Original Research

Statin (Mevalotin) preconditioning decreases infarct size in senile rat myocardial infarction model

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Abstract

Aim: Although statins have been proved to have cardioprotective effects, it remains to be investigated whether “statin-induced preconditioning (PC)” can reduce the infarct size for senile animals.

Methods: Senile Wistar rats (weighing 350–450 g) were randomly assigned to one of 14 experiment groups (n ≤ 5 for each group). All hearts underwent 30 minutes of coronary artery occlusion and 2 hours of reperfusion (control procedure). Ischemic PC was elicited by three cycles of 5-minute occlusion and 5-minute reperfusion. Statin PC was performed by administering Mevalotin (1 mg/kg, the optimal dose being determined by a dose–effect study) 30 minutes prior to the control procedure. In addition, pretreatment with glibenclamide, 5-hydroxydecanoic acid, chelerythrine, genistein, and NG-nitro-4-arginine methyl ester, or their combination, was also performed to test possible signaling mechanisms.

Results: Statin PC decreased the infarct size significantly compared with the control group (22 ± 4% vs. 53 ± 4%, p < 0.01). Pretreatment with NG-nitro-4-arginine methyl ester completely blocked the effects of Mevalotin (infarct size: 48 ± 4% vs. 22 ± 4%, p < 0.01). The cardioprotective effects of statin PC could be blunted by antagonism of mitochondrial adenosine triphosphate (ATP)-sensitive potassium and sarcolemmal ATP-sensitive potassium channels (glibenclamide: 50 ± 4% vs. 22 ± 4%, p < 0.01; 5-hydroxydecanoic acid: 50 ± 4% vs. 22 ± 4%, p < 0.01). In addition, protein kinase C antagonist (chelerythrine) and tyrosine kinase antagonist (genistein) also have similar effects (chelerythrine: 48 ± 4% vs. 22 ± 4%, p < 0.01; genistein: 45 ± 4% vs. 22 ± 4%, p < 0.01).

Conclusion: It is established in this study the myocardial PC caused by statins, that is involved cellular mechanisms including mainly the pathway of endothelial nitrous oxide synthase (eNOS)—nitrous oxide donation—ATP-sensitive potassium channels. Protein kinase C and tyrosine kinase have regulatory effects.

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1. Introduction

Preconditioning (PC) involves a pretreatment or pre-maneuver that is able to decrease myocardial damage after a severe decrease in coronary flow. We have demonstrated many modes of PC in previous experiments to show cardioprotective effects.1–3 The term “preconditioning” means a powerful experimental tool that can reduce the infarct size (IS) after ischemia—reperfusion or other pretreatments such as adrenergic stimulation,4 activation of adenosine receptors5 and adenosine triphosphate (ATP)-sensitive potassium (K_{ATP}) channels,6 and induction of heat shock proteins6 and oxidative stress.8 Some investigators have further studied the possible effector cells of such PC. Besides neutrophils, which we earlier proposed to be affected by PC,1 endothelial cells are the main target cells at present.2,9–11

Activation of G protein-linked phospholipase C, tyrosine kinase pathways, and protein kinase C, and generation of
oxygen radicals are usually involved in PC. In addition, KATP channels are considered the most possible triggers and effectors of PC. Recent studies suggested that mitochondrial KATP channels (MitoKATP channels), in addition to sarcolemmal KATP channels, might account mainly for the protective mechanism. MitoKATP channel opening has been proved to be related to mitochondrial swelling and optimization of respiration, prevention of mitochondrial calcium overload, and control of reactive oxygen radicals, all of which are possibly linked to cardioprotection during severe myocardial ischemia and infarction.

As mentioned above, cellular targets of PC seem to be heterogeneous. Although saline-perfused isolated hearts or isolated cardiomyocytes can be preconditioned, the so-called vascular component of PC has been presented. The concept is concerned with the reduction of postsischemic inflammation or mitigation of leukocyte adhesion in the perfused vascular bed after PC. Endothelial cells, the interface between parenchymal tissue and blood cells, are involved in the significant pathophysiological process.

