Emerging Non-Statin LDL-Lowering Therapies for Dyslipidemia and Atherosclerosis

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Elevated low-density lipoprotein cholesterol is an important risk factor for atherothrombotic arterial disease. HMG-CoA reductase inhibitors, or statins, are very effective in lowering cholesterol levels, and several trials using statins have shown reductions in mortality and cardiovascular events, leading to the recent recommendations that all patients with known vascular disease, or who are at high risk for vascular disease, should be considered candidates for statin therapy. Yet statins reduce cardiovascular events by only about 20%–40%. Nonstatin therapies (either as monotherapy or in addition to statins) to reduce LDL cholesterol by mechanisms that do not involve inhibition of HMG-CoA reductase are likely to be useful for patients in need of LDL reduction; particularly those who either cannot take statins or respond only partially or not at all to statins alone. These therapies include cholesterol absorption inhibitors, Acyl-CoA cholesterol acyl transferase inhibitors, farnesoid X receptor antagonists, sterol-regulating binding protein cleavage activating protein, and microsomal triglyceride transfer protein.

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Elevated cholesterol, specifically low-density lipoprotein (LDL) cholesterol, is an important risk factor for atherothrombotic arterial disease. Statins (HMG-CoA reductase inhibitors) are very effective in lowering cholesterol and LDL cholesterol levels, representing an extremely important development in the pharmacotherapy of dyslipidemia and atherothrombotic vascular disease.[1,2] Several prospective randomized trials using statins have shown reductions in mortality and cardiovascular events in a variety of clinical settings, leading to the recent recommendations that all patients with known vascular disease or who

Table 1
Limitations of Statin Therapy

- Intolerance and adverse effects in some patients
- Ineffective or only partially effective lowering of cholesterol levels, in tolerated doses, in some patients
- Only 20%–40% risk reduction observed

are at high risk for vascular disease should be considered candidates for statin therapy.[1,2] Yet, despite the established clinical benefits of statins, cardiovascular events continue to occur in both men and women.

Multiple reasons account for the continuing epidemic of cardiovascular disease: underutilization of statins and less-than-optimal long-term compliance, intolerance of statins in some patients because of adverse effects; inability to achieve target cholesterol levels in many patients at tolerable doses and relative ineffectiveness in patients with homozygous familial hypercholesterolemia who lack the LDL receptor gene or carry a defective LDL receptor gene. Furthermore it should be recognized that even statins reduce cardiovascular events by only about 20%–40%, leaving at least 60% of the events continuing to occur (Table 1).[3,4] These findings underscore the complexity and multifactorial nature of atherosclerosis and suggest need for additional interventions,[3,4] which are likely to include a variety of regimens to augment HDL levels and HDL function but will likely also include nonstatin drugs to reduce LDL cholesterol (Table 2) by mechanisms that do not involve inhibition of HMG-CoA reductase. These LDL-lowering therapies (either as monotherapy or in addition to statins) are likely to be useful for patients in need of LDL reduction who either cannot take statins or respond only partially or not at all to statins alone.

Cholesterol Absorption Inhibitors

On average, 300 mg of cholesterol is absorbed daily from the gut and another 800 mg of cholesterol produced by the body is transported from the liver into the intestine. To maintain cholesterol homeostasis, about 1100 mg of cholesterol is excreted from the gut (Figure 1). Bile acid sequestering resins bind bile acids and prevent their absorption, leading to hepatic conversion of cholesterol to bile acids and thereby leading to a modest reduction in circulating total and LDL cholesterol levels; however, resins produce a number of annoying side effects that reduce their widespread applicability.

Other intestinally active agents include plant stanols. Oil-based products enriched with plant stanol esters can lower LDL cholesterol concentrations by 10%–14%.[5] Plant stanols are believed to act by preventing micellar cholesterol formation, thereby inhibiting intestinal absorption of cholesterol. Recent data also suggest that stanols may increase cholesterol efflux from intestinal cells into the intestinal lumen by activating intestinal activity of ABCA-(ATP binding cassette transporter) activity.[6] Newer agents to block intestinal transport of cholesterol have been developed and include agents that inhibit the bile acid transporter or the putative intestinal cholesterol permease on the intestinal brush border, leading

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Table 2
Novel Non-Statin LDL-Lowering Agents

