Review

Vascular effects of HMG CoA-reductase inhibitors (statins) unrelated to cholesterol lowering: new concepts for cardiovascular disease

Allan M. Lefera,*, Rosario Scalib, David J. Leferb

aDepartment of Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107, USA
bDepartment of Molecular and Cellular Physiology, Louisiana State University Medical Center, Shreveport, LA 71130, USA

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1. Introduction

In 1976, Endo et al. [1] reported on the discovery of a specific competitive inhibitor of the enzyme 3-hydroxy-3-methylglutaryl (HMG) CoA reductase. This compound, isolated from fungi, was called mevastatin. In 1988, Alberts [2] described a more potent HMG CoA reductase inhibitor, lovastatin, isolated from Aspergillus terreus. These two compounds started the development of a series of cholesterol lowering agents, now commonly called ‘statins’. Statins block the conversion of HMG CoA to mevalonic acid and thus dramatically attenuate the biosynthesis of cholesterol. These effects occur largely in the liver, where the statins primarily distribute [2]. The major effect of these pharmacologic agents is a marked reduction in LDL-cholesterol levels in the blood.

On the basis of these and other findings, the statins have been widely prescribed in humans with elevated serum cholesterol levels. These statins are well tolerated, are very safe, and effectively lower serum LDL-cholesterol levels and maintain this effect over periods of many years [3]. In one clinical trial, 4444 patients were carefully studied and not only was simvastatin shown to be safe and effective in lowering circulating cholesterol, it also significantly improved survival in patients with coronary heart disease (CHD) [4], and carried a 37% reduction in the risk of treated patients undergoing myocardial revascularization procedures [4]. These findings speak eloquently to the clinical usefulness of the statins. For many years, all the beneficial effects of the statins were attributed to their cholesterol lowering effect. Nevertheless, quite recently, the statins were found to exert direct cardiovascular effects which clearly are independent of their cholesterol lowering effects, and are not directly attributable to a reduction in serum cholesterol levels.

2. Endothelial dysfunction

The purpose of this review is to describe, evaluate, and discuss the major mechanisms underlying these non-lipid-lowering effects of the statins. These effects relate primarily to attenuating and blunting endothelial dysfunction in cardiovascular disease states including coronary artery disease, myocardial ischemia/reperfusion, cerebral ischemia, and diabetes. The major factor contributing to the endothelial dysfunction in these circulatory disorders appears to be reduced stability of the enzyme generating nitric oxide (NO) in endothelial cells (i.e. endothelial nitric oxide synthase, eNOS). Statins improve the stability of the mRNA for eNOS, and by so doing enhance the generation of NO from the endothelium.

Endothelial dysfunction has long been shown to be a critical early component of organ injury following myocardial or cerebral ischemia, hemorrhage, as well as other disease states including hypercholesterolemia and diabetes [1,5]. In 1989, in a seminal study of the effect of hypercholesterolemia on endothelial function, Osborne et al. [6] showed that a moderate degree of hypercholesterolemia in rabbits (i.e. to 300 mg/dl) produced a marked degree of coronary and aortic endothelial dysfunction in the absence of any atherosclerotic plaque formation.
Moreover, administration of lovastatin for 2 weeks markedly attenuated the endothelial dysfunction in both vessel types [7]. At that time, it wasn’t clear whether enhancement of prostacyclin, nitric oxide, adenosine, or some other endothelium-derived mediator could account for this endothelial preservation.

It wasn’t until the early 1990s that some of the pieces in the puzzle began coming together. Around that time, several multicenter clinical trials on the role of cholesterol in coronary heart disease were completed including the MRFIT study and the Framingham Heart Study. In the MRFIT study [8] conducted in 361 662 men, serum cholesterol was found to be closely correlated with death from ischemic heart disease. At a serum cholesterol of 300 mg/dl the coronary heart disease death rate was 17 per 1000, whereas at 150 mg/dl it was only three per 1000, a decline of almost 600% [9]. By 1995, several small clinical trials with statins began appearing [10,11]. In one case, Shepherd et al. [11], showed that pravastatin decreased the mortality rate from coronary heart disease by 28%. Treasure et al. [10] actually studied the effect of a statin on coronary vasodilator responses of patients having atherosclerosis. These results suggested that the statins preserved NO-dependent functions of the coronary vascular endothelium, a finding rapidly confirmed by the work of Kinlay et al. [12]. These studies coupled with the larger 4S study (Scandinavian Simvastatin Survival Study) involving 4444 patients, reinforced the concept that statins significantly reduced the incidence of coronary heart disease [4]. Moreover, the mechanism for the protective effects of the statins was thought to involve endothelial preservation related to enhancing NO release from the endothelium.