On the other hand, statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) have been available for decades. Some clinical trials have indicated that statins appear to have beneficial effects independent of their cholesterol-lowering properties, when compared with other agents. These so-called pleiotropic properties include anti-inflammatory effects, plaque stabilization, improved endothelial function, and inhibition of vascular smooth muscle cell proliferation. The beneficial pleiotropic effects of statins in protecting the isolated murine hearts against lethal reperfusion injury via activation of phosphorylidyinositide 3-kinases (PI3K)/Akt prosurvival signaling pathway and its downstream effectors, endothelial nitrous oxide synthase (eNOS), were also demonstrated. In addition, phosphorylation of multiple prosurvival pathways involving p44/p42 Mitogen-activated protein kinases (MAPK), p38 MAPK, and heat shock protein (HSP) 27 were also demonstrated in a later study.

It is well known that statins are associated with nitrous oxide synthase (NOS) activation. Nitrous oxide (NO) is a unique intra/intercellular messenger involved in the regulation of many physiological functions. Any compounds that modulate NO release may display a great therapeutic value.

Different statins such as lovastatin, atorvastatin, pravastatin, and simvastatin demonstrate variable potency to enhance the release of nitrous oxide (NO). The role of nitrous oxide synthase (NOS) activated by statins. In addition, some tyrosine kinase, their antagonists, chelerythrine (2 mg/kg, 30 minutes prior to ischemic PC with or without statin pretreatment, and also prior to statin PC), or their combination for synergic effects, were also given as a pretreatment in various groups. To test the role of NOS in statin-induced cardioprotection, we also determined the effects of the NOS inhibitor NG-nitro-4-arginine methyl ester (L-NAME). L-NAME (50 mg/kg) was administered 30 minutes prior to ischemic PC with or without statin pretreatment, and also prior to statin PC.

In summary, the rats were assigned to the following groups for carrying out the experiments:

1. Control group: 30-minute occlusion + 2-hour reperfusion (control procedure)
2. Ischemic PC: (5-minute occlusion + 5-minute reperfusion) × 3 + control procedure
3. Statin PC: Mevalotin 30 minutes + control procedure

We designed the following in vivo study to investigate the presence of the phenomenon “statin-induced PC” and its cellular mechanisms.
4. L-NAME + ischemic PC: L-NAME + (5-minute occlusion + 5-minute reperfusion) × 3 + control procedure
5. L-NAME + statin PC: L-NAME + Mevalotin 30 minutes + control procedure
6. Glibenclamide + ischemic PC: glibenclamide + (5-minute occlusion + 5-minute reperfusion) × 3 + control procedure
7. Glibenclamide + statin PC: glibenclamide + Mevalotin 30 minutes + control procedure
8. 5-HD + ischemic PC: 5-HD + (5-minute occlusion + 5-minute reperfusion) × 3 + control procedure
9. 5-HD + statin PC: 5-HD + Mevalotin 30 minutes + control procedure
10. Chelerythrine + ischemic PC: chelerythrine + (5-minute occlusion + 5-minute reperfusion) × 3 + control procedure
11. Chelerythrine + statin PC: chelerythrine + Mevalotin 30 minutes + control procedure
12. Genistein + ischemic PC: genistein + (5-minute occlusion + 5-minute reperfusion) × 3 + control procedure
13. Genistein + statin PC: genistein + Mevalotin 30 minutes + control procedure
14. Sham group

2.3. Determination of IS

As previously described, the IS and the area at risk of ventricular myocardium were measured by planimetry after incubation with 1% triphenyltetrazolium (Sigma Co., St Louis, MO, USA) in phosphate buffer (pH 7.4) and 10% formalin. The volume of the left ventricle, the infarct volume, and the volume of the area at risk were then calculated by multiplying each area by the slice thickness and summing the products. The IS was expressed as a percentage of the area at risk.

