

Antioxidative effects of statins

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Received: 17 June 2008 / Accepted: 15 July 2008 / Published online: 1 August 2008
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Abstract Abstract HMG CoA reductase inhibitors (statins) have been shown to be effective lipid lowering agents and are beneficial in the primary and secondary prevention of coronary heart disease. However, the overall benefits observed with statins appear to be greater than what might be expected from changes in lipid levels alone and the positive effects have only partially been reproduced with other lipid lowering drugs, suggesting effects in addition to cholesterol lowering. In experimental models, many of the cholesterol-independent effects of statins are mediated by inhibition of isoprenoids, which serve as lipid attachments for intracellular signalling molecules such as small Rho guanosine triphosphate-binding proteins, whose membrane localization and function are dependent on isoprenylation. This review summarizes the effects of statins on endothelial function and oxidative stress.

Keywords Statin · Prevention · Endothelial dysfunction · Oxidative stress · Review

Introduction

The primary mechanism of HMG CoA reductase inhibitors (statins) is the lowering of serum cholesterol levels via inhibition of hepatic cholesterol synthesis and subsequent upregulation of low-density lipoprotein (LDL)-receptors in the liver (Goldstein and Brown 1990). However, recent

evidence suggests that statins have beneficial effects beyond cholesterol lowering and in extra-hepatic tissues. Experimental and clinical evidence revealed that several important regulators of the cardiovascular system can be regulated by statins. Prominent candidates are the endothelial NO synthase (eNOS), endothelin, free oxygen radicals, MHC-II, protein kinase Akt and the metalloproteinases. This article reviews the effects of statins on endothelial dysfunction and oxidative stress.

Properties of HMG-CoA reductase inhibitors

The rate-limiting enzyme in cholesterol biosynthesis in the liver is HMG-CoA reductase (Goldstein and Brown 1990) (Fig. 1). HMG-CoA reductase catalyses the four-electron reductive deacylation of HMG-CoA to CoA and mevalonate which is the committed and rate-limiting step in cholesterol biosynthesis (Rodwell et al. 1976). All HMG-CoA reductase inhibitors (statins) share an HMG-like moiety and inhibit the reductase by the same mechanism. The bulky, hydrophobic compounds of statins blocks the access of the substrate HMG-CoA to the reductase by occupying the HMG-binding pocket and part of the binding surface for CoA (Istvan and Deisenhofer 2001). The tight binding of statins is due to the large number of van der Waals interactions between inhibitors and HMG-CoA reductase. The liver is the primary place of action of all statins. The extra-hepatic plasma concentration and permeability, e.g. into vascular cells, differs between statins and depends mainly on their lipophilicity (McTaggart et al. 2001; Lea and McTavish 1997; Corsini et al. 1995; Blum 1994). Inhibition of cholesterol synthesis in hepatocytes upregulates the expression of the hepatic LDL receptor. As a consequence, LDL and its precursors are cleared from the circulation

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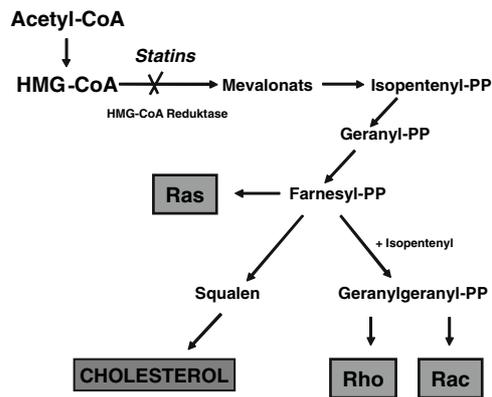


Fig. 1 Inhibition of mevalonate synthesis not only blocks the synthesis of cholesterol but also the isoprenoid-intermediates of the cholesterol pathway. The isoprenoid geranylgeranylpyrophosphate plays an important role for the posttranslational modification of proteins. The membrane translocation and activity of the small GTP-binding proteins Rho and Rac depend on their geranylgeranylation. Ras translocation from the cytoplasm to the plasma membrane is dependent on farnesylation. Statins inhibit small G protein isoprenylation and function

(Goldstein and Brown 1990). Furthermore, statin treatment also increases the plasma concentration of antiatherogenic HDL and apo A-I (Vega and Grundy 1998). In addition to lowering cholesterol synthesis, inhibition of the HMG-CoA reductase reduces the synthesis of intermediates of the mevalonate pathway (Goldstein and Brown 1990).

