Published in final edited form as:

Nat Rev Cardiol. 2011 May ; 8(5): 266–277. doi:10.1038/nrcardio.2010.200.

Novel HDL-directed pharmacotherapeutic strategies

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Abstract

The burden of atherothrombotic cardiovascular disease remains high despite currently available optimum medical therapy. To address this substantial residual risk, the development of novel therapies that attempt to harness the atheroprotective functions of HDL is a major goal. These functions include the critical role of HDL in reverse cholesterol transport, and its anti-inflammatory, antithrombotic, and antioxidant activities. Discoveries in the past decade have shed light on the complex metabolic and antiatherosclerotic pathways of HDL. These insights have fueled the development of HDL-targeted drugs, which can be classified among four different therapeutic approaches: directly augmenting apolipoprotein A-I (apo A-I) levels, such as with apo A-I infusions and upregulators of endogenous apo A-I production; indirectly augmenting apo A-I and HDL-cholesterol levels, such as through inhibition of cholesteryl ester transfer protein or endothelial lipase, or through activation of the high-affinity niacin receptor GPR109A; mimicking the functionality of apo A-I with apo A-I mimetic peptides; and enhancing steps in the reverse cholesterol transport pathway, such as via activation of the liver X receptor or of lecithin-cholesterol acyltransferase.

Introduction

Individuals with manifest atherosclerotic disease, or a collection of associated risk factors, continue to endure a high incidence of adverse cardiovascular events despite the use of aggressive combination medical therapy. In the best-case scenario of intensive treatment and vigilant monitoring, the incidence of ‘hard’ cardiovascular events in secondary prevention cohorts is 20% after 4–5 years.^{1,2} To address the substantial residual risk of atherothrombotic events that exists even after optimum medical therapy, the development of novel therapies that attempt to make use of the atheroprotective functions of HDL, from its critical role in reverse cholesterol transport to its anti-inflammatory, antithrombotic, and antioxidant activities (Figure 1),³ is a major goal. Discoveries in the past decade, including

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Competing interests

D. J. Rader declares associations with the following companies: Abbott, AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Johnson & Johnson, Merck, Novartis, and Resverlogix. See the article online for full details of the relationships. E. M. deGoma declares no competing interests.

Author contributions

E. M. deGoma and D. J. Rader contributed equally to the discussion of content for the article, the research of data to include in the manuscript, writing the article and the reviewing and editing of the manuscript before submission.

the identification of the high-affinity niacin receptor GPR109A, the development of assays that enable the assessment of HDL functionality (such as those that assess the efficiency of macrophage-specific reverse cholesterol transport), and the results of massive genome-wide association studies, have shed light on new and complex metabolic and antiatherosclerotic pathways in which HDL is involved.^{4,5} These insights, in turn, have fueled the development of new HDL-targeted drugs, which can be classified according to four different therapeutic approaches: directly augmenting the concentration of apolipoprotein A-I (apo A-I), the major protein constituent of HDL; indirectly augmenting the concentration of apo A-I and HDL cholesterol; mimicking the functionality of apo A-I; and enhancing reverse cholesterol transport (Box 1). In this Review, we aim to discuss the hypothesized mechanisms of these pharmacotherapies, changes in HDL mass and function and, where available, surrogate atherosclerosis end points from pre-clinical and clinical studies.

Box 1

HDL-directed pharmacotherapeutic strategies

Directly augmenting apo A-I

Intravenous apo A-I therapy

- Recombinant apo A-I Milano/phospholipids (ETC-216)
- Purified native apo A-I/phospholipids (CSL-111/112)
- Autologous delipidated HDL

Oral upregulators of endogenous apo A-I production

- RVX-208

Indirectly augmenting apo A-I and HDL-cholesterol

Cholesteryl ester transfer protein inhibitors

- Dalcetrapib
- Anacetrapib

Niacin receptor (GPR109A) agonists

Endothelial lipase inhibitors

Mimicking apo A-I functionality

Apo A-I mimetic peptides

Enhancing reverse cholesterol transport

Liver X receptor agonists

Lecithin-cholesterol acyltransferase activators

Abbreviation: ApoA-I, apolipoproteinA-I.