2.4. Measurement of eNOS messenger RNA by reverse transcriptase-polymerase chain reaction

Myocardial tissue samples were obtained from the rats injected with Mevalotin (~1 mg/kg) or saline vehicle and rapidly frozen in liquid nitrogen. Total RNA was extracted from mouse heart, and reverse transcriptase-polymerase chain reaction (RT-PCR) was performed as described previously. The mouse eNOS fragment was amplified using the sense primer (5′-GCAGAAGAGTCCAGCGAACA-3′) and the antisense primer (5′-GCCAGCCCAACACACAAAGTC-3′). Glycerinaldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal control. The mouse GAPDH fragment was amplified using the sense primer (5′-CGGAGTCAACG GATTTGGTCGTAT-3′) and the antisense primer (5′-AGCCT TCTCCATGGTTGGTAAGAC-3′). The expression of eNOS messenger RNA (mRNA) was normalized to the level of GAPDH mRNA in each experiment.

2.5. Chemicals

All chemicals were purchased from Sigma Co. except those that were particularly mentioned. Mevalotin was obtained from the Laboratory of Sankyo Co., Inc. (Tokyo, Japan). Mevalotin was injected intraperitoneally as a single dose of 1 mg/kg prior to the corresponding experiments.

2.6. Statistical analysis

All values are expressed as mean ± the standard error of the mean. Analysis of variance, using Newman–Keuls post hoc test, was used to determine whether any significant differences existed among groups with regard to the IS and area at risk. A value of p < 0.05 was considered to be statistically significant.

3. Results

3.1. Cardioprotection from statin PC

We determined the decrease of the IS after statin PC using the triphenyltetrazolium method, as described above. Pretreatment with statin reduces the IS significantly (Fig. 1; 22 ± 4% vs. 53 ± 4%, p < 0.01). The cardioprotective effect provided by statin PC is comparable to that provided by ischemic PC (Fig. 1; 24 ± 4% vs. 53 ± 4%, p < 0.01).

3.2. Mechanisms of statin PC

To investigate the cellular mechanisms of statin PC, we applied various agents prior to statin PC. The main results are depicted in Figs. 1 and 2. Pretreatment with L-NAME completely blocked the effects of IS reduction provided by statin PC (Fig. 1; 48 ± 4% vs. 22 ± 4%, p < 0.01). Both MitokATP and sarcolemmal KATP channels can antagonize the cardioprotective effects of statin PC (Fig. 1; glibenclamide: 50 ± 4% vs. 22 ± 4%, p < 0.01; 5-HD: 50 ± 4% vs. 22 ± 4%, p < 0.01). In addition, protein kinase C antagonist (chelerythrine) and tyrosine kinase antagonist (genistein) also have
similar effects (Fig. 1; chelerythrine: 48 ± 4% vs. 22 ± 4%, p < 0.01; genisteine: 45 ± 4% vs. 22 ± 4%, p < 0.01).

On comparison with Fig. 2, we found that the cellular mechanisms of statin PC are similar to those of ischemic PC. These observations imply that the donation of NO to the downstream KATP channels remains the key pathway involved in myocardial PC.

### 3.3. Cardiomyocyte eNOS activation by statin PC

Activation of myocardial eNOS activation was observed in the myocardium treated with statin PC (1.60 ± 0.2-fold vs. control, p < 0.05). L-NAME can block the statin-induced eNOS mRNA expression completely. However, antagonism of sarcolemmal K<sub>ATP</sub> channels, tyrosine kinase C, MitoK<sub>ATP</sub> channels, and protein kinase C all restore and even enhance the activation of eNOS mRNA (see Fig. 3). These results may imply that eNOS—NO donation—MitoK<sub>ATP</sub> form a positive feedback.

To prove these points, we investigated the eNOS mRNA expression by RT-PCR. Surprisingly, the results were almost the same as those observed in the case of statin PC. Activation of myocardial eNOS was observed in the myocardium treated with ischemic PC (1.57 ± 0.2-fold vs. control, p < 0.05). L-NAME can block the statin-induced eNOS mRNA expression completely. Antagonism of sarcolemmal K<sub>ATP</sub> channels, tyrosine kinase C, MitoK<sub>ATP</sub> channels, and protein kinase C restores and even enhances the activation of eNOS mRNA. These results may further confirm that there is a positive feedback in eNOS—NO donation—MitoK<sub>ATP</sub> channels.