All of the above clinical and basic research studies were conducted in hypercholesterolemic or atherosclerotic subjects. The general feeling among these investigators was that these beneficial effects on the endothelium, possibly related to NO, were solely due to the cholesterol lowering effect of the statins (i.e. it was the cholesterol lowering per se that resulted in vascular protection).

### 3. Key cardiovascular actions of the statins

It wasn’t until 1997 [13] that evidence was obtained showing that (a) statins directly enhance the biosynthetic machinery to produce NO, and (b) these effects of statins occur in normocholesterolemic cells. This landmark study showed that simvastatin and lovastatin increased the half-life of the mRNA for eNOS from 13 to 38 h. This remarkable effect occurred following exposure to 1 μM statin treatment for only 48 h. In a follow-up study, statins were shown to exert these salutary NO promoting effects by inhibiting the biosynthesis of mevalonate (the major precursor of cholesterol) and of the isoprenoid geranylgeranylporphosphate (GGPP) [14]. GGPP is important in the post-translational modifications of a variety of proteins including eNOS, and Ras-like proteins such as Rho. Inhibition of Rho results in a 3-fold increase in eNOS and nitrite generation, since Rho is an inhibitor of NO generation [14].

The above mentioned molecular effects are certainly of interest, but lack clinical relevance, unless they have a therapeutic significance in either the treatment of or the prevention of disease states. The significance of the acute effects of the statins in normocholesterolemic subjects became apparent shortly thereafter when Endres et al. [15] and Lefer et al. [16] showed that simvastatin protects against life-threatening disorders. Simvastatin was found to attenuate brain injury and cerebral infarct size in mice subjected to cerebral ischemia [15], and markedly limited cardiac contractile dysfunction in rat hearts subjected to global myocardial ischemia and reperfusion [16]. In both of these studies, the cytoprotective effects of simvastatin were related to enhanced NO. In the former study, the effect was abrogated in mice having their eNOS gene deleted (i.e. eNOS−/−), and in the latter study, vascular NO levels were measured directly with a NO electrode.

These studies called attention to the fact that the NO preserving effects of acute statin therapy actually translate into important tissue preserving actions in two vital organs, the brain and the heart. In the case of cerebral ischemia a significant component of the beneficial effect was attributed to increased cerebral blood flow [15] and in the case of myocardial ischemia/reperfusion injury, a key effect appeared to be attenuation of neutrophil infiltration into the reperfused myocardium [16]. In both cases, these key actions could be attributed largely to enhanced endothelial NO generation. These studies provided strong evidence that statins could be considered as therapeutic agents in acute life-threatening disorders independently of their cholesterol lowering effects, even in the setting of normocholesterolemia. In fact, the statins can now be considered as ‘endogenous NO donors’ or NO enhancing agents. There is an abundant literature demonstrating that physiological levels of exogenous NO either administered as authentic NO, NO donors, or NO precursors (e.g. L-arginine) ameliorate the pathophysiology of ischemia–reperfusion, shock states and hypercholesterolemia [17,18].

### 4. Statins enhance NO Production

The NO enhancing effect of the statins have attracted widespread interest. There is universal agreement on the fact that statins enhance the machinery for synthesizing NO (i.e. eNOS is upregulated). This enhanced expression of eNOS was first shown by Liao’s laboratory [13,14] for simvastatin and mevastatin, and was confirmed in bovine aortic endothelial cells [19]. This work was extended to lovastatin [20], atorvastatin [19], and fluvastatin [21]. Moreover, these positive results were found to occur in human cells [13,14,19] in mice [22], and in rats [21]. All
of these studies show clear upregulation of eNOS mRNA, and several of them show eNOS protein to be significantly increased. Relevant to these data is the finding of Endres et al. [15] that simvastatin increased the catalytic activity of calcium-dependent NOS in simvastatin-treated mice by 2- to 3-fold. However, it is apparent that for the NO mediated hypothesis of statin action to be validated, actual enhanced NO levels need to be shown. This has recently been done using a direct NO electrode which specifically measures NO release from cells [23]. This has been reported by two separate groups using a specific NO electrode coupled with a NO meter. Both groups studied pravastatin and simvastatin [16,24], one using cultured bovine aortic endothelial cells (BAECs) [24] and the other using explanted rat aortic segments [16]. In both cases, statins were found to significantly increase NO generation by 2- to 3-fold, a response which was totally inhibited by the NOS inhibitor L-NAME. Thus, the NO releasing action is a common property of these two naturally occurring statins.