Name	Mechanism of action
Ezetimibe	Inhibition of intestinal cholesterol absorption
Avasimibe	Inhibition of ACAT leading to inhibition of cholesterol esterification and intestinal cholesterol absorption
Guggulsterone	FXR antagonism leading to reduced cholesterol synthesis
GW532	Activation of SCAP leading to increased LDL receptor gene expression
MTP Inhibitors	Inhibition of VLDL assembly and secretion through inhibition of MTP
Apo E–related peptide	Increased hepatic uptake of LDL and VLDL

ACAT, Acyl-CoA cholesterol acyl transferase; FXR, farnesoid X receptor; SCAP, sterol regulatory binding protein cleavage activating protein; VLDL, very-low-density lipoprotein; MTP, microsomal triglyceride transfer protein; GW532, GlaxoSmithKline compound.

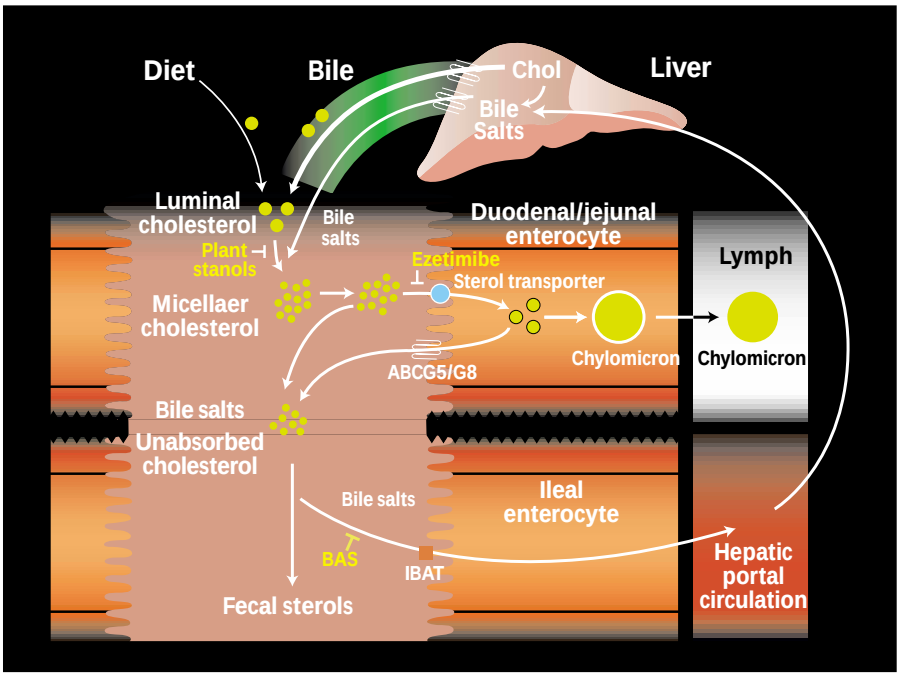

Figure 1. *Schematic shows the mechanism and site of action of various cholesterol-lowering agents that act in the gut. Plant stanol esters inhibit micellar formation, thereby preventing cholesterol absorption. Bile acid sequestrants (BAS) inhibit bile acid absorption which eventually leads to greater conversion of cholesterol in the liver to bile acids and subsequent elimination through the gut. Ezetimibe acts on the putative intestinal sterol transporter to selectively inhibit intestinal cholesterol absorption.*

to selective inhibition of cholesterol absorption with little effect on fat-soluble vitamin absorption.[7-10]

One such agent, ezetimibe, was recently approved by the FDA for use in the United States. Ezetimibe has been shown to reduce total cholesterol levels by 15%–20%, and its cholesterol-lowering effects are synergistic with those of statins.[10-15] In patients with homozygous familial hypercholesterolemia, ezetimibe produced a significant reduction in LDL cholesterol when combined with high-dose statin therapy, compared to high-dose statin alone, and was well tolerated.[13] Ezetimibe (Zetia®, Merck/Schering-Plough Pharmaceuticals, Kenilworth, NJ) is a welcome addition to our armamentarium as monotherapy for patients who need modest LDL lowering or as combination therapy with statins (or fibrates) for patients who need greater LDL lowering but are unable to tolerate high doses of statin or respond poorly to statins alone. A combination of low-dose statin (10 mg of atorvastatin) and 10 mg of ezetimibe has been shown to produce the same magnitude of LDL lowering as monotherapy with high-dose atorvastatin (80 mg).