Mechanism mediating cholesterol-independent effects of HMG-CoA reductase inhibitors

An important mechanism underlying cholesterol independent effects relates to the inhibition of isoprenoid intermediates of the cholesterol synthesis pathway (Liao and Laufs 2005). Statins work by reversibly inhibiting HMG-CoA reductase through side chains that bind to the enzyme's active site and block the substrate-product transition state of the enzyme (Istvan and Deisenhofer 2001). Thereby statins competitively inhibit the synthesis of L-mevalonic acid, the immediate product of HMG-CoA reductase. At the same time, statins prevent the synthesis of other important isoprenoid intermediates of the cholesterol biosynthetic pathway, such as farnesylpyrophosphate and geranylgeranylpyrophosphate (Goldstein and Brown 1990) (Fig. 1). Isoprenoids are important lipid attachments for the posttranslational modification of a variety of proteins, like small guanosine triphosphate (GTP)-binding protein Ras; and Ras-like proteins, such as Rho, Rab, Rac, Ral, or Rap for example (Van and D'Souza-Schorey 1997). Thus, protein isoprenylation permits the covalent attachment, subcellular localization, and intracellular trafficking of membrane-associated proteins. Members of the Ras and Rho GTPase

family are major substrates for posttranslational modification by prenylation (Hall 1998; Van and D'Souza-Schorey 1997). Rac, Ras and Rho are small GTP-binding proteins, which cycle between the inactive GDP-bound state and active GTP-bound state. In endothelial cells, Rho translocation is dependent on geranylgeranylation, whereas Ras translocation from the cytoplasm to the plasma membrane is dependent on farnesylation (Laufs et al. 1998; Laufs and Liao 1998). Statins inhibit Rac, Ras and Rho isoprenylation, leading to the accumulation of inactive Rac, Ras and Rho in the cytoplasm. Because Rho is major target of geranylgeranylation, inhibition of Rho and its downstream target, Rho-kinase, is a likely mechanism mediating some of the cholesterol-independent effects of statins on the vascular wall (Laufs et al. 2000). The members of the Rho GTPase family, which consists of RhoA, Rac and Cdc42, serve specific functions in terms of cell shape, motility, secretion and proliferation, although overlapping functions between the members could be observed in overexpressed systems. The distinct but complementary functions of Rho family members also extend to their effects on cell signaling. It is therefore not surprising to find that Rho-induced changes in the actin cytoskeleton and gene expression are related (Tapon and Hall 1997).

Endothelial function

Hypercholesterolemia causes endothelial dysfunction, which is an early manifestation of atherosclerosis and occurs even in the absence of angiographic evidence of disease (Libby et al. 1991, Libby 1995). Rapid lowering of plasma LDL cholesterol by apheresis improves endothelium-dependent vasodilatation (Tamai et al. 1997). These observations along with many other studies show that lowering of serum cholesterol levels is an important mechanism of the beneficial vascular effects of statins. Interestingly, in some studies with statins, restoration of endothelial function occurs before significant reduction in serum cholesterol levels (O'Driscoll et al. 1997; Anderson et al. 1995; Treasure et al. 1995), suggesting that there are additional effects on endothelial function beyond cholesterol reduction. Improvement of NO-dependent endothelial function is one of the clinical hallmarks of statin treatment and can be observed very rapidly. E.g., treatment with statins has been shown to improve coronary endothelial function within one day, significantly before serum cholesterol starts to fall (Liao and Laufs 2005; Wassmann et al. 2003; Laufs et al. 2001). An important characteristic of endothelial dysfunction is the impaired synthesis, release and activity of endothelial-derived nitric oxide (NO). Endothelial NO has been shown to inhibit several components of the atherogenic process. For example, endothelium-derived

NO mediates vascular relaxation and inhibits platelet aggregation, vascular smooth muscle proliferation and endothelial-leukocyte interactions. Furthermore, inactivation of NO by superoxide radical (O_2^-) limits the bioavailability of NO and leads to nitrate tolerance, vasoconstriction and hypertension (Harrison 1997; Munzel et al. 1995).