### 4. Discussion

This study demonstrated that preadministration of statin can provoke cardioprotective effects and reduce the IS in a rat myocardial infarction model. Cardioprotective effects are, at least partially, related to the activation of the myocardial eNOS system and K<sub>ATP</sub> channels, which are modified by an additional signaling pathway such as the tyrosine kinase and protein kinase C system. There are positive feedbacks between the tyrosine kinase/protein kinase C system and eNOS—NO—MitoK<sub>ATP</sub> channels.

Statins are well known to be associated with NOS activation. NO is a unique intra/intercellular messenger involved in the regulation of many physiological functions such as central and peripheral neurotransmission, smooth muscle contractility, platelet reactivity, and angiogenesis, among others. Inappropriate production of NO has been linked to a number of pathologies; therefore, compounds that modulate its release may display a great therapeutic value. NO is generated by a family of enzymes known as NOS, named after the tissues in which they were originally described. However, eNOS and neuronal NOS are constitutively expressed not only in endothelia and neurons, but also in many other cell types. They release NO in response to receptor stimulation after activation of the Ca<sup>2+</sup>/calmodulin-dependent pathway. More recently, different kinds of stimuli have been proved to activate eNOS in a Ca<sup>2+</sup>/calmodulin-independent way. It has been reported that basic fibroblast growth factor (FGF), similar to what was reported for bradykinin, is able to activate eNOS in Chinese hamster ovary-k1 cells, via the sphingomyelinase-dependent generation of ceramide, causing translocation of eNOS from the plasma membrane, where it is bound to caveolin-1, to the cytosol in its active form. Among the different roles attributed to NO in physiology and pathology, its involvement in carcinogenesis and angiogenesis has achieved great importance recently.

NO has been reported to induce vascular endothelial growth factor expression in carcinoma cells and neovascularization in tumors. Moreover, angiogenic factors, including both basic FGF and vascular endothelial growth factor, are powerful NOS activators. Thus, identification of compounds with antiangiogenic properties is of pivotal relevance for the inhibition of tumor growth, and substances able to block NO production may be active in this respect. The tested statins, i.e., lovastatin, atorvastatin, pravastatin, and simvastatin, demonstrate variable potency to enhance the NO/oxygen concentration ratio after stimulation of NOS, resulting in an increase of NO bioavailability in endothelial cells.
It has been proved that early administration of statins can minimize the deteriorating effect of transient cerebral or myocardial ischemia in animal models.31,32 These and other studies elucidate a role for NOS activated by statins.33 In addition, some statins have been proved to attenuate oxidant-induced mitochondrial dysfunction in cardiac myocytes.34,35 These basic studies supported the clinical observation that statins have certain cardioprotective effects. For example, the incidence of myonecrosis in patients undergoing rotational revascularization after coronary artery bypass grafting was significantly lower among patients pretreated with statins. This difference remained significant after multivariable and propensity adjustment.35

Our study has certain limitations. The first is that there is a dose–effect relationship between the glibenclamide dose and its effect on ischemic PC and statin PC, as evident from our data. As mentioned previously, the doses of glibenclamide that proved to provide cardioprotective effects mimicking ischemic preconditioning animals were used for experiments. Further study designs among animals of different age groups. The results of this study elucidate a role for NOS activated by statins.33 IN vivo pharmacokinetics may be complicated and even individualized. However, we believe that such a model can mimic actual pathophysiological conditions in clinical settings.

The second limitation is that we cannot demonstrate whether the cellular mechanisms of statin PC are different among animals of different age groups. The results of this study are similar to those of previous studies in which younger animals were used for experiments. Further study designs comparing the degree of cardioprotective effects of statin and the differences in possibly involving signaling pathways may be needed.

In this study, we have focused upon the acute cardioprotective effects of statin PC. It remains to be answered whether such protective effects persist or whether delayed statin PC can occur.

In summary, Mevalotin, similar to other statins, has been proved to provide cardioprotective effects mimicking ischemic PC. Activated myocardial eNOS system and K\textsubscript{ATP} channels are the main signaling pathways.

Conflict of interest

The author has no conflicts of interest to declare.

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References