Acyl-CoA Cholesterol Acyl Transferase Inhibitors

Acyl-CoA cholesterol acyl transferase (ACAT) is an enzyme that is responsible for cholesterol esterification in the macrophages, liver, and intestines.[16] Two forms of ACAT, ACAT-1 and ACAT-2, have been described encoded by two different genes.[16] Macrophages express predominantly ACAT-1, whereas intestines express predominantly ACAT-2. Human liver expresses both ACAT-1 and ACAT-2, with a predominance of ACAT-1.

Cholesterol ester formation by macrophages through the activity of ACAT-1 results in foam cell formation in atherosclerotic lesions, an event critical to formation and progression of atherosclerosis (Figure 2). Therefore inhibition of cholesterol ester formation by ACAT-1 inhibition may produce antiatherogenic effects. In addition, inhibition of cholesterol ester formation in the intestine through inhibition of ACAT-2 may reduce cholesterol absorption, resulting in a reduction in circulating cholesterol levels. Complete elimination of ACAT-1 activity through gene targeting or transplantation of ACAT-1-null bone marrow has not been shown to reduce atherosclerosis in apo E or LDL-receptor-null mice.[17,18] In fact, increased lesion size and macrophage necrosis (presumably through the toxic effects of unesterified free cholesterol) have been demonstrated, along with accumulation of cholesterol deposits, in the skin and brain.[17,18]

These observations have raised concerns about selective and complete inhibition of ACAT-1. On the other hand, partial inhibition of ACAT activity by using nonselective inhibitors of both ACAT-1 and ACAT-2 has shown promising results in murine hypercholesterolemia atherosclerosis models.[19-21] Similarly complete absence of ACAT-2 through gene targeting results in resistance to diet-induced hypercholesterolemia in mice, suggesting that selective ACAT-2 inhibition may be another

Ezetimibe is a welcome addition to our armamentarium as monotherapy for patients who need modest LDL lowering.

approach to reducing dietary absorption of cholesterol.[22] A number of ACAT inhibitors are currently undergoing evaluation.[23] One such compound, avasimibe, has entered clinical trials.[24] Avasimibe has been shown to reduce LDL and total cholesterol levels without major adverse effects.

Farnesoid X Receptor Antagonists

Resin extract of the Indian guggul tree (Commiphora mukul) has been in use in India since 600 B.C. for a variety of ailments and was approved for cholesterol lowering in India in 1987. It is available as a dietary supplement in the United States as guggulipid and has been shown to reduce LDL cholesterol by about 15%–18% and triglycerides by about 25%–30%.[25,26] The active ingredient appears to be guggulsterone.

Recently guggulsterone was shown to inhibit hepatic cholesterol synthesis by blocking the bile acid–induced activation of farnesoid X receptor (FXR) and to lower cholesterol levels in cholesterol-fed mice.[27] In addition, guggulsterone has been shown to have thyroid-stimulating activity, which may lead to blood-lipid lowering as well as weight loss. The dosage of guggulsterone is 25 mg three times a day, which can be obtained from 500 mg of guggulipid, standardized to contain 5% guggulsterone, given thrice daily. Long-term safety and efficacy have not been fully established.

Sterol-Regulatory Binding Protein Cleavage Activating Protein Ligands

The hepatic expression of LDL receptors is regulated by intracellular sterol concentrations (Figure 3). The LDL receptor gene contains sterol-responsive elements (SRE) that can be activated by the nuclear translocation of an active form of sterol-regulating binding proteins (SREBP-1 and SREBP-2) from the Golgi apparatus.[28,29] SREBP are normally located in the sarcoplasmic reticulum and require transport to the Golgi apparatus, where two proteases (site 1 protease and site 2 protease) cleave SREBP into active forms which then translocate to the nucleus to activate LDL receptor gene transcription. The transport of SREBP to the Golgi apparatus requires the activity of a chaperone protein called the sterol-regulatory element binding protein cleavage activating protein (SCAP), which contains a sterol-sensing mechanism. When SCAP senses a decrease in cellular cholesterol, SCAP is activated to transport SREBP to the Golgi apparatus for activation, and from there the active SREBP moves into the nucleus to stimulate LDL receptor gene transcription, leading to enhanced expression of LDL receptor. Increased LDL receptor density clears circulating LDL cholesterol, leading to decrease in serum LDL cholesterol levels.

