Emerging mechanisms for secondary cardioprotective effects of statins

Steven J. Miller*

Division of Experimental Pathology, Methodist Research Institute, Clarian Health Partners Inc. (Methodist, Indiana University, Riley Hospitals), 1701 N. Senate Blvd., Indianapolis, IN 46202, USA

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See article by Li et al. [11] (pages 130–135) in this issue.

1. Introduction

A widely accepted risk factor for the development of coronary artery disease is an elevated level of plasma cholesterol. Early on, it was recognized that inhibition of de novo synthesis of cholesterol could be an effective method to reduce plasma cholesterol, and thereby potentially reduce the incidence of atherosclerosis. The rate-limiting step in cholesterol synthesis is the reduction of 3-hydroxy-3-methylglutaryl (HMG)-CoA to mevalonate, which is catalyzed by HMG-CoA reductase. This enzyme is an obvious target for inhibition in order to bring about a reduction in cholesterol synthesis and a corresponding drop in plasma cholesterol. The first specific inhibitor of HMG-CoA reductase to be discovered was a fungal metabolite named compactin [1,2], closely followed by mevinolin [3,4]. Synthetic derivatives of these compounds, along with other naturally occurring compounds, have spawned a wide variety of clinically useful HMG-CoA reductase inhibitors collectively referred to as ‘statins’. The ability of these inhibitors to effectively lower plasma cholesterol and reduce cardiovascular morbidity and mortality in patients with and without cardiovascular disease has been documented in numerous studies [5–7]. However, beneficial effects of statins on vascular function beyond their ability to lower serum cholesterol have been noted, although whether these effects were truly unrelated to their lipid lowering abilities has been unclear [8–10]. These vascular effects include improved endothelial function, decreased coagulation, increased fibrinolysis, decreased inflammation, and increased plaque stability, among other effects. Recent in vitro and in vivo evidence suggests that initial observations noting these secondary statin effects were correct; however, the mechanisms responsible have only recently begun to come to light. In this issue of Cardiovascular Research, Li et al. [11] demonstrate that simvastatin and atorvastatin are able to modulate the effects of oxidized-low density lipoprotein (LDL) on LOX-1 expression and protein kinase B (PKB) activity. Their results indicate that these statins inhibit oxidized-LDL-mediated increases in LOX-1 expression, as well as uptake of oxidized-LDL, in human coronary artery endothelial cells. The authors also demonstrate that oxidized-LDL causes a decrease in the phosphorylation state of PKB and that this effect was blocked by the two statins. The authors conclude that part of the cardioprotective effect of statins is due to their ability to decrease oxidized-LDL uptake by LOX-1 and that this effect is mediated by an increase in PKB activity. This work is significant in that it demonstrates for the first time the ability of at least two statins to reverse the negative effects of oxidized LDL on LOX-1, and because of the novel observation that these effects are associated with modulation of PKB activity.

2. Pleiotropic effects of statins

Although many studies have documented the ability of statins to effectively lower plasma cholesterol levels [5–7], evidence has steadily mounted for secondary beneficial effects of therapeutic doses of statins on cardiovascular disease that are apparently unrelated, or indirectly related, to their lipid-lowering abilities [9,12]. These are the so-called ‘pleiotropic’ effects, and include, but are not limited to, activities that show major clinical benefits such as endothelial function, inflammation, coagulation and plaque vulnerability [8].

Endothelial function can be improved by increased release of nitric oxide (NO), which results in protective effects such as inhibition of platelet adhesion, inflammation and smooth muscle cell proliferation. Recent results...
suggest that the cardioprotective effects of statins can be at least partially explained by the regulation of endothelial nitric oxide synthase (eNOS) expression and that these effects are independent of lipid-lowering actions [13]. Wassmann et al. [14] showed that a 30 day treatment with atorvastatin in normocholesterolemic, spontaneously hypertensive rats improved endothelial function as assessed by effects on vasorelaxation and vasoconstriction. Reactive oxygen species decreased 62% from controls, along with a 138% increase in eNOS mRNA and a 209% increase in vessel wall eNOS activity. mRNA for aortic angiotensin type I receptor and the NADPH oxidase subunit p22phox also were significantly reduced compared to controls. Increases in inducible nitric oxide synthase (iNOS) expression in cardiac myocytes also have been shown to occur with statin treatment [15]. These effects were reversible with exogenous mevalonate or geranylgeranyl-pyrophosphate, and were attributed to inhibition of Rho.

