Effect of Lipid-Lowering Therapy on Vasomotion and Endothelial Function

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The recent clinical trials of lipid lowering have established the benefit of this therapy in men and women with, or at high risk for, cardiovascular disease. It is now thought that most of the reduction in the risk of clinical events is due to functional rather than anatomic changes in atherosclerotic arteries. Cholesterol-lowering drugs improve endothelial vasomotor function and vascular nitric oxide in patients with coronary artery disease over several months. These changes in vasomotor function may reflect other beneficial changes that are regulated by nitric oxide such as the reduced recruitment and activation of inflammatory cells and a shift in the coagulation balance to favor thrombolysis. These mechanisms may contribute to the reduction in myocardial ischemia and clinical events observed with lipid lowering in patients with vascular disease. Lipid-lowering therapy decreases cardiovascular events and is an important adjunct to coronary revascularization most likely because an improvement in endothelial function prevents the development and destabilization of new atherosclerotic lesions and subsequent ischemic events.

Introduction

Numerous clinical trials in the past decade have established that lipid-lowering therapy reduces the risk of coronary events and mortality in men and women with cardiovascular disease or at high risk of disease [1,2••,3,4,5••]. Clinical trials now underway have turned towards new questions that address the optimal treatment target for low-density lipoprotein (LDL), and the therapeutic goals for primary prevention. The final proof of the cholesterol hypothesis and atherosclerosis has coincided with an increased understanding of the mechanisms responsible for atherogenesis, atherosclerosis, and their clinical sequela. These have provided new insights on how lipid lowering reduces cardiac events, while also raising several new questions.

The initial enthusiasm for lipid-lowering therapy stemmed from a concept that it would remove cholesterol from atherosclerotic plaques and thus decrease the luminal stenosis. This hypothesis was supported by experimental studies in animal models that had demonstrated substantial regression of intimal thickening with cholesterol lowering treatment [6]. Surprisingly, a large number of subsequent angiographic trials in patients with coronary artery disease failed to show a substantial change in the lumen of coronary arteries with cholesterol lowering (1%–2% on average over 2–4 years) [7]. These studies compared the progression of specified coronary lesions determined by quantitative coronary angiography among those receiving lipid lowering treatment for 2 to 4 years. In contrast, these regression studies showed a striking reduction in clinical events (ranging from 40%–80%) [7].

Because most acute coronary events are caused by the sudden rupture of mild or moderate stenoses [7], the concept that the gradual accumulation of atherosclerosis ultimately leads to occlusion and myocardial infarction demanded reevaluation. It is now thought that the benefits from lowering lipids do not arise from the small changes observed in the lumen of arteries, but rather from functional changes that stabilize the plaque and reduce the chance of rupture and the development of an occlusive thrombus [8–10]. These functional changes include a reversal of abnormal vasoconstrictive forces to various stimuli (*eg*, shear stress), a reduction in the recruitment and activation of inflammatory cells into the atherosclerotic plaque, and a change in the balance of coagulation favoring thrombolysis over thrombus formation [11]. One of the unifying aspects of these various effects of lipid-lowering on the biology of the atherosclerotic lesion is the central role of the endothelium in modulating these responses.

Endothelial Function

Although the endothelium is a single layer of cells lining the lumen of blood vessels, basic research and clinical studies have established that the endothelium is a highly active regulatory organ. Part of this role derives from the production of nitric oxide by endothelial cells. Nitric oxide is produced by the enzymatic action of nitric oxide synthase, which converts the amino acid L-arginine to nitric oxide and L-citrulline [12–14] (Fig. 1). Nitric oxide has many biologic effects that may influence the development and the clinical expression of cardiovascular disease [11,15].