Endothelial dysfunction is an important and early marker of atherosclerosis (Liao 1998). The loss of NO contributes to the atherogenic process. On the other hand, hypercholesterolemia plays an important role in decreasing endothelial production and in increasing degradation of NO (Hernandez-Perera et al. 1998; Laufs et al. 1997; Liao 1994; Liao et al. 1995). One of the earliest effects of statin treatment is a rapidly increased bioavailability of NO (John et al. 2001; Tsunekawa et al. 2001; Kaesemeyer et al. 1999; O'Driscoll et al. 1997; Anderson et al. 1995) mediating improvement of endothelium-dependent vasodilatation. Increased endothelial NO release has been reported as early as 3 days after initiation of treatment (John et al. 2001; Tsunekawa et al. 2001). Upregulation of eNOS expression is independent of cholesterol levels (Feron et al. 2001; Amin-Hanjani et al. 2001; Wagner et al. 2000; Laufs et al. 1999; Tannous et al. 1999; Kaesemeyer et al. 1999; Hernandez-Perera et al. 1998; Williams et al. 1998; Laufs et al. 1998). On the molecular level, upregulation of endothelial NO production is mediated by several complementary mechanisms: reduction of LDL improves endothelial NO, in addition, statins increase eNOS mRNA stability by inhibiting the rhoA GTPase-dependent actin cytoskeleton (Laufs and Liao 1998, 2000). Additional important effects of statin treatment on eNOS function include the activation of protein kinase Akt (Kureishi et al. 2000), inhibition of calveolin (Laufs 2003) and activation of the phosphatidylinositol 3-kinase/protein kinase Akt (PI3K/Akt) pathway (Kureishi et al. 2000; Fulton et al. 1999). The important role of NO on endothelial dysfunction is also influenced by superoxide radicals. An imbalance between the production of superoxide radicals and NO in the vessel wall leads to an attenuated vasodilator response and is known to promote the development of endothelial dysfunction (Harrison 1997).

Oxidative stress

Reactive oxygen species (ROS) include superoxide, hydroxyl (OH.) and hydrogen peroxide (H_2O_2). Free radicals can induce oxidation and damage to DNA, membranes, proteins and other macromolecules, if they are present in excess. Diverse specific and non-specific antioxidant defence systems therefore exist to scavenge and degrade ROS to non-toxic molecules (Li and Shah 2004). The “redox state” of a cell describes the balance between ROS

production and their removal by antioxidant systems; a pathological imbalance in favour of excess ROS is termed oxidative stress. A small amount of O_2^- is normally produced as a by product of the use of molecular oxygen during mitochondrial oxidative phosphorylation. A family of superoxide dismutase (SOD) enzymes rapidly converts O_2^- to H_2O_2 , which is itself broken down by glutathione peroxidase (GPX) and catalase (CAT) to water. The pathophysiological effects of ROS depend on the type, concentration and specific site of production. At lower concentrations local targeted production of ROS serves as a second-messenger system that transmits biological information through the highly specific modulation of intracellular signalling molecules, enzymes and proteins which is called redox signalling. These processes are involved in the activation of many signal transduction protein kinases and transcription factors, the stimulation of DNA synthesis and expression of growth-related genes, (Li and Shah 2004; Finkel 1999) and the regulation of myocardial excitation–contraction coupling (Gao et al. 1996). High levels of ROS tend to react with numerous protein centres, DNA, cell membranes and other molecules, causing considerable cellular damage as well as generating other more reactive radicals. Another ROS-related pathophysiological mechanism involves the reaction of O_2^- with the signalling molecule nitric oxide, which in health has a central role in vascular homeostasis as well as in modulating cardiac function. The reaction between O_2^- and nitric oxide leads to inactivation of nitric oxide and loss of its biological activity (Li and Shah 2004). Nitric oxide synthase enzymes normally generate nitric oxide, but may instead generate O_2^- if they become “uncoupled”, a state that is especially likely to occur in the setting of deficiency of the NOS cofactor BH4 or the NOS substrate L-arginine (Verhaar et al. 2004). NOS uncoupling and subsequent O_2^- production are implicated in the genesis of vascular endothelial dysfunction in patients with heart failure (Dixon et al. 2003).

