Potential Role of Statins in Inflammation and Atherosclerosis

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The mevalonate pathway plays a crucial role in regulation of cellular cholesterol synthesis and isoprenoid groups. The entire pathway is closely regulated by feedback from an enzyme in the cascade, 3-hydroxy-3-methyl-CoA (HMG-CoA) reductase, as well as LDL receptors. Clinically, inhibition of this pathway by statins, potent inhibitors of HMG-CoA reductase, has been shown to reduce plasma levels of LDL cholesterol and several clinical trials with this group of drugs have demonstrated a remarked improvement in cardiovascular risk reduction. Interestingly, the improvement in cardiovascular end points in those trials was superior to estimations calculated from the effect on LDL cholesterol lowering. These findings support the idea of non-lipid effects of statins in atherosclerosis. Further, recent observations using in vivo and in vitro models of atherosclerosis have shed light on their potential role for manipulation of various cellular functions via inhibition of the mevalonate pathway. Among them, recently identified inhibitory effects of statins on monocyte-endothelial interaction suggest their effect on inflammation. Herein, we discuss recent progress in this area of study, with special focus on the biological function of statins.


Key words: atherosclerosis, statin, inflammation, hypercholesterolemia

Introduction

The beneficial effects of 3-hydroxyl-3-methyl-CoA (HMG-CoA) reductase inhibitors, or statins, on cardiovascular diseases are now widely recognized from an increasing large body of clinical trials that have documented a significant reduction of coronary events with their use (1,2). Most of these effects can be attributed to strong LDL cholesterol-lowering effects, however, results of several recent clinical trials have exhibited surprisingly fewer incidences of clinical events in statin-treated groups, which have led to the notion of non-lipid effects for statins (2). The mevalonate pathway is a crucial mechanism within cells to regulate the dynamic balance between extracellular and intracellular cholesterol levels (3). Cholesterol is derived from plasma LDL that enters the cell by receptor mediated endocytosis, and is also synthesized from Acetyl-CoA within cells via this pathway. Balancing of these two independent cholesterol synthesis pathways is provided by a finely regulated feedback mechanism between HMG-CoA reductase activity and LDL cholesterol receptor expression. Therefore, the primary effect of statins to inhibit HMG-CoA reductase activity leads to an up-regulation of LDL receptors on the surface of hepatocytes, thereby enhancing LDL cholesterol uptake that results in a reduction of plasma levels of LDL cholesterol. It is known that mevalonate is involved in the production of sterols and is crucial in the generation of non-sterol isoprenoids, such as Ras or Rho (4,5). The results of numerous recent laboratory and clinical studies point to non-lipid effects of statins with various cell types, including vascular endothelium, smooth muscle cells (6-8), and monocyte/macrophages (7,9-12),
which may explain the unexpected benefits seen with their use.

**Statins and immune function**

As noted above, recent studies point to additional benefits of statins, mainly through their non-sterol effects. One such effect is as an immunomodulator (13). Considering evidence showing that the immune system plays an important role with atherosclerosis (14,15), it is reasonable to study such an effect for statins. Kwak and colleagues found that statins inhibit interferon-γ induced MHC-II expression in T lymphocytes (16). These inhibitory effects were found with inducible MHC-II expression, however, not with the constitutive expression of MHC-II, suggesting that a potential molecular target of statins is the CIITA promoter responsible for the inducible expression of MHC-II (16). The proliferation of T lymphocytes by activation of CD3 has been shown to be modulated by mevalonate metabolism, thus, statins inhibit this proliferation. Further, Montro et al. recently found that statins could be immunoprotective by enhancing Th1 cytokine (IFN-γ, IL-18) production, which resulted in the stabilization of atherosclerotic plaque (17). In earlier clinical observations, treatment with statins was related to less frequent rejection in cardiac transplantation (18,19) and better survival in renal transplantation (20). Although the serum lipid profiles were markedly improved in statin-treated patients in these transplantation studies (21), a direct immunomodulator role for this group of drugs, as noted above, may be operative in this phenomenon. Moreover, these immunomodulator roles for statins shed light on other complex physiological mechanisms such as leukocyte-endothelial dynamic interaction (22,23).

**Leukocyte endothelial interaction in atherosclerosis**

The molecular mechanisms responsible for leukocyte-endothelial adhesive interactions have been extensively studied during the past decade owing to the development of molecular and cellular biology techniques, including genetically engineered mice models. As noted in previous studies, atherosclerotic lesions contain numerous inflammatory cells including macrophages and lymphocytes (24). Therefore, leukocyte-endothelial interaction is regarded as a primary focus point by researchers in this field. Leukocyte-endothelial interactions consist of several steps, and each is operated by different groups of adhesion molecules that are expressed on both endothelial cells and leukocytes (25,26). The trigger of this dynamic cascade requires activation of endothelial cells by pro-inflammatory cytokines such as interleukin-1 and tumor necrosis factor-α. These stimuli lead to transcriptional up-regulation of E-selectin on the surface of endothelial cells, which supports leukocyte rolling. Rolling leukocytes are slowed until they finally stick to the endothelium, and this firm adhesion to endothelium is mainly mediated by intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Subsequently, the leukocyte becomes flattened and starts to migrate toward the endothelial cell-cell junction for diapedesis to be subendothelial tissues (23). The process of diapedesis is thought to be mediated by soluble factors such as chemokines (IL-8, MCP-1), platelet activating factor (PAF), and adhesion molecules such as platelet endothelial cell adhesion molecule-1 (PECAM-1) and vascular endothelial cadherin (VE-cadherin). Meanwhile, heterodimeric integrins are constitutively expressed on the leukocyte surface, and their regulation is primarily modulated by changing the affinity of the extracellular ligand-binding domain.