Directly augmenting apo A-I levels

Lipid-poor apo A-I, also termed nascent HDL or pre β -HDL, initiates reverse cholesterol transport by activating macrophage ATP-binding cassette sub-family A member 1 (ABCA1) and accepting effluxed cholesterol. From a pharmacodynamic standpoint, direct augmentation of lipid-poor apo A-I concentration arguably represents the most validated HDL-related therapeutic approach in terms of antiatherogenic potential. Lipid-poor apo A-I-phospholipid complexes, sometimes referred to as recombinant HDL (rHDL), have been

studied extensively in animals and in preliminary studies in humans. Preclinical studies have demonstrated that the administration of apo A-I is associated with the inhibition or regression of atherosclerosis,^{6–10} enhanced macrophage-specific reverse cholesterol transport,⁵ and the inhibition of vascular inflammatory pathways,¹¹ endothelial adhesion molecule expression¹² and phospholipid oxidation.¹³ Moreover, short exploratory clinical studies of rHDL infusion have yielded decreases in coronary atherosclerosis, as assessed by coronary imaging, comparable with those obtained with long-term statin use at doses associated with improved clinical outcomes. These findings support the therapeutic potential of intravenous apo A-I infusion (Table 1).^{14–17}

Recombinant apo A-I Milano/phospholipid

The recombinant apo A-I Milano protein differs from wild-type apo A-I by a cysteine to arginine substitution at amino acid 173. The effects of apo A-I Milano complexed with phospholipid (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine), also known as ETC-216, have been studied in animals and humans. The apo A-I Milano mutation was first identified in a cohort of Italian patients who exhibited a decreased prevalence of atherosclerosis despite very low levels of HDL cholesterol (10–20 mg/dl).¹⁸ In support of an antiatherogenic activity for this drug, infusions of ETC-216 in rabbit and mouse models of atherosclerosis were associated with considerable reductions in the lipid and macrophage content of plaque.^{10,19,20} The superiority of apo A-I Milano over native apo A-I, however, remains uncertain. Using genetic mouse models of atherosclerosis, cholesterol efflux assays *in vitro*, and examination of macrophage-specific reverse cholesterol transport *in vivo*, some studies suggested that apo A-I Milano had greater antiatherogenic effects than wild-type apo A-I,^{21,22} whereas others indicated equivalence to the wild-type protein.^{23–26} Head-to-head intervention studies comparing administration of the two types of apo A-I are lacking.

In a small clinical study of 47 patients with acute coronary syndrome, five weekly doses of 15–45 mg/kg of ETC-216 significantly reduced total atheroma volume by 14.1 mm³ compared with baseline (4.2%; $P < 0.001$), as measured by coronary intravascular ultrasonography (IVUS) (Table 1).¹⁴ Total atheroma volume did not change significantly with the administration of placebo (–0.2 mm³; $P = 0.97$), and HDL-cholesterol and apo A-I levels were not reported. Two of 15 patients (9%) in the high-dose ETC-216 group were withdrawn from the study owing to adverse events, one because of symptomatic cholelithiasis and the other because of a hypersensitivity reaction.¹⁴ A subsequent dose-ranging study in a rabbit model suggested that five infusions of ETC-216 at doses equivalent to 8–15 mg/kg in humans might be adequate to maximize plaque stabilization or regression.⁸ Substantial interest remains in investigating the therapeutic potential of rHDL infusion containing apo A-I Milano. Clinical development of this drug, which was halted owing to manufacturing difficulties, is scheduled to resume in 2011 following completion of technology transfer to a different pharmaceutical company.²⁷

Purified native apo A-I/phospholipid

CSL-111 consists of complexes of native apo A-I and phosphatidylcholine, the former isolated from precipitates obtained by cold ethanol fractionation of human plasma and the latter derived from soybean.^{16,28} A randomized, placebo-controlled study in which serial intravascular ultrasonography was used to assess 145 patients with acute coronary syndrome revealed that administration of four weekly 40 mg/kg infusions of CSL-111 reduced atheroma volume by 5.3 mm³ compared with baseline (3.4%; $P < 0.001$) (Table 1).¹⁶ No statistically significant difference in atheroma volume was observed when comparing patients receiving placebo and those treated with CSL-111 ($P = 0.48$). Quantitative coronary angiography, on the other hand, demonstrated that CSL-111 use was associated with less progression of coronary atherosclerosis than placebo (–0.039 mm and –0.071 mm,

respectively; $P = 0.03$), as measured by the coronary score (per-patient mean of the minimal lumen diameter for all lesions measured) among patients with less-severe stenoses at baseline.¹⁶ Lower coronary scores have been associated with an increased risk of incidence of coronary events.²⁹ By comparison, 40 mg daily doses of pravastatin or 40–80 mg daily doses of lovastatin administered for 2 years attenuated coronary atherosclerosis progression as assessed by coronary score to a similar degree as only 4 weeks of CSL-111 treatment.^{30,31} One-third of patients randomly assigned to receive 80 mg of CSL-111 had reversible alanine aminotransferase elevations exceeding 10 times the normal upper limit, but the group administered CSL-111 at the lower dose of 40 mg/kg did not differ from the placebo group with regard to transaminase levels. More participants randomly assigned to 40 mg/kg of CSL-111 exhibited hypotension compared with those receiving placebo (13.8% and 7.1%, respectively). Further details regarding the observed changes in blood pressure were not provided in the original publication.¹⁶