Confirmation of the NO hypothesis of statin action is provided by the fact that the statins do not protect in eNOS deficient mice. This was first shown in mice subjected to stroke [15], and later confirmed in mice subjected to myocardial ischemia/reperfusion [25]. Moreover, the increase in cerebral blood flow in mice given simvastatin was abolished in eNOS deficient mice [22] as was the amelioration of leukocyte adherence to the microvascular endothelium [26]. Thus, there are ample and unequivocal data showing that statins significantly enhance NO release from the vascular endothelium. This NO enhancing action is consistent with several studies showing the beneficial effects of NO or NO donors in some of the same disease states (i.e. myocardial ischemia/reperfusion) [17,18]. Moreover, this NO enhancing effect of statins occurs at clinically relevant doses of the statins. In one study [16], the cardioprotective effect of simvastatin occurred at 25 μg/rat, a dose equivalent to 10 mg in a human subject, the initial starting dose of this statin.

5. Effects of statins on leukocyte–endothelial interactions

One of the key effects of the statins appears to be inhibition of leukocyte–endothelium interaction, a crucial anti-inflammatory action. This effectively attenuates the infiltration of leukocytes (particularly neutrophils) into inflamed regions, thus curtailing tissue injury in these areas. Several examples of local inflammation occur following ischemia/reperfusion of organs (e.g. myocardial ischemia/reperfusion, mesenteric ischemia/reperfusion, cerebral ischemia/reperfusion). In this regard, both fluvastatin [27] and simvastatin [28] were found to markedly attenuate leukocyte adherence to mesenteric post-capillary venules. This occurred in response to a variety of pro-inflammatory stimuli including thrombin, leukotriene B₄ (LTB₄), L-NAME, and platelet activating factor (PAF). Thrombin and L-NAME would be expected to primarily activate the endothelium, whereas PAF and LTB₄ would primarily activate leukocytes. The net result of statins would be a significant reduction in the number of transmigrated leukocytes which is precisely what was found by both groups. In terms of the mechanism of this effect, Pruefer et al. [28] showed that simvastatin attenuated the up-regulation of the cell adhesion molecule P-selectin on the mesenteric endothelium, an effect also observed for simvastatin on the coronary endothelium in ischemia/reperfusion [16]. Consistent with these findings was a reduction in leukocyte rolling flux [27] and leukocyte rolling velocity [28]. Both of these events are early components of enhanced leukocyte–endothelium interaction and are regulated by the selectin family of adhesion glycoproteins, particularly P-selectin. Moreover, Romano et al. [29] recently observed that fluvastatin reduces soluble P-selectin levels in hypercholesteremic patients. In addition to modulating P-selectin, statins appear to exert similar down-regulatory effects on another endothelial cell adhesion molecule, ICAM-1 [29,30]. However, while statins exert an anti-inflammatory effect on endothelial cell adhesion molecules (i.e. P-selectin, ICAM-1), they also appear to have similar effects on leukocyte adhesion molecules. Thus, simvastatin down-regulates CD18 (i.e. the β-chain of the β₂-integrins) expression on rat neutrophils [16] and both lovastatin and simvastatin decrease CD11b (i.e. the α-chain of β₂-integrins). These actions effectively attenuate adherence of leukocytes to the endothelium. Moreover, these anti-adhesion molecule effects appear to be dependent upon NO [16,29] since they were accompanied by either increased NO or increased nitrite levels indirectly related to enhanced NO. However, the most convincing demonstration that reduced leukocyte–endothelium interaction by statins is dependent upon NO, is that the statins failed to ameliorate the high leukocyte rolling and adherence in eNOS deficient mice [25].

