Regulation of endothelial function in coronary microcirculation by HMG-CoA reductase drugs

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Introduction

First introduced into clinical practice in the late 1980s, several years after the discovery of their lipid-lowering effects[1], the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) reduce cholesterol synthesis through a competitive inhibition of HMG-CoA reductase, an enzyme catalyzing the rate-limiting conversion step of HMG-CoA to mevalonate[2]. This leads to increased expression of low-density lipoprotein (LDL) receptors[3], ultimately leading to reduction of LDL plasma concentration.

Ample epidemiological data have suggested that hypercholesterolemia is a powerful risk factor for coronary heart disease[4,5]. In addition, different cholesterol-lowering drugs or non-pharmacological treatments can significantly reduce morbidity from coronary heart disease[6-14], thus proving a causal role for cholesterol in coronary events. For many years, all the beneficial effects of statins were attributed to their cholesterol-lowering effect. Nevertheless, quite recently, statins were found to exert direct cardiovascular effects which are independent of their cholesterol-lowering effect, and are not directly attributable to a reduction in serum cholesterol levels.

Regarding cardiovascular effects of statins, an important emerging role is represented by their regulatory effect in coronary microcirculation and endothelial function (Fig. 1).

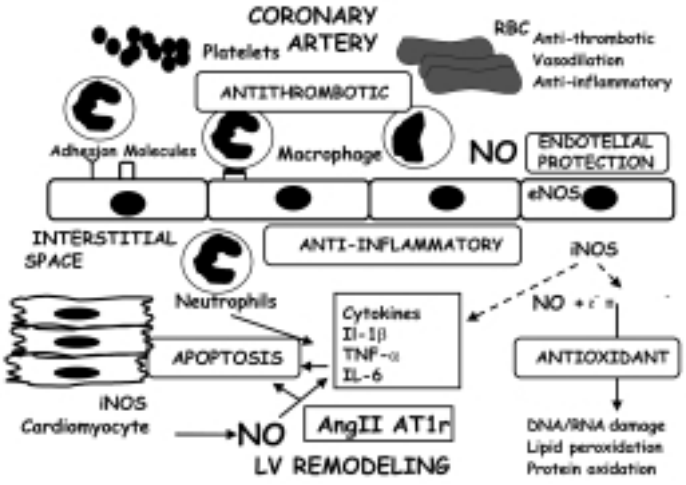

Figure 1. *Schematic representation of the effects of statins on coronary circulation and cardiac function. Statins preserve endothelial function and exert antioxidant, anti-inflammatory, antiapoptotic and antithrombotic effects that are endothelium-protective during hypoxia, ischemia and reperfusion and in the presence of endothelial dysfunction. This cardioprotection could be also relevant in preventing or reducing the remodeling process of the left ventricle. AngII = angiotensin II; AT1r = angiotensin type 1 receptors; eNOS = endothelial nitric oxide synthase; IL = interleukin; iNOS = inducible nitric oxide synthase; LV = left ventricular; NO = nitric oxide; O_2^- = superoxide anion; $ONOO^-$ = peroxynitrite; RBC = red blood cells; TNF = tumor necrosis factor.*

Effects of statins on endothelial function and coronary microcirculation

Statins exert beneficial effects on coronary microcirculation due to either cholesterol-dependent and cholesterol-independent mechanisms. The main pathophysiological mechanisms are summarized in table I.

Statins are able to restore endothelial dysfunction and improve coronary microcirculation by their antithrombotic, antioxidant, antiatherosclerotic, anti-inflammatory and endothelial protective effects. They also significantly contribute to plaque stabilization and, sometimes, regression[15,16].

All these effects attenuate endothelial dysfunction in cardiovascular disease states including coronary artery disease, myocardial ischemia/reperfusion, cerebral ischemia, and diabetes.