A subsequent study in patients with lower extremity peripheral artery disease demonstrated that treatment with a single CSL-111 infusion was associated with significant reductions in lipid content ($P < 0.05$; assessed by oil red O staining) and endothelial adhesion molecule expression ($P < 0.05$; assessed by vascular cell adhesion molecule 1 [VCAM-1] expression) in plaque excised by atherectomy.³² These changes were observed at the time of percutaneous superficial femoral artery revascularization, which occurred 5–7 days following CSL-111 infusion, indicating a rapid onset of action of the drug. In addition, CSL-111 considerably increased the levels of HDL cholesterol and enhanced the capacity of plasma depleted in apolipoprotein B (apo B) to promote cholesterol efflux from macrophages. Among patients with diabetes mellitus, infusion of CSL-111 increased the levels of HDL cholesterol up to 40%, inhibited platelet aggregation *ex vivo*, and reduced monocyte activation and neutrophil adhesion.^{33,34} A reformulated version of CSL-111, called CSL-112, has been reported in preclinical studies to provide greater cholesterol efflux capacity and have less hepatotoxicity than CSL-111.³⁵ A phase I study of intravenous CSL-112 has been initiated.³⁵

Autologous delipidated HDL

A novel HDL-directed pharmacotherapeutic approach utilizes the infusion of autologous delipidated HDL.¹⁷ The Plasma Delipidation System-2 (PDS-2) from Lipid Sciences involves the collection of 1 l of plasma by apheresis over 1.5–2.0 h followed by the selective removal of lipids from HDL particles using organic solvents; the lipid-poor pre β -HDL, an efficient acceptor of cholesterol from ABCA1, is subsequently reinfused over 1 h.

Preclinical evaluation of selective delipidated HDL in a nonhuman primate model of dyslipidemia achieved a significant 6.9% reduction in aortic atheroma volume, assessed by IVUS.³⁶ In a small clinical study, among 28 patients with acute coronary syndrome, seven weekly infusions of autologous delipidated HDL increased pre β -HDL levels approximately 30-fold and decreased total atheroma volume by 12 mm³ (5.2%) from baseline (Table 1).¹⁷ As in the studies of intravenous apo A-I therapy discussed above, no statistically significant differences were observed between actively-treated patients and control groups. Autologous delipidated HDL infusions did not induce transaminitis or hypersensitivity reactions; however, apheresis resulted in hypotension in a third of the participants undergoing this treatment.

Oral upregulator of endogenous apo A-I

A small synthetic molecule (termed RVX-208) belonging to the quinazoline family, which are known primarily for their antimalarial properties, was reported in cellular screening assays to raise apo A-I production.³⁷ In a monkey model of atherosclerosis, administration

of a compound containing RVX-208 over approximately 2 months increased plasma apo A-I and HDL-cholesterol levels up to 60% and 97%, respectively, in a dose-dependent manner. Monkeys treated with RVX-208 had enhanced cholesterol efflux from macrophage foam cells *ex vivo*. A doubling in the levels of triglycerides was observed with the highest dose of the drug; however, the mid-range dose led to a 43% increase in apo A-I concentration without affecting triglyceride levels.³⁷ No effect on apo B or LDL-cholesterol levels was observed with any dose of RVX-208. A short exploratory study in humans demonstrated increases in total plasma apo A-I and pre β -HDL levels (10% and 42%, respectively), as well as augmentation of cholesterol efflux with the use of RVX-208.³⁷ Findings from the ASSERT study were presented at the 2010 AHA conference, and the full results are anticipated to be released in 2011. Modest changes in HDL-C and apo A-I were reported to have been observed in patients with stable coronary artery disease on chronic statin therapy.³⁸ According to Dr Stephen J. Nicholls, a study of RVX-208 is being planned in patients with acute coronary syndrome using coronary intravascular ultrasonography and is currently scheduled to begin in 2011.³⁸

Indirectly augmenting apo A-I levels

Increasing apo A-I and HDL-cholesterol levels can also be achieved indirectly by manipulating HDL metabolism, particularly through approaches that slow the rate of catabolism of apo A-I or HDL cholesterol, or both. Approaches within this category include inhibition of cholesteryl ester transfer protein (CETP) or endothelial lipase and, potentially, stimulation of the niacin receptor GPR109A.