Antioxidative effects of statins in the vascular system

Statins may also improve endothelial function through their antioxidant effects. For example, statins enhance endothelium-dependent relaxation by inhibiting production of ROS from aortas of cholesterol-fed rabbits (Rikitake et al. 2001). Importantly, lipid lowering by itself lowers vascular oxidative stress (Cai and Harrison 2000). In addition, other antioxidant effects of statins appear to be cholesterol independent. The major source of ROS in the vascular wall is the NAD(P)H oxidase complex. The small GTP binding protein Rac1 is important for the assembly of the NAD(P)H oxidase enzyme complex. Inhibition of Rac1 isoprenylation by statin treatment prevents the activation of NAD(P)H

oxidase and ROS release (Wassmann et al. 2001a, 2001b; Endres et al. 1998; Williams et al. 1998; Laufs et al. 1997, 1998). These enzymes catalyse electron transfer from NADPH to molecular oxygen, resulting in the formation of O_2^- . NADPH oxidase activity has been found to be increased in experimental models of LVH and CHF as well as in end-stage failing human myocardium (Dixon et al. 2003; Heymes et al. 2003; Maack et al. 2003; Li et al. 2002). Interestingly, ROS produced by NADPH oxidases can promote ROS generation by other sources, thereby amplifying total levels of ROS. For example, O_2^- from NADPH oxidase may oxidize and degrade BH4, thereby leading to NOS uncoupling (Verhaar et al. 2004). Similarly, NADPH oxidase derived ROS may also activate xanthine oxidase (Li and Shah 2004). Wassmann et al. (Wassmann et al. 2001b) evaluated the effect of atorvastatin on the vascular production of ROS. After treatment of spontaneously hypertensive rats with atorvastatin, carbachol-induced vasorelaxation in aortic segments was significantly improved. Furthermore, vascular production of ROS was reduced. Interestingly, statin therapy reduced blood pressure in this rat model and downregulated the angiotensin II type 1 (AT1) receptor expression. Moreover, the expression of the eNOS expression and activity was enhanced.

Besides ROS generating enzymes, antioxidative defense systems are important for the oxidative stress that ultimately results. The SOD isoforms, GPX and CAT are enzymes residing within the vasculature that finally lead to the elimination of free radicals by the generation of water and oxygen (Strehlow et al. 2003; Andreoli 2000; Gutteridge and Halliwell 2000). The ultimate oxidative stress within vascular cells is determined by ROS production and corresponding elimination processes. The latter are realized by the radical scavenging enzymes GPX, the SOD isoforms and CAT. Whereas atorvastatin had no influence on the expression of GPX and SODs, CAT expression and activity were profoundly upregulated in vitro and in vivo. Physiologically, the upregulation of CAT can be observed after an increase of hydrogen peroxide concentrations. However, reduced superoxide production by NAD(P)H oxidase after decreased expression of essential subunits leads to reduced concentrations of hydrogen peroxide when SOD levels are not altered (Lassegue et al. 2001; Ushio-Fukai et al. 1996). Because CAT is used in the elimination of hydrogen peroxide (Andreoli et al. 2000; Gutteridge and Halliwell 2000), increased levels of CAT further reduce the concentrations of this radical, thereby accelerating the turnover of superoxide to hydrogen peroxide. Finally, this leads to a decrease of the overall intracellular free radical load in VSMCs. Therefore, upregulation of CAT may represent another antioxidative action of statins (Wassmann et al. 2003).

