Cracking Down on Caveolin
Role of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Inhibitors in Modulating Endothelial Cell Nitric Oxide Production

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One of the most effective approaches in the treatment of atherosclerosis has been the use of 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors (statins) to treat hypercholesterolemia. During the past decade, numerous studies involving >20,000 individuals have shown that these drugs dramatically reduce cardiovascular death, myocardial infarction, unstable angina, and stroke. Statin therapy prevents events in individuals with established cardiovascular disease and is also effective in primary prevention.1

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A major mechanism by which lipid lowering is thought to improve outcome is by preventing the development of new atherosclerotic lesions and by depleting lipids from established plaques (ie, plaque stabilization).2,3 A striking finding is that statins seem to decrease clinical events within a few months of the onset of therapy.4 This suggests that they may have beneficial effects beyond those of plaque stabilization and lesion prevention.

One such beneficial effect might be the restoration of nitric oxide production by the endothelium. Endothelium-derived nitric oxide, previously known as the endothelium-derived relaxing factor, modulates vasodilatation and prevents platelet adhesion, the expression of adhesion molecules, and smooth muscle cell proliferation.5 Nitric oxide has also been shown to have antioxidant effects by enhancing the expression of superoxide dismutase,6 preventing lipid-chain reactions,7 and reacting with the superoxide anion.8 Thus, nitric oxide seems to play a major role as an endogenous protective factor against atherosclerosis. Indeed, virtually every atherosclerosis risk factor is associated with decreased endothelial cell nitric oxide production, and it is likely that this loss of nitric oxide is a major reason why these conditions predispose to vascular lesion development.

Given this central role of nitric oxide in protection from atherosclerosis, several groups have become interested in the potential effects that the statins may have on its production. One recently documented effect of the statins is an upregulation of the expression of endothelial nitric oxide synthase (eNOS).8,9 An important and often overlooked property of the statins is that they reduce the production of both cholesterol and a number of isoprenoid intermediates. Among these are geranylgeranyl pyrophosphate (GGPP), farnesyl pyrophosphate, and isopentyl pyrophosphate.10 These molecules are not simply cholesterol precursors; they also play important roles in cell signaling. For example, GGPP and farnesyl pyrophosphate are used as lipid anchors for many membrane-associated proteins,11 and GGPP allows the small G-protein rho to attach to the cell membrane. Importantly, the activation of rho signals the destabilization of eNOS mRNA, resulting in a decrease in eNOS protein expression and, ultimately, nitric oxide production. Statins prevent this activation of rho by preventing the production of GGPP.12 Recent studies in intact animals have elegantly demonstrated that this phenomenon is responsible for the decrease in stroke size mediated by simvastatin and lovastatin.12

An additional beneficial effect of the statins is to decrease endothelial cell superoxide production. Superoxide rapidly reacts with nitric oxide, leading to a loss of nitric oxide bioavailability. Recent studies by Wagner et al13 have shown that statins prevent the isoprenylation of p21 rac, a small G-protein involved in the assembly and function of the superoxide-forming NADPH oxidase.

The above effects of the statins are independent of their lipid-lowering properties and have led to the concept that these agents may have benefits beyond that of cholesterol reduction. This notion is attractive, because it may explain why these drugs seem to have benefits in individuals with only modest elevations of cholesterol or early on, before plaque stabilization is likely to occur.