CETP inhibitors

CETP shuttles cholesteryl esters from HDL to LDL and VLDL in exchange for triglycerides.³⁹ Genetic CETP deficiency causes marked elevation in HDL-cholesterol levels and modest elevation in apo A-I levels. Some,^{40–44} but not all,^{45–47} epidemiological studies indicate that CETP has an atherogenic role, with reduced plasma CETP mass or activity associated with reduced incidence of atherothrombotic cardiovascular disease; however, this issue remains far from settled. Preclinical studies involving reduction in CETP activity through various means, including the use of small-molecule inhibitors, antisense oligonucleotides, and vaccine-induced antibodies, have generally demonstrated decreased plaque formation with these agents, lending further support to CETP inhibition as a viable therapeutic strategy for the prevention and management of atherothrombotic disease.^{48–52}

Despite these supporting data, the demise of the CETP inhibitor torcetrapib raised doubts about the concept of CETP inhibition. In the Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE),⁵³ which involved patients receiving atorvastatin, torcetrapib worsened the primary combined cardiovascular end point (death from coronary heart disease, nonfatal myocardial infarction, stroke, and hospitalization for unstable angina) (hazard ratio [HR] 1.25, 95% CI 1.09–1.44, $P = 0.001$) and all-cause mortality (HR 1.58, 95% CI 1.14–2.19, $P = 0.006$) compared with placebo after 12 months despite increasing HDL-cholesterol levels by 72% and decreasing LDL-cholesterol levels by 25%.⁵³ The worrisome results of ILLUMINATE, paralleled by negative imaging findings in trials in which coronary and carotid ultrasonography were used,^{54,55} have been at least partly attributed to the off-target effects of torcetrapib, such as the raising of systolic blood pressure by an average of 5.4 mmHg.^{56,57} As suggested by aldosterone measurements from participants in ILLUMINATE,⁵³ findings from animal models⁵⁸ and human adrenal cell assays⁵⁹ indicate that these off-target effects are related to stimulation of aldosterone synthesis by torcetrapib via pathways independent of CETP inhibition.

Could inhibition of CETP activity explain some of the adverse effects observed in the clinical development program of torcetrapib? Initial concerns about CETP inhibition highlighted the possibility that the formation of large cholesterol-rich HDL particles resulting from CETP inhibition might be associated with impaired cholesterol efflux from peripheral macrophages to these particles.⁶⁰ However, studies of patients either deficient in CETP or treated with a CETP inhibitor demonstrated not reduced, but rather enhanced, efflux of cholesterol via ATP-binding cassette sub-family G member 1 (ABCG1) to HDL.^{59,61,62} An important arm in the proximal step of reverse cholesterol transport, therefore, remains intact, if not improved, in the setting of CETP inhibition. On the other hand, the net effect of CETP inhibition on reverse cholesterol transport might depend, in part, on the effects of CETP on the hepatic uptake of HDL-derived cholesterol. The hepatic uptake of HDL cholesterol can be direct, or occur after transfer of HDL cholesterol via CETP to apo B-containing lipoproteins, which are then taken into the liver via the LDL receptor. As illustrated by macrophage-specific reverse cholesterol transport assays, in the setting of highly effective LDL clearance, CETP can actually enhance reverse cholesterol transport and have an atheroprotective role; however, when LDL clearance is impaired, CETP can slow down reverse cholesterol transport and thus be proatherogenic.⁶³ The interdependence of terminal reverse cholesterol transport pathways suggests that the hypothesis that the individuals who are most likely to benefit from CETP inhibition are those with suboptimal LDL-receptor-mediated hepatic uptake of cholesterol. Fortunately, at least two novel compounds apparently lacking the off-target effects of torcetrapib enable further testing of the CETP-inhibition strategy.

