pretreatment with 5 μmol/L simvastatin significantly attenuated the number of cells that lost high levels of fluorescence, indicating preservation of ΔΨₘ (Figure 1B). As shown in Figure 1C, the effectiveness of simvastatin in maintaining ΔΨₘ was not evident at lower concentrations of simvastatin. At concentrations greater than 1 μmol/L, simvastatin exerted protective effects that appeared maximal by 5 μmol/L. These data demonstrate that
Simvastatin preserves mitochondrial membrane potential in a dose-dependent manner in response to oxidative stress. It has previously been demonstrated that acute treatment with statins produces cardioprotective effects in vivo in a NO-dependent manner. We tested the role of NOS in simvastatin-induced cardioprotection by determining the effects of the NOS inhibitor N\textsubscript{G}-nitro-L-arginine methyl ester (L-NAME). Figures 2A and 2B show that L-NAME blocks the beneficial effects of simvastatin in a cardiomyocyte-specific system. Previous studies have identified the signal transduction pathway(s) through which acute administration of statins can posttranslationally enhance endothelial nitric oxide synthase (eNOS) activity. Namely, Kureishi et al\textsuperscript{14} found that simvastatin induced the phosphorylation (activation) of Akt, and subsequently eNOS, within 1 hour of a 1 \mu\text{mol}/L treatment. Considering the timing of the simvastatin treatment in the present study (1 hour), our findings are temporally consistent with such a posttranslational process\textsuperscript{14} and likely are independent of transcriptional changes.

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Considering prior findings indicating that NO donors can activate mitoK\textsubscript{ATP} channels,\textsuperscript{15} we questioned whether the protective effects of statins were also mitoK\textsubscript{ATP}-dependent. In Figure 2B, blockade of mitoK\textsubscript{ATP} by 5-hydroxydecanoate (5-HD) abrogated the protective effects of simvastatin. These data indicate that statins may, at least partially, operate through activation of mitoK\textsubscript{ATP} channels in cardiac myocytes. While the actual existence of mitoK\textsubscript{ATP} is not universally accepted,\textsuperscript{16} the present results link statins to the well-accepted mitochondrial mechanisms of pharmacological preconditioning.\textsuperscript{17}

The use of an isolated cardiac myocyte system demonstrates the potential of simvastatin to exert protective effects directly in cardiac myocytes in the absence of other cell types. This suggests that cardiac myocytes per se can serve as the
triggers and effectors of the pleiotropism of statins. This possibility does not exclude a role for the vasculoprotective hypothesis of the cholesterol-independent effects of statins. It is indeed likely that statins exert protective effects both in endothelial cells and in cardiac myocytes, and that interactions between the two cell types may occur in vivo.

The use of isolated neonatal myocytes may limit the application of these findings with respect to the intact adult heart. Challenging myocytes with oxidative stress is unlikely to be a solitary cause of cardiac injury in vivo. The dose of simvastatin presented here is also higher than the therapeutic concentration expected in humans. Accordingly, we performed additional experiments to evaluate whether lower (nmol/L) doses of simvastatin for longer durations (24 hours) would produce similar effects to the high-dose acute administration shown here. Such a dosing regimen was unsuccessful in protecting cardiac myocytes (data not shown). However, it is impossible to make direct dosage comparisons between species and between isolated cells and plasma. Furthermore, the doses of simvastatin used in the present study are similar to those used by others in isolated cell models.14,18,19

Presently, we demonstrate a novel paradigm whereby simvastatin, via NOS and mitoKATP channels, results in cardioprotection in cardiac myocytes. These data provide additional insight into the mechanisms of the protective effects of statins. According to the current paradigm, statins activate NOS and promote the vascular bioavailability of NO. The protective effects of NO on the vasculature are well characterized and widely appreciated. In the intact heart, it is likely that statins are exerting effects on multiple cell types. Here, we provide primary evidence that cardiac myocytes may also be beneficiaries of the protective effects of statins. Previous work supports a scenario in which statins act at the vascular level to prevent the occurrence of ischemia and improve perfusion after ischemia both by preventing thrombotic events and maintaining vasorelaxation. Our findings augment this understanding by demonstrating that myocytes are protected at the mitochondrial level, an idea for which significant evidence exists in vivo.20

The clinical evidence21 for cholesterol-independent effects of statins is extensive and growing. The pleiotropic effects of statins may be largely mediated by NO,2 which can activate mitoKATP channels15 and induce cardioprotection.6 The possibility that statins exert effects similar to preconditioning in the clinical setting is intriguing. This may lead one to question whether potential clinical ramifications of pharmacological preconditioning with statins are currently overlooked, a possibility worthy of further examination.

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References

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