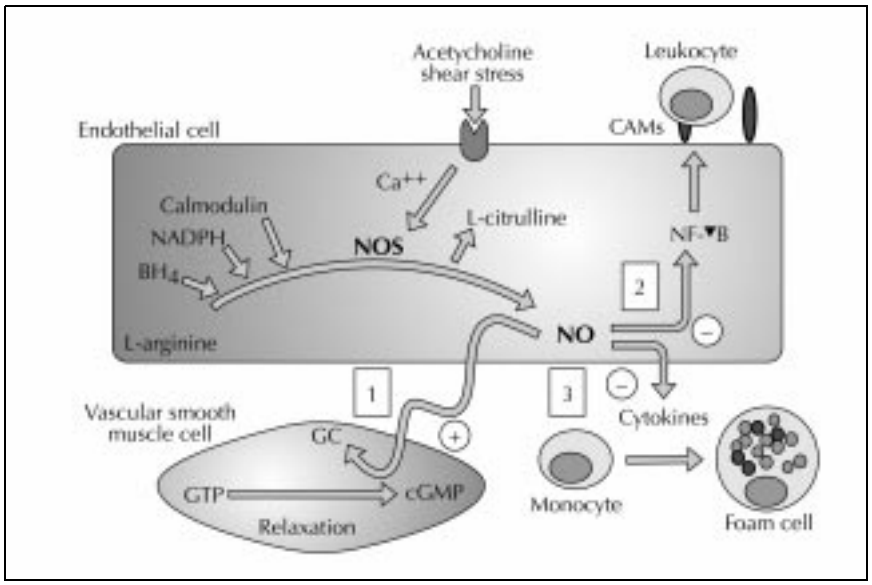

Figure 1. Acetylcholine, shear stress, and other stimuli increase the activity of nitric oxide synthase (NOS) to produce nitric oxide (NO) from L-arginine in the endothelial cell. **1**) Nitric oxide diffuses across vascular wall to the vascular smooth muscle cell activating guanylate cyclase to produce cyclic GMP and vasorelaxation. **2**) Nitric oxide reduces the expression of cytokines that activate leukocytes within the vascular wall. **3**) Nitric oxide reduces the activation of the nuclear factor-κB complex that is responsible for the expression of cellular adhesion molecules (CAMS) that assist the migration of leukocytes into the vascular wall. BH_4—Tetrahydrobiopterin; cGMP—cyclic guanosine monophosphate; GC—guanylate cyclase; GTP—guanosine triphosphate; NADPH—nicotinamide-adenine dinucleotide phosphate; NF—nuclear factor.

For example, nitric oxide generated by the endothelium acts on the adjacent vascular smooth muscle cells in the arterial media to raise cyclic GMP levels, thus causing arterial relaxation [11,15]. In this way, the endothelium can modulate vascular tone by changing the amount of nitric oxide produced. Typically nitric oxide production by endothelial cells increases in response to physiologic stimuli such as increased shear stress from augmented blood flow, and vasoactive mediators such as acetylcholine, bradykinin, serotonin and thrombin [16–20]. In contrast, when the bioavailability of nitric oxide is reduced, vasoconstrictive forces have a dominating effect. Nitric oxide bioavailability is reduced by oxidized LDL and oxygen-derived free radicals [15,21,22], which are typically found in abundance in atherosclerotic plaques. Even mild vasoconstriction, when superimposed on obstructive atheroma, can markedly increase stenosis resistance [17,18,23,24]. This is likely an important mechanism for angina in patients with coronary artery disease.

Although initial research focused on the vasomotor effects of nitric oxide, more recent work has elaborated the broader effects of nitric oxide. Nitric oxide exerts several actions that influence inflammatory signals, pathways that are increasingly recognized as playing a critical role in atherosclerosis. For example, nitric oxide inhibits the activity of nuclear factor (NF)-κB, a transcriptional regulatory complex within the endothelial cell [25–27] (Fig. 1). One of the downstream results of NF-κB activation is an increase in surface leukocyte adhesion molecule expression [28,29], an early step in increasing the number of inflammatory cells within the atherosclerotic lesion. Nitric oxide also inhibits the expression of the chemoattractants monocyte chemoattractant protein-1 and macrophage colony stimulating factor [30–32] (Fig. 1). These cytokines attract and activate leukocytes within atherosclerotic plaques. Thus, nitric oxide inhibits the recruitment and activation of inflammatory cells in the vessel wall. This inhibition is likely to be important because inflammatory cells like monocytes/macrophages are thought to contribute to plaque rupture by secreting matrix degrading enzymes that weaken the fibrous cap of atherosclerotic plaques increasing the likelihood of plaque rupture [8,15].

Platelet aggregation and adhesion, and the balance of thrombolysis and thrombosis at the vessel wall-blood interface, are also modulated by nitric oxide. Nitric oxide reduces platelet aggregation [33–35], and the healthy endothelium appears to maintain an appropriate balance of plasminogen activator inhibitor-1 (PAI-1) and tissue-type plasminogen activator (t-PA) that favors thrombolysis over thrombosis [36,37]. In the event of plaque rupture, these factors could play an important role in limiting the extent of thrombus formation and reducing the chance or severity of a clinical event. Therapies that increase the availability of nitric oxide in atherosclerotic arteries are therefore likely to prevent clinical events by opposing vasoconstriction, stabilizing atherosclerotic plaques and by reducing coronary thrombosis.