The major factor contributing to endothelial dysfunction in these pathological conditions appears to be the reduced production or bioavailability of nitric oxide (NO) in endothelial cells. NO produced by the constitutive endothelial NO synthase (eNOS, NOS III) has a protective role in ischemic conditions, regulating leukocyte and platelet adhesion and activation[17], inducing vasodilation and reducing post-ischemic hyperpermeability, and maintaining the antithrombotic properties of the vessel wall[18,19]. The inducible form of NOS (iNOS, NOS II), produced by neutrophils and cardiomyocytes after stimulation with a series of proinflammatory mediators, also contributes to the inflammatory response in conjunction with cytokines such as tumor necrosis factor-α, interleukin-1β and interleukin-6[18]. An excessive NO production following inflammatory stimulation, leads to an enhanced reaction of NO with superoxide anion, with increased production of peroxynitrite, and may in turn be responsible for increased peroxidative damage in the vascular wall[20,21]. This occurs through enhanced cardiomyocytic and endothelial cell apoptosis and the promotion of oxidative damage of cellular DNA/RNA, proteins and membrane lipids. Statin therapy seems to favorably modify the balance of NO production. It was not until 1997 that evidence was obtained showing that statins directly enhance the biosynthesis of NO by increasing the half-time of the mRNA for eNOS from 13 to 38 hours[22]. This effect also occurs in normocholesterolemic conditions. Statins were also shown to exert this NO promoting effects by inhibiting the biosynthesis of L-mevalonate (precursor of cholesterol) and of the isoprenoids, including geranylgeranylpyrophosphate and farnesilpyrophosphate. Isoprenoids are important agents in the post-translational modifications of a variety of proteins including eNOS, and Ras-like proteins such as Rho proteins. The inhibition of Rho results in a 3-fold increase in eNOS and nitrite generation, since Rho is a relevant inhibitor of NO generation[23]. Farnesylation is necessary for the anchoring of G-proteins such as p21^{ras} to cell membrane. This modulates receptor-mediated eNOS activity through effects on membrane fluidity and cell growth[24]. Farnesylation of the γ-subunit of certain G-proteins may also be involved in the modulation of Ca^{2+} entry, an important step in reperfusion injury[25-27].

In normocholesterolemic rat hearts, statins decrease polymorphonucleate adhesion after ischemia/reperfusion[28]. In isolated perfused working hearts from normocholesterolemic rats we have recently demonstrated that simvastatin administration before ischemia reduces myocardial damage after ischemia and reperfusion by increasing eNOS and reducing iNOS overexpression that characterized reperfusion period[29,30]. The inhibition of iNOS overexpression may suppress the inflammatory response accompanying acute ischemia, which contributes to the ischemic damage. The modulation of NOS activity induces an improvement in coronary flow, a reduction in coronary resistances and in

Table I. Main protective properties of statins in coronary circulation.

Cholesterol-dependent mechanisms
Stabilization/regression of atherosclerosis
Favorable hemorrheological and antithrombotic properties
Cholesterol-independent mechanisms
- Endothelium
Modulation of eNOS, nNOS and iNOS
Inhibition of leukocyte and platelet adhesion
Vasodilation
Maintenance of a thromboresistant interface between the bloodstream and the vessel wall
- Inflammatory processes
Reduction of monocyte adhesion molecules (CD11b/CD18-isoprenylation of leukocyte G-proteins)
Reduced isoprenoid-dependent anchoring
Reduced dimerization of adhesion molecules such as CD11b/CD18 on monocytes
Modulation of cytokine production by decreasing isoprenylation of proteins involved in intracellular signaling and inflammation
Modulation of adhesion molecule expression on endothelium and inflammatory cells
Reduced inhibition of inflammatory cell migration
Reduction of thrombogenesis through inhibition of tissue factor expression
Reduction of IL-1β, TNF-α-induced apoptosis
- Antioxidant effects
Reduction of lipoprotein oxidation and amelioration of free radical injury
Increase of α-tocopherol/total cholesterol ratio
Preservation of SOD activity
Preservation of the paraoxonase system
- Left ventricular remodeling
Reduction of angiotensin II-induced hypertension, cardiac hypertrophy and fibrosis
Down-regulation of angiotensin type 1 receptor

eNOS = endothelial nitric oxide synthase; IL = interleukin; iNOS = inducible nitric oxide synthase; nNOS = neuronal nitric oxide synthase; SOD = superoxide dismutase; TNF = tumor necrosis factor.

vascular post-ischemic hyperpermeability. The causal role of NO in simvastatin-induced myocardial and vascular protection in our system is proven by the loss of these favorable effects in the presence of the NOS inhibitor N$^{\omega}$-nitro-L-arginine methyl ester[30].