**Statins and leukocyte-endothelial interaction**

Atherosclerosis manifestation is a complicated process involving multiple factors such as lipid metabolism, inflammatory cytokines (27), coagulation cascades, and interaction between circulating leukocytes and vascular endothelium (28). In recent studies, our group provided evidence supporting the idea that statins are important modulators of leukocyte-endothelial interaction under physiological conditions (12,29,30). When mononuclear U937 cells were pretreated with statins, their adhesion (but not rolling) to the cytokine-activated human umbilical vein endothelial cell (HUVEC) monolayer was significantly attenuated (12). Interestingly, pretreatment of activated HUVEC with statin failed to demonstrate a similar inhibitory effect on the adhesion of U937 cells (M Yoshida, personal observation). Although moderate down-regulation of cell surface integrins (CD11a, CD18, VLA-4) were observed by flow cytometric analysis of U937 cells after statin treatment, it was insufficient to explain the marked reduction of adhesion found by our simulated flow chamber system (12). It is known that statins inhibit the synthesis of sterols, including cholesterol, as well as isoprenoids, including RhoA GTPase (3). In fact, we have been able to show that RhoA GTPase was inactivated after statin treatment in U937 cells, and that inactivation of RhoA GTPase by transfection of a dominant-negative RhoA cDNA or treatment with C3 toxin was sufficient to reduce U937 adhesion to HUVEC. These inhibitory effects were all restored by co-incubation with mevalonic acid (12). We have also shown that inactivation of RhoA GTPase leads to deregulation of the actin-cytoskeleton network, followed by inactivation of focal adhesion kinase, resulting in inactivation (but not down-regulation) of integrins (29). These molecular mechanisms seem to operate to reduce monocyte-endothelial interactions when monocytes are treated with statins.

**Statins and vascular inflammation**

An understanding of the participation of T lymphocytes and monocytes/macrophages in atherosclerosis lesions has led to the concept of atherosclerosis as an inflammatory disease, which was first described by Ross and
colleagues, and is now widely accepted among researchers with abundant supporting evidence (15,28,31). Notably, recent progress in the elucidation of the mechanism responsible for the formation and rupture of atherosclerotic plaque clearly suggests a critical role for the inflammatory response in the process. Recent studies suggest that statins possess an anti-inflammatory property in addition to their lipid lowering effects (13). Moreover, a microarray analysis utilizing cDNA prepared from cultured endothelial cells treated with statin suggested their dramatic effects on atherosclerosis-relevant molecules (32). However, it is still undetermined whether they have putative pleiotropic effects to control the progress of atherosclerosis. Nonetheless, several in vitro and in vivo studies have demonstrated that treatment with a statin can improve vascular function, such as the expression of endothelial nitric oxide synthase.

Clinical markers for inflammation, such as high sensitive C-reactive protein (hs-CRP) and interleulin-6, or WBC counts (33) have been suggested to be novel risk factors for cardiovascular diseases (34,35). Ridker et al. recently reported that C-reactive protein was superior to LDL cholesterol in predicting the risk of cardiovascular diseases among cohorts in a Women’s Health Study (36). As for therapy, treatment with statins significantly reduced the plasma levels of hs-CRP in several large-scale clinical trials among patients with high levels of LDL cholesterol (37). Therefore, a large-scale trial to test the efficacy of statins in patients with high C-reactive protein and low LDL cholesterol may be needed to fully elucidate their role in modulating inflammation to stabilize atherosclerosis. Although the mechanisms responsible for this anti-inflammatory role have not been fully revealed, the striking efficacy of statins, as summarized in Fig. 1, for cardiovascular risk management likely involves direct (independent from lipid lowering) or indirect (through lipid lowering) actions by these compounds to modulate vascular inflammation, including anti-adhesive effects.

Closing remarks

When considering the complex nature of atherosclerosis, it is impossible to point out a single factor that is responsible for all cardiovascular events. Established risk factors such as blood pressure, plasma level of LDL cholesterol, diabetes, and smoking, as well as other relatively novel risk factors such as inflammation, are equally important for cardiovascular risk reduction. Although it seems rather complicated to treat these factors individually, recent observations from the field of vascular biology have pointed out similarities in the molecular mechanisms behind these pathological conditions. Therefore, we have to control total risk management of patients using an approach that focuses on the molecular targets behind the clinical manifestations. In that regard, statins with their additional potential may become part of important therapeutic strategies in the future.

**Fig. 1.** Potential effects of statins on cells in vasculature. The cholesterol-independent effects of statins towards various cell type relevant to atherosclerosis and vessel wall were summarized.
References


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