Statins work by reducing intracellular cholesterol synthesis through inhibition of HMG-CoA reductase, the rate-limiting step in cholesterol biosynthesis, thereby activating the SCAP-SREBP pathway and ultimately increasing LDL receptor expression. Recently, GlaxoSmithKline (Research Triangle Park, NC) has developed

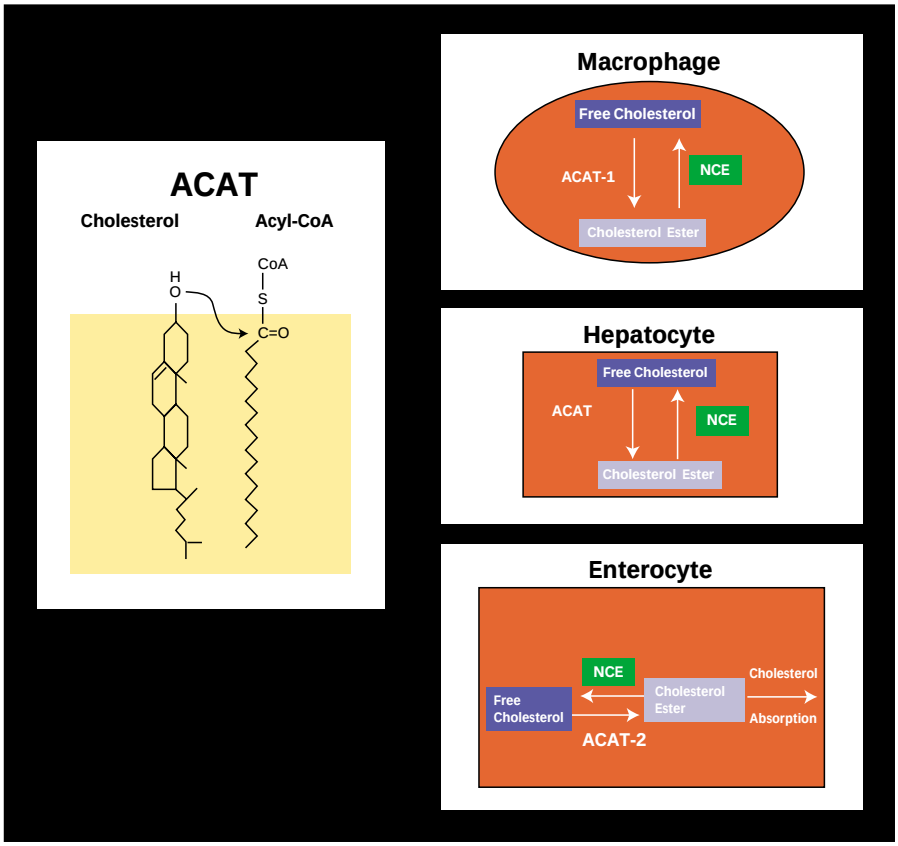

Figure 2. *Acyl-CoA cholesterol acyl transferase (ACAT) catalyzes the esterification of cholesterol by adding an acyl-CoA chain. This enzyme plays an important role in lipoprotein assembly in the liver, dietary absorption of cholesterol, and intracellular cholesterol ester formation in macrophages and smooth muscle cells of the vessel wall. Inhibition of ACAT may reduce atherosclerosis by reducing absorption of dietary cholesterol, interfering with lipoprotein assembly in the liver, and reducing cholesterol ester accumulation in the cells of the vessel wall. NCE, neutral cholesterol esterase.*

compounds which act as direct activating ligands for SCAP, simulating the effects of reduced cellular cholesterol and leading to overexpression of LDL receptors, thereby inducing a large reduction in circulating LDL and very-low-density lipoprotein (VLDL) cholesterol levels.[28,29] This is an exciting development that opens up the possibility of novel compounds to reduce LDL cholesterol levels.