In the current issue of Circulation, Feron et al14 illustrate another mechanism by which statins may influence endothelial cell nitric oxide production. This phenomenon relates to the fact that the activity of the eNOS enzyme is regulated by its interaction with the scaffolding protein caveolin-1. Caveolin-1 is present in high amounts in small, cholesterol-rich invaginations in the cell wall, termed caveolae, and it serves as a docking station for numerous signaling proteins.15 Among these is eNOS and, interestingly, caveolin-1 potently inhibits eNOS function by preventing its interaction with calcium/calmodulin.16 Feron et al14 make the important observation that atorvastatin dramatically inhibits caveolin-1 expression. These investigators previously showed that high levels of LDL increase caveolin-1 expression17 and, in the present study, they show an antagonistic interplay between concentra-
tions of LDL and statins on caveolin-1 expression. In the setting of no added LDL, a tiny dose of atorvastatin (0.01 μmol/L) completely inhibited caveolin-1 expression. When endothelial cells were coincubated with LDL, the effect of atorvastatin on caveolin-1 expression was less impressive, in part due to the fact that the LDL increased the baseline levels of caveolin-1. Using communoprecipitation, the investigators proceeded to show that the atorvastatin dose dependently inhibited the amount of caveolin-1 bound to eNOS. In keeping with these findings, atorvastatin also seemed to affect both the basal and stimulated release of nitric oxide.

A minor concern regarding this article is that the cysteine protease inhibitor N-acetyl-leu-leu-norleucine (ALLN) was used to reduce catabolism of the sterol response element–binding protein. The problem is that ALLN may prevent the catabolism of many proteins. Thus, although ALLN was previously shown to potently inhibit caveolin-1 transcription,18 its effect in the present study may have occurred via a variety of other pathways. Nevertheless, the findings are internally consistent with the concept that sterols (in the form of LDL) stimulate the transcription of caveolin-1 and that statins inhibit this effect.

Unlike the effects of statins on eNOS expression and cellular superoxide production mentioned above, the effect of atorvastatin on caveolin-1 expression is clearly dependent on its ability to lower cholesterol. Feron et al14 showed that the effect of statins on caveolin-1 could be overwhelmed by the addition of LDL. Thus, in the intact animal or human, one might expect that a similar effect on caveolin-1 expression, and a reciprocal effect on eNOS function, could be achieved by other mechanisms of cholesterol lowering, such as dietary restriction or the use of other lipid-lowering drugs. Indeed, the first study showing that cholesterol lowering improved endothelium-dependent vascular relaxation used a dietary intervention rather than statins.19 Likewise, early studies in humans also showed that cholesteryamine and diet effectively improved coronary endothelium-dependent vasodilatation.20

Over the past decade, there has been an enormous effort from many laboratories to understand how lipids and hypercholesterolemia alter endothelium-dependent vasodilatation using both animal models and humans. Several abnormalities of nitric oxide biosynthesis/bioavailability have been identified.5 There is substantial evidence that excessive production of superoxide or, perhaps, other free radicals can lead to inactivation of nitric oxide. Treatment of vessels and intact animals with membrane-permeable forms of superoxide dismutase corrects endothelium-dependent vasodilation in animals with experimental atherosclerosis.21 In these models, there has been ample documentation that the vascular production of superoxide is increased. In keeping with these findings, intra-arterial infusion of ascorbic acid corrects endothelium-dependent vasodilation in the forearm of hypercholesterolemic humans.22 The treatment of hypercholesterolemic humans with intravenous tetrahydrobiopterin, a critical cofactor for eNOS, has also been shown to improve endothelium-dependent vasodilation in hypercholesterolemic humans.23 Recent studies have shown that peroxynitrite potently oxidizes tetrahydrobiopterin,24 demonstrating another oxidant-based mechanism that may impair endothelium-dependent vasodilatation. Finally, in advanced human atherosclerosis, there is a clear loss of eNOS expression in endothelial cells overlaying atherosclerotic lesions.25

It is unclear how caveolin-1/eNOS interactions, defined in the present and previous studies by Feron et al,14,17 might be involved with any of these more established mechanisms that are thought to underlie endothelial dysfunction. A major challenge for all investigators interested in this problem is to demonstrate how present and prior studies related to caveolin-1 and eNOS interactions, performed entirely in cultured cells, relate to the altered production of nitric oxide in the setting of hypercholesterolemia and atherosclerosis in vivo.

References


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