Thus, statins appear to exert a significant portion of their cytoprotective effects via enhancing NO release and the resulting attenuation of leukocyte–endothelium interaction. This effectively prevents neutrophil and monocyte mediated injury and retards atherosclerotic plaque formation. Furthermore, statins exert these effects independently of their well known lowering of circulating cholesterol levels.

6. Other potential vasculoprotective and cytoprotective actions of statins

Although it is clear that a major anti-inflammatory action of the statins is to promote basal release of endothelium-generated NO, this is not the only important cellular or molecular effect of the statins [31]. Other
important effects of the statins can be categorized as follows:

(a) Anti-oxidant
(b) Anti-thrombotic
(c) Vasculoprotective
(d) Angiogenic
(e) Membrane transport

One important action of the statins is their ability to scavenge oxygen-derived free radicals. A variety of statins exhibit this property including simvastatin [32], fluvastatin [33,34], atorvastatin, pravastatin and cerivastatin [35], although pravastatin has been reported to be not so effective under all conditions [33]. Generally, the statins scavenge superoxide in a dose-dependent manner [33,35]. Others have reported that fluvastatin also scavenges hydroxyl radicals [34]. Some of the superoxide scavenging actions of statins may be due to their reduction in the biosynthesis of isoprenoids, and since some of the superoxide generators (e.g. NADPH oxidase) are isoprenylated proteins, this effect of the statins would reduce the effectiveness of these free radical generators.

Of course, the reduction in superoxide radical formation by statins would act to enhance the bioavailability of NO, and thus potentiate the effectiveness of endothelium-generated NO, a key aspect of the tissue-protective effects of the statins.

A second additional effect of the statins can be categorized as an anti-thrombotic effect. This action occurs in several ways. First, simvastatin has been shown to reduce levels of plasminogen activator inhibitor-1 (PAI-1) in human vascular smooth muscle and endothelial cells and to increase expression of tissue plasminogen activator (tPA) in these same human vascular cells [36]. The net result of these two effects would be to markedly tilt the fibrinolytic balance in the blood vessel wall toward increased fibrinolytic activity. Additionally, simvastatin has been shown to reduce in vivo clotting activation in healthy patients presumably due to reduced tissue factor (TF) expression on monocytes [37]. This effect also occurred in a dose-dependent manner over the clinical range of statins used in hyperlipidemic patients (i.e. 10–80 mg). Moreover, these anti-thrombotic effects can synergize with the anti-platelet aggregating effect of NO, which also occurs in response to statin therapy.

Thirdly, the statins exert additional effects on vascular cells (i.e. smooth muscle and endothelial cells). One such effect is the reduction in the precursor of endothelin-1 (i.e. pre-proET-1) as well as in the synthesis of ET-1 [19]. This endothelin blunting effect occurred in response to either atorvastatin or simvastatin, and was reversed by mevalonate but not by cholesterol. However, in a clinical study, fluvastatin failed to reduce circulating levels of ET-1 while it lowered circulating cholesterol levels [38]. Along these lines, plasma from fluvastatin-treated patients has been found to attenuate smooth muscle cell proliferation [39]. This effect could be important in reducing atherogenesis and in limiting the extent of restenosis in blood vessels following angioplasty. A recent study has clearly demonstrated that simvastatin induces angiogenesis via an Akt-dependent mechanism [40]. The therapeutic potential of angiogenesis in cardiovascular diseases such as myocardial ischemia and stroke is great. Simvastatin was also shown to attenuate endothelial cell apoptosis, another cytoprotective effect of the statins [40]. Once again, these properties are also in common with those of nitric oxide [41]. Very recently, statins were shown to influence sarcolemmal Na⁺/K⁺ pump function, an effect which may influence contractility of cardiac myocytes via modulation of Na⁺/K⁺ pump current [42]. Thus, most of these additional anti-inflammatory effects of the statins are consistent with their NO enhancing action, and in fact these effects could be partially due to the NO enhancing action of the statins.

### 7. Potential usefulness of statins in other cardiovascular disorders

The statins of course are designed to reduce LDL cholesterol levels and thus to exert an atherosclerotic preventing or delaying effect. This has been amply demonstrated in major clinical trials in humans, mentioned earlier in this review (Section 1). What has emanated from these studies is a clear consensus that cardiovascular events are reduced in patients on a daily maintenance dose of a statin (i.e. usually 20–80 mg per day). These findings have recently been extended in the laboratory to animals having normal circulating cholesterol levels. Some of these key effects (e.g. attenuation of myocardial ischemia/reperfusion injury and amelioration of stroke induced neurological deficits) have been discussed in Section 3 of this paper. These effects are related to a significant blunting of the endothelial dysfunction occurring early in these disorders. Clearly, endothelial dysfunction is a prominent feature in other cardiovascular diseases, including diabetes mellitus, hypertension, and graft vessel disease following organ transplantation.