Assessing Endothelial Function

The assessment of endothelial function in vivo most commonly differentiates endothelium-dependent from endothelium-independent vasodilation. These methods were developed from the original experimental observations of Furchgott and Zawadski [14] who first described the critical role of the normal endothelium in regulating the relaxation of isolated arteries.

Endothelial function of the coronary arteries can be studied during cardiac catheterization and provides a direct measure of nitric oxide bioavailability in healthy or diseased arteries that are responsible for angina or myocardial infarction [17,18,24,38,39]. In these studies, endothelium-dependent vasomotion of the conduit epi-

cardial arteries is assessed using infusions of acetylcholine or other stimuli that increase the production and release of nitric oxide. A direct vasodilator such as nitroglycerin is used to evaluate endothelium-independent vasomotion. The subsequent changes in the diameter of the epicardial coronary artery under study are measured using quantitative coronary angiography. Coronary microvascular endothelial function can be measured by assessing the change in coronary blood flow to endothelium-dependent and endothelium-independent vasodilators that act on the coronary resistance arterioles [40,41]. Such changes in coronary blood flow are most commonly determined by Doppler techniques.

The systemic arteries can also be studied to assess endothelial function noninvasively. Two-dimensional (B-mode) ultrasound is used to measure the diameter of conduit arteries such as the brachial and femoral arteries in response to the endothelium-dependent vasodilation from reactive hyperemia and the endothelium-independent vasodilation from sublingual nitroglycerin [19,42]. The brachial artery endothelial responses correlate well with the coronary responses in patients who have had both studies performed. The noninvasive techniques are more widely applicable and represent a useful surrogate for studying coronary arteries. Another assessment of the systemic arteries uses the technique of venous occlusion plethysmography. This method is used to assess forearm blood flow as a measure of the microvascular responses in the forearm usually to pharmacologic stimuli of nitric oxide synthase [20]. It is assumed that the endothelium-dependent vasomotor responses are related to the other biologic actions of nitric oxide mentioned earlier, that determine plaque vulnerability and the balance of thrombosis and thrombolysis [15].

Effect of Lipid-Lowering Therapy

Studies of human coronary arteries have demonstrated impaired function of the endothelium in patients with atherosclerosis [17] or those with coronary risk factors but free of angiographically detectable disease, [24,40,41,43]. Several interventions that reduce blood cholesterol and LDL also improve endothelial vasomotor function. In patients with hyperlipidemia, drug therapy, particularly with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), can improve the vasomotor function of the epicardial coronary arteries to the endothelium-dependent vasodilator acetylcholine within 6 months to 1 year of therapy [44–47]. The endothelium also modulates vasomotor tone of the resistance vessels and thereby, regulates coronary blood flow. In patients with hyperlipidemia, lipid-lowering therapy improves the coronary blood flow response to acetylcholine [44]. These effects which should decrease angina or myocardial ischemia, occur in a timeframe that is generally too short for important changes in the lumen diameter of arteries with atherosclerosis.

These responses to pharmacologic agents indicating preserved nitric oxide bioavailability in the coronary epicardial and resistance vessels complement recent studies that have examined the effect of lipid-lowering on myocardial ischemia. In one study of patients with coronary stenoses who had segmental impaired myocardial perfusion by positron emission tomography (PET) scanning, statin therapy for 4 months improved myocardial blood flow to adenosine [48••]. Because the improvement in myocardial blood flow was localized to the abnormal segments, it is likely that statin therapy had a greater impact in improving flow mediated dilation of the stenotic epicardial arteries rather than causing a general improvement in microvascular function [48••]. Ambulatory Holter monitoring has also been used to assess the changes in myocardial ischemia due to activities of daily living. In two studies, lipid lowering therapy was associated with a reduction in myocardial ischemic episodes on Holter monitoring [49,50]. These changes were unlikely to occur within the time frame of angiographic regression and are more likely explained by improvements in coronary endothelial vasomotor function. The vasomotor responses to exercise, a nonpharmacologic assessment of endothelial function, were examined in another study of lipid-lowering. In men with abnormal epicardial vasoconstriction in response to exercise, 7 months of treatment with bezafibrate improved vasodilator responses, and the improvement was correlated with the improvement in serum cholesterol [51].