In addition to NO modulation, an important contribution in coronary microcirculation modulation can be ascribed to the anti-inflammatory and antiatherothrombotic effects of statins. HMG-CoA reductase inhibitors have been shown to inhibit several pathways in inflammatory processes occurring in plaque destabilization, myocardial hypoxia, ischemia and reperfusion. Statins reduce leukocyte-endothelium interactions occurring either in hypercholesterolemic[31] or in normocholesterolemic conditions[32,33], and inhibit monocyte adhesion to the endothelium by reducing the endothelial expression of ICAM-1 and VCAM-1, as well as the number of monocytes expressing Mac-1, one of the ICAM-1 ligands[32]. In hypercholesterolemic humans, simvastatin and lovastatin reduce monocyte expression of CD11b/CD18, another ICAM-1 ligand[31]. These effects, mediated through reduced isoprenylation of leukocyte G-proteins or reduced isoprenoid-dependent anchoring or dimerization of adhesion molecules such as CD11b/CD18 on monocytes, are involved in reducing ischemic cell damage after coronary artery occlusion. Anti-inflammatory effects of statins have recently been confirmed by an analysis of the CARE study showing a reduction in cerebral and coronary ischemic events, during pravastatin treatment, related to reduced C-reactive protein levels[34].

Statins also exert beneficial antithrombotic effects. Statins reduce enhanced platelet reactivity accompanying hypercholesterolemia, although it is not clear if such an effect occurs independent of cholesterol reduction[35] and the thrombogenic potential of atheroma[36], also independent of cholesterol levels[37]. Statins also directly influence the local fibrinolytic balance [inhibiting plasminogen activator inhibitor-1 (PAI-1) expression and increasing tissue-type plasminogen activator (t-PA) within the vessel wall], promoting fibrinolysis and thus reducing the thrombotic risk after plaque rupture[38,39]. By reducing the local expression of PAI-1 by smooth muscle cells within vascular lesions while increasing t-PA expression by luminal endothelial cells, statins may tip the local fibrinolytic balance towards increased fibrinolysis, which would limit the extent of thrombus formation that follows plaque rupture. On the other hand, increased local fibrinolysis may also promote extracellular matrix degradation (activation of matrix metalloproteinases) that, in turn, may destabilize advanced atherosclerotic plaques[39].

Recently, in normocholesterolemic conditions, the statin-mediated improvement of endothelial function was associated with a reduced production of reactive oxygen species and a down-regulation of angiotensin type 1 receptor in hypertension and with an improved left ventricular remodeling and function in rats with heart failure. This effect was associated with an attenuated left ventricular expression of fetal myosin heavy chain isoenzymes and collagen I. Statin treatment may retard the progression of chronic heart failure, ameliorating angiotensin II-induced hypertension, cardiac hypertrophy, fibrosis, and remodeling independently of cholesterol reduction. Although the clinical significance remains uncertain, the results suggest that statins interfere with angiotensin II-induced signaling and transcription factor activation, thereby ameliorating remodeling and reducing progression to heart failure[40,41].

Conclusions

Statins are widely used lipid-lowering agents; they are effective in reducing serum cholesterol level and attenuate atherosclerosis and endothelial dysfunction and all of its circulatory effects. However, statins are also able to exert a variety of important cardiovascular protective effects independently of this antiatherosclerotic effect. Many of these effects are related to the preservation of vascular endothelium in many clinical conditions, including myocardial ischemia or infarction. In the preservation of coronary microcirculation a pivotal role is represented by the modulation of NO synthesis and bioavailability. This maintains a physiological level of NO in the endothelial cell which preserves vascular reactivity, down-regulates thrombotic mechanisms and exerts important anti-inflammatory actions by attenuating leukocyte-endothelial cell interaction. In addition, statins seem able to reduce the iNOS overexpression that characterizes the reperfusion period after an ischemic event, limiting the following peroxidative stress and mechanical dysfunction of myocardial tissue. For all these reasons, statins could represent a valid, novel pharmacological approach able to restore endothelial function in various pathological conditions which have in the endothelial dysfunction a common denominator like hypertension, hypercholesterolemia, diabetes, ischemic disease and heart failure.

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