Statins also show effects on reduced inflammation and adhesion to the endothelium. Yoshida et al. [16] has recently shown that cerivastatin reduced monocyte adhesion to vascular endothelium under physiological flow conditions by decreasing expression of integrins and actin polymerization via RhoA inactivation. Inoue et al. [17] showed that four different statins decreased mRNA and protein for IL-1β, IL-6, and cyclooxygenase-2. These statins also induced peroxisome proliferator-activated receptor alpha (PPARalpha) and PPARgamma mRNA and protein expression in HUVEC and hepatocytes. In addition, expression of p22phox and p47phox, subunits of NADPH oxidase, were decreased by treatment with statins, and this effect was reversed by mevalonate, geranylgeraniol, farnesol, and cholesterol. Clinical studies have shown that pravastatin significantly decreases plasma concentrations of C-reactive protein [18,19], and similar results have been found using simvastatin and atorvastatin [20]. Statins are increasingly being employed to counteract inflammatory responses in transplantation, and pravastatin has been shown to decrease plasma markers of inflammation and improve endothelial cell function in human heart transplant recipients [21]. In a recent, significant study Sparrow et al. [22] utilized a murine model to confirm that simvastatin directly inhibits acute and chronic inflammatory conditions and shows antiatherosclerotic activity independent of its cholesterol lowering effect.

Statins also may reduce the incidence of vascular disease by minimizing thrombogenic events. Simvastatin has been shown to depress blood clotting by inhibiting activation of prothrombin and factor XIII, reducing rates of factor Va generation, and by enhancing factor Va inactivation [23]. These effects were unrelated to lipid lowering effects. Pravastatin therapy was shown to cause a reduction in thrombogenicity in hyperlipidemic patients due to tissue plasminogen activator- and plasminogen activator inhibitor-1-mediated effects [24]. Interestingly, these fibrinolytic/antithrombotic actions were determined not to be proportional to the magnitude of LDL-C reduction.

Angiogenesis is another aspect of vascular function that may be regulated by statin action, and Kureishi et al. [25] has demonstrated that simvastatin stimulated phosphorylation and activation of the protein kinase Akt, which mediates the angiogenic activity of VEGF and NO. This ability holds great promise for the use of statins to treat ischemic damage.

A significant new area of vascular function that has been shown to be affected by statins is that of immunomodulation. Statins were shown to decrease induction of major histocompatibility class II (MHCII) antigen expression by primary macrophages and endothelial cells in response to interferon gamma; however, constitutive expression of MHCII antigens was not affected [26]. This effect was due to inhibition of the inducible promoter IV of the transactivator CIITA.

### 3. Regulation of LOX-1 receptor by statins

Perhaps not surprisingly, statins affect other pathways related to the prevention of vascular disease. The work by Li and coworkers on the LOX-1 receptor demonstrates effects of statins that may affect multiple pathways independent of lipid-lowering effects [11]. LOX-1 is a lectin-like receptor on endothelial cells that facilitates uptake of oxidized-LDL (ox-LDL) [27]. Increased expression of LOX-1 promotes pathobiological effects of ox-LDL such as increased apoptosis, suppression of constitutive nitric oxide synthase (cNOS) activity and increased cell adhesion [28]. Protein kinase B (PKB), a homologue of v-Akt, has been shown to be important for the expression of cNOS activity. Since ox-LDL decreases cNOS expression, changes in PKB activity may be involved in this effect of ox-LDL. Previous work suggested that statins may affect an increase in PKB activity [25], thus it is likely they are involved in regulation of LOX-1 expression. The current work of Li et al. [11] shows that in human coronary artery endothelial cells ox-LDL upregulated expression of LOX-1 protein and mRNA, which enhanced ox-LDL uptake, and at the same time decreased phosphorylation (activity) of PKB without affecting PKB protein levels. Simvastatin or atorvastatin, in a concentration dependent manner, decreased the ox-LDL-mediated increase in LOX-1 expression, which resulted in less ox-LDL uptake. These statins by themselves had no effect on LOX-1 expression. Statins also increased activity of PKB in the presence of ox-LDL, but this effect was reversed when cells were treated with an upstream (PI 3-kinase) inhibitor of PKB. Since the PKB pathway is involved in other areas related to vascular disease, such as inflammation, effects of statins on PKB could provide a common mechanism and at least partially explain these effects.

One criticism of much of the work to date on secondary effects of statins is that it has been done using in vitro models. Many findings await confirmation in animal models, and ultimately these mechanisms of statin action...
will need to be demonstrated in humans. Such confirmation seems highly likely to occur. Additional secondary effects of statins may be discovered in the future, but those already described suggest that there is enormous potential for their use to counteract the various deleterious effects of vascular disease.

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