More aggressive and rapid lipid lowering may improve endothelial function very quickly over the course of hours to days. Studies using regular LDL-apheresis, which can reduce LDL concentrations by 60% within hours, have suggested that greater improvements in endothelial function can be achieved with more aggressive lipid reduction. In one recent study, LDL apheresis was no better than statin therapy at preventing the progression of angiographic atherosclerosis, but had a significantly better effect on reducing ischemia in response to bicycle exercise testing [52].

These beneficial effects of lipid lowering on vasomotor function are likely to extend beyond the coronary circulation as shown by studies of peripheral artery endothelial function. In patients with hyperlipidemia, lipid lowering can lead to improvements in peripheral artery endothelial function over a period of weeks [53], or even over a period of hours with the rapid reduction in LDL cholesterol achieved by LDL apheresis [54•]. Evidence that these changes are related to clinical improvements in claudication and ambulatory distance are not yet available but are under investigation in several studies.

One logical extension of the lipid-lowering effects on endothelial function might be a decrease in the need for coronary revascularization in some patients with chronic stable angina. Several studies have or are examining the benefit of aggressive lipid lowering as an alternative or as an adjunct to coronary revascularization. Successful LDL lowering may become a required step before considering

coronary revascularization for a patients with stable angina. Indeed, one could make such an argument based simply on the evidence already existing for a reduction in clinical events. The recently reported AVERT (Atorvastatin Versus Revascularization Treatment) study [55] randomized 341 patients to 80 mg of atorvastatin or coronary angioplasty with usual care lipid lowering (about 70% of the latter group received some form of lipid-lowering therapy). This trial, although somewhat controversial, has highlighted the importance of lipid-lowering therapy among patients being considered for coronary angioplasty. In this study, the time to a first ischemic event was significantly delayed in the lipid-lowering group compared to the angioplasty group. This result was principally due to differences in the need for further revascularization or the development of worsening angina requiring hospitalization. These end-points are likely to be higher after coronary angioplasty because of the finite risk of restenosis after angioplasty. Furthermore, angioplasty is a focal therapy whereas atherosclerosis is typically a diffuse disease. As such, angioplasty will have no effect on the stability of other mild atherosclerotic plaques that may precipitate acute coronary syndrome at a later date. In contrast, lipid lowering has a generalized effect to stabilize atherosclerosis throughout the coronary tree. Future studies, such as the COURAGE (Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation) trial, will determine the additional effect of lipid- lowering therapy with simvastatin in patients who are suitable for coronary revascularization. This strategy would use coronary angioplasty to treat a flow-limiting lesion responsible for the stable angina symptoms, and employ lipid lowering therapy to prevent the sudden rupture of plaques elsewhere in the coronary arteries thus reducing the risk of future ischemic events. Regardless of the outcomes of these trials, the improvement in vasomotor function and the prevention of ischemia with LDL-lowering demonstrated in earlier studies, establishes the importance of lipid-lowering as an adjunct to all revascularization modalities.

Lipid-Independent Effects of Statins

The statin class of drugs may exert beneficial effects beyond their ability to lower cholesterol. Recent laboratory work, as well as some clinical studies, have suggested that statins might have a direct effect on the expression of the gene for endothelial nitric oxide synthase, the enzyme that produces nitric oxide [56•,57,58•]. These studies indicate that the change in nitric oxide synthase levels may result from stabilization of the endothelial cell nitric oxide synthase mRNA. The clinical significance of these findings in the treatment of atherosclerotic arteries has yet to be determined. Other studies also suggest possible lipid-independent effects of statins [59], including work in primates [60].

Conclusions

Studies of endothelium-dependent vasomotor function demonstrate that lipid lowering therapies have a powerful effect on increasing the bioavailability of nitric oxide and restoring the normal vasomotor responses that increase myocardial blood flow after various stimuli. These observations likely reflect changes in other cellular functions regulated by endothelium-derived nitric oxide. These include a reduction in the recruitment and activation of inflammatory cells, and a shift in the thrombus-thrombolytic balance in favor of thrombolysis. These functional changes in arteries likely contribute to the impressive benefits seen with lipid-lowering in clinical trials. Further understanding of the role of the endothelium in regulating these responses, and delineating the boundaries of benefit from lipid-lowering drugs, will help refine our therapeutic approaches towards preventing and treating coronary artery disease [59].

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