Antioxidative effects of statins in the myocardium

Although the main impact of statin therapy in cardiovascular disease appears to be predominantly vascular, recent animal and human studies suggest that statins may also have direct beneficial effects on the myocardium. Animal studies suggest that a phagocyte-type NADPH oxidase may be a relevant source of ROS in the ventricular myocardium (Bendall et al. 2002; MacCarthy et al. 2001; Aikawa et al. 2000). In the cardiomyocytes, three of its five components, p40*phox* PHOX (for phagocyte oxidase), p47*phox*, and p67*phox*, exist in the cytosol, forming a complex. The other two components, p22*phox* and gp91*phox*, are bound to the membranes. Various stimuli lead to the phosphorylation of the cytosolic components, and the entire cytosolic complex then migrates to the membrane. Importantly, not only the core subunits but also two low-molecular-weight guanine nucleotide-binding proteins, Rac1 and Rap, are required for activation. During activation, Rac1 binds GTP and migrates to the membrane with the core cytosolic complex. Therefore, Rac1 is critically involved in the activation of cardiovascular NADPH oxidase. NADPH oxidase-dependent ROS production is involved in cardiac hypertrophy in response to pressure overload (Li et al. 2002; MacCarthy et al. 2001), stretch (Aikawa et al. 1999), angiotensin II-infusion (Bendall et al. 2002), and α -adrenergic stimulation (Xiao et al. 2002).

Because Rac1 is required for NADPH oxidase activity and cardiac hypertrophy is mediated, in part, by myocardial oxidative stress, it is likely that statins could inhibit cardiac hypertrophy through an antioxidant mechanism involving inhibition of the association of RhoGDI α with Rac1 which is mediated by phosphatidylinositol-3 kinase and depends on geranylgeranylation (Custodis et al. 2006) (Fig. 2). Indeed, statins inhibit angiotensin II-induced oxidative stress and cardiac hypertrophy in rodents (Takemoto et al.

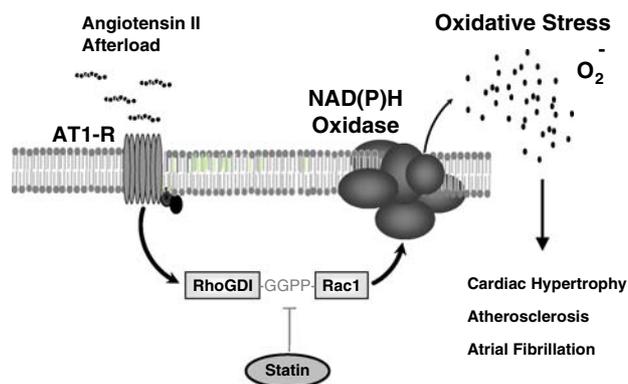


Fig. 2 Antioxidative mechanism of statins. Activation of NAD(P)H oxidase by angiotensin II via Rac1 GTPase. Statins could inhibit NAD(P)H oxidase through inhibition of the association of RhoGDI α with Rac1 which depends on geranylgeranylation

2001). This has also been observed in clinical studies where statins inhibit cardiac hypertrophy in humans with hypercholesterolemia (Lee et al. 2002). NADPH-oxidase-mediated ROS are increased in left ventricular myocardium from patients with heart failure and correlate with an increased activity of Rac1 GTPase, and oral statin treatment is able to decrease Rac1 function in the human heart (Maack et al. 2003).

Furthermore plays increased atrial oxidative stress an important role in inducing and maintaining atrial fibrillation (AF) (Cai et al. 2002; Nattel et al. 2002; Mihm et al. 2001; Carnes et al. 2001). AF induced by rapid atrial pacing in pigs is characterized by increased NAD(P)H oxidase activity and superoxide production in the left atrium (Dudley et al. 2005). In isolated atrial myocytes from human right atrial appendages, NADPH oxidase is the main source of atrial superoxide production (Kim et al. 2005). NADPH-stimulated superoxide release was higher in patients with AF. NO synthase contributed to atrial superoxide production in fibrillating atria, suggesting that increased oxidative stress may lead to NOS “uncoupling”. These findings indicate that NADPH oxidase significantly contributes to superoxide production in AF (Kim et al. 2005). Indeed, left atrial tissue of patients with AF is characterized by upregulation of Rac1-GTPase and the superoxide-producing NADPH-oxidase compared to patients with sinus rhythm (Adam et al. 2007). In mice with cardiac specific overexpression of Rac1 under the control of the α MHC promoter (RacET), we observed AF with aging, which was associated with an increased NADPH oxidase activity. Notably, treatment with HMG-CoA reductase inhibitors inhibits