It is therefore not surprising that statins ameliorate the pathogenesis of these disease states. Earlier studies have shown that statins lower cardiovascular event risk reduction in diabetic patients, to even a greater extent than in non-diabetic controls [43,44]. More recently, further analysis of the Scandinavian Simvastatin Survival Study has revealed that simvastatin treated diabetic patients exhibited a 42% reduction in risk of major coronary events, and a 43% reduction in coronary event related mortality [45]. This is related to improved endothelial function and higher NO bioavailability in patients with type II diabetes mellitus [46]. Finally, in genetically diabetic mice (db/db mice), simvastatin was found to attenuate the severity of myocardial ischemia reperfusion injury as well as to reduce...
Fig. 1. Schematic diagram of mechanism of the vasculoprotective effects of statins via enhancement of endothelial NO release. Statins inhibit the biosynthesis of cholesterol at the mevalonic acid step. Mevalonic acid destabilizes the mRNA for endothelial nitric oxide synthase (eNOS) thus effectively reducing synthesis of NO from L-arginine to L-citrulline and the subsequent release of NO. Statins inhibit the formation of mevalonic acid preventing this reduction in NO release. NO is essential in retarding platelet aggregation and polymorphonuclear (PMN) leukocyte adherence to the endothelium via the endothelial cell adhesion molecules, P-selectin and intercellular adhesion molecule-1 (ICAM-1). Statins promote these anti-inflammatory actions of physiological concentrations of endothelium-derived (NO).
leukocyte–endothelium interaction [47]. Recently, lovastatin has been shown to preserve vascular reactivity in the cerebral circulation in spontaneously hypertensive rats an effect which would moderate the hypertension [48]. Moreover, Wenke et al. [49] has reported that simvastatin significantly reduces graft vessel disease in patients, a serious complication of heart transplantation. Simvastatin-treated patients exhibited a significantly longer graft survival rate. Perhaps related to these vasculoprotective effects of simvastatin is its ability to reduce smooth muscle cell proliferation and attenuate neointimal formation in vivo following vascular injury (i.e. retard restenosis) [50]. Of course, caution must be applied to these data until large prospective clinical trials confirm these earlier findings.

Careful analysis of the clinical trials and basic science studies clearly point to an impressive array of potentially beneficial effects of the statins in a variety of circulatory disorders involving both elevated and normocholesterolemic conditions. This subject has recently been reviewed by Bellosta et al. [51]. The new data have prompted one group to conclude that ‘nonlipid properties of statins may help to explain the early and significant cardiovascular event reduction reported in several clinical trials of statin therapy’ [52].

8. Summary

The statins are widely used lipid lowering agents possessing an impressive safety record. They are effective in reducing LDL cholesterol in human subjects, and as such effectively attenuate atherosclerosis and all of its attendant circulatory pathophysiology. However, statins also exert a variety of important cardiovascular protective effects independently of this anti-atherosclerotic effect. Many of these effects are related to preservation of the vascular endothelium. Fig. 1 illustrates the endothelial preserving effect of the statins which represents a class effect of these agents. As can be seen, the crucial effect on the endothelium is the enhancement of NO synthesis. This is accomplished by increasing the stability of the mRNA for eNOS and by enhancing NO release from endothelial cells. This maintains a physiologic level of NO in the endothelial cell and on the endothelial surface which preserves vascular reactivity, down-regulates thrombotic mechanisms, and exerts important anti-inflammatory actions by attenuating leukocyte–endothelial cell interaction. The net result of these important endothelial preserving actions is a cardiovascular system that is capable of responding to stress, and to cope with challenges to circulatory homeostasis. One must also be aware that the statins, by virtue of their lipid-lowering effects per se, can improve endothelial dysfunction [53]. The ultimate result is a reduction in both the incidence and severity of coronary and cerebral events, and thus an impressive reduction in mortality rates.

References