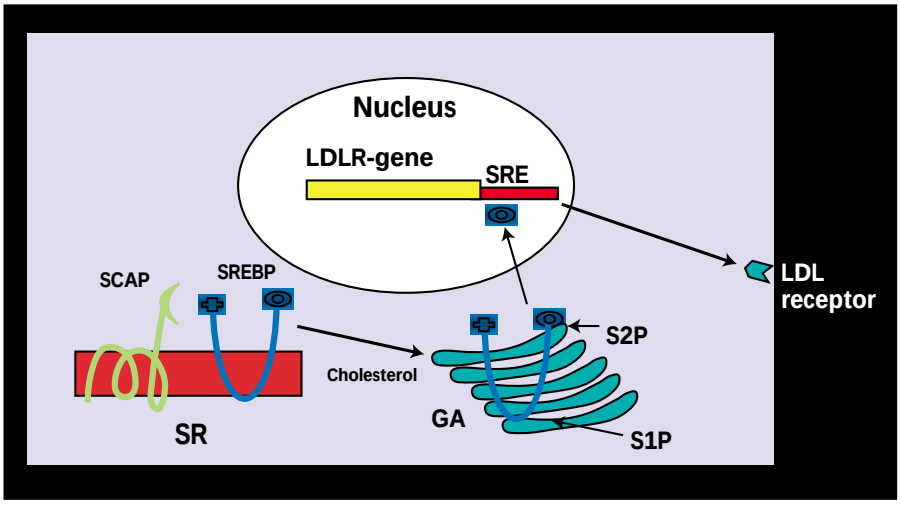

Figure 3. *Schematic illustrating the regulation of hepatocyte LDL-receptor gene transcription by intracellular cholesterol through sterol regulatory binding protein and SREBP-cleavage activating protein. SREBP, sterol regulatory binding protein; SCAP, SREBP-cleavage activating protein; SR, sarcoplasmic reticulum; GA, Golgi apparatus; LDLR, low-density lipoprotein receptor; SRE, sterol regulating element. S1P, site 1 protease; S2P, site 2 protease.*

Microsomal Triglyceride Transfer Protein Inhibitors

Microsomal triglyceride transfer protein (MTP) is a heterodimeric lipid transfer protein present in the endoplasmic reticulum of hepatocytes and intestinal cells. A defect in MTP gene (producing a severe deficiency in MTP) causes marked reductions in plasma triglycerides, LDL, and VLDL cholesterol (abetalipoproteinemia). These findings suggest that synthetic inhibitors of MTP capable of producing a partial deficiency in MTP function might be therapeutically useful for inhibiting the production of VLDL and chylomicrons, thereby reducing the levels of atherogenic lipoprotein.[30] Bristol-Myers Squibb Company (Princeton, NJ) has designed and tested small molecules capable of inhibiting MTP that can produce marked reductions in atherogenic lipoproteins in hamsters and Watanabe heritable hyperlipidemic rabbits that lack a functional LDL receptor.[31] Although these preclinical observations look quite promising, the safety and clinical efficacy of MTP inhibitors have not yet been fully established.

Synthetic Apo E-Related Peptide

Datta and colleagues have recently synthesized a dual-domain peptide, Ac-hE18A-NH(2), in which the arginine-rich heparin-binding domain of apolipoprotein E (apo E)(residues 141-150) is covalently linked to an 18 amino acid class A amphipathic helix with a high lipid affinity.[32] This peptide, when administered intravenously to apo E knockout

Main Points

- HMG-CoA reductase inhibitors, or statins, reduce cardiovascular events by only about 20%–40%.
- Nonstatin low-density lipoprotein (LDL) lowering therapies, either as monotherapy or in addition to statins, may help patients in need of LDL reduction who either cannot take statins or respond only partially or not at all to statins alone.
- Oil-based products enriched with plant stanol esters can lower LDL cholesterol concentrations by 10%–14%.
- Ezetimibe can reduce total cholesterol levels by 15%–20%, and its cholesterol-lowering effects are synergistic with those of statins; in patients with homozygous familial hypercholesterolemia, ezetimibe produced a significant reduction in LDL cholesterol when combined with high-dose statin therapy, compared to high-dose statin alone, and was well tolerated.
- Avasimibe, an acyl-CoA cholesterol acyl transferase inhibitor, has been shown to reduce LDL and total cholesterol levels without major adverse effects.
- Resin extract of the guggul tree has been in use in India since 600 B.C. for a variety of ailments and was approved for cholesterol-lowering in India in 1987; it has been shown to reduce LDL cholesterol by about 15%–18% and triglycerides by about 25%–30%.

mice, reduced plasma cholesterol level by 88% at 6 hours and by 30% at 24 hours.[32] This peptide associates with LDL and VLDL and results in their rapid uptake by liver cells via a heparin sulfate proteoglycan facilitated pathway, opening up a novel approach to treating hypercholesterolemia.[32] Further investigation of this peptide will be necessary to determine its safety and efficacy. ■

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