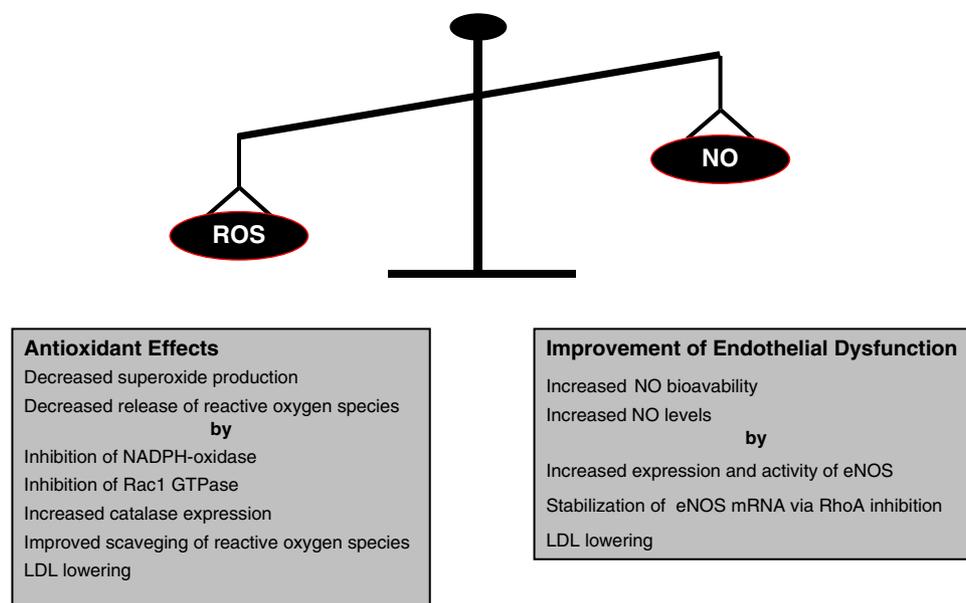
Rac1 activation by inhibiting its geranylgeranylation and membrane translocation (Adam et al. 2007; Laufs et al. 2002). Indeed oral treatment of the Rac1-overexpressing mice with statins inhibited Rac1, thereby lowered NADPH-oxidase activity and markedly reduced the incidence of AF (Adam et al. 2007).

Open questions

In experimental models, statins improve endothelial function through increased NO production and decreased oxidative stress (Fig. 3) in addition to cholesterol lowering. However, in clinical studies the quantitative contribution of cholesterol-independent compared to cholesterol-dependent effects is difficult to determine. Indirect evidence from small studies comparing statins with ezetimibe suggest a potential clinical relevance of these effects, however, the data are not fully conclusive (Landmesser et al. 2004). Another issue is the evidence suggesting that an abrupt discontinuation of statin medication may exert negative effects in patients with acute coronary syndromes or stroke while in stable vascular patients discontinuation may be safe. Withdrawal of statin treatment confers overshoot activation of small G-proteins Rho and Rac causing production of ROS and suppression of NO bio-availability (Endres and Laufs 2006).

Based on the data showing a rapid beneficial effect and the evidence for a potential harmful effect of withdrawal for certain patients, it may be of interest to evaluate an intravenous application of statins. Potential indications for an i.v.

Fig. 3 Effects of statins on endothelial function and oxidative stress



statin formulation could include the administration in patients with acute vascular syndromes such as ischaemic stroke and acute coronary syndromes as well as critically-ill patients pre-treated with statins whenever enteral administration is not possible (e.g., patients with vascular disease, those undergoing major surgery or trauma patients). Additional indications for i.v. treatment that have to be tested are the pre-treatment of patients undergoing surgery with high-vascular risk and peri-operative treatment of patients with high risk of AF [for review see (Endres and Laufs 2008)].

Summary

In summary, evidence from a variety of experimental and clinical studies show that statins have the potential to exert effects in addition to the lowering of serum cholesterol levels. These additional properties include beneficial effects on endothelial function and oxidative stress. Recent evidence show that many of these effects are mediated inhibition of isoprenoid synthesis, in particular Rho and Rac GTPases in vascular and myocardial cells. Despite the broad experimental evidence for beneficial vasoprotective cholesterol-independent and extra-hepatic actions of statins, the clinical importance of the pleiotropic effects of statins in addition to cholesterol-lowering remains to be clarified in more detail.

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