Dalcetrapib—Dalcetrapib binds CETP irreversibly and is considerably less-potent than torcetrapib.⁶⁴ A study in a hamster model suggested that dalcetrapib promotes reverse cholesterol transport.⁶⁵ In a human study, among individuals with mean baseline HDL-cholesterol and LDL-cholesterol levels of 47 mg/dl and 144 mg/dl, respectively, monotherapy with 600 mg of dalcetrapib daily increased HDL-cholesterol levels by 23% compared with placebo administration, after 4 weeks of treatment.⁶⁶ Among patients with type II dyslipidemia receiving 40 mg of pravastatin daily whose baseline levels of HDL cholesterol and LDL cholesterol were 48 mg/dl and 120 mg/dl, respectively, the addition of 600 mg of dalcetrapib daily increased HDL-cholesterol levels by 28% and decreased LDL-cholesterol levels by 7% compared with placebo administration, after 4 weeks of treatment.⁶⁷ After 24 weeks of therapy with 900 mg of dalcetrapib daily, HDL-cholesterol levels increased by 33% (mean baseline level of 42 mg/dl) in patients at high risk of coronary heart disease events who were treated with 10–80 mg of atorvastatin daily, when compared with HDL levels in participants receiving placebo plus atorvastatin.⁶⁸ LDL-cholesterol levels, however, did not differ in the two patient groups (mean baseline level of 74 mg/dl).⁶⁸ Importantly, no changes in blood pressure or aldosterone levels were observed with the use of high-dose dalcetrapib (900 mg).⁶⁸

Two ongoing vascular imaging studies, DAL-Plaque⁶⁹ and DAL-Plaque 2,⁷⁰ will assess changes in atherosclerotic plaque (morphology, composition, and inflammatory activity) among patients with coronary heart disease treated with 600 mg of dalcetrapib daily. A wide variety of modalities will be used, including coronary and carotid ultrasonography, PET, and MRI. Another study, DAL-Vessel,⁷¹ will examine a different functional surrogate, arterial reactivity, by assessing brachial flow-mediated dilatation. Finally, the phase III clinical trial DAL-Outcomes⁷² will evaluate the effects of adding 600 mg of dalcetrapib daily to optimum pharmacotherapy in patients with acute coronary syndrome over a follow-up period of at least 2 years, with results expected in 2013.⁶⁴

Anacetrapib—Anacetrapib potently inhibits CETP by forming a tight reversible bond with this protein.⁷³ Healthy individuals with mean baseline HDL-cholesterol and LDL-

cholesterol levels of 51 mg/dl and 138 mg/dl, respectively, exhibited changes in the levels of these lipoproteins of +129% and -38%, respectively, after receiving 300 mg of anacetrapib daily for 10 days.⁷⁴ No changes in blood pressure, assessed through 24 h ambulatory monitoring, were observed. Among patients with dyslipidemia who had mean baseline HDL-cholesterol and LDL-cholesterol levels of 50 mg/dl and 141 mg/dl, respectively, addition of 300 mg of anacetrapib to 20 mg of atorvastatin daily for 8 weeks increased HDL-cholesterol levels by 120% and decreased LDL-cholesterol levels by 30% compared with statin monotherapy.⁷⁵ Interestingly, lipoprotein(a) levels, which were unchanged by statin therapy, decreased by up to 50% following anacetrapib administration.⁷⁵

Beyond a beneficial effect on standard lipid-mass parameters, anacetrapib can improve measures of HDL functionality. Compared with HDL isolated from patients receiving niacin or placebo, HDL sampled from patients who received 300 mg of anacetrapib daily for 8 weeks promoted greater cholesterol efflux from foam cells in culture, independent of HDL-cholesterol levels.⁶² In the same experimental set-up, lipid-A-induced cytokine and chemokine messenger RNA (mRNA) transcription (markers of inflammation) were comparable when the cells were treated with HDL from patients who received anacetrapib and when they were treated with control HDL, suggesting that the anti-inflammatory activity of HDL remained preserved with anacetrapib administration.

The phase III Determining the Efficacy and Tolerability of CETP Inhibition with Anacetrapib (DEFINE) randomized, placebo-controlled trial examined the effect of 100 mg of anacetrapib administered daily for 18 months to 1623 patients with coronary heart disease or equivalent conditions (peripheral artery disease, cerebrovascular disease, diabetes, or a 10-year Framingham risk score >20%) who had achieved LDL-cholesterol treatment goals with statin therapy.⁷⁶ The primary end points were the percent change in LDL-cholesterol at 24 weeks and the safety profile of anacetrapib at 76 weeks. HDL-cholesterol and other lipid parameters including lipoprotein(a) were assessed as secondary endpoints.⁷⁷ Treatment with anacetrapib was associated with a 40% reduction in LDL-cholesterol from 81 mg/dl to 45 mg/dl ($P < 0.001$) and a 138% increase in HDL-cholesterol from 41 mg/dl to 101 mg/dl ($P < 0.001$) compared with placebo.⁷⁷ Lipoprotein(a) decreased 36% compared with placebo from 27 nmol/l to 15 nmol/l. No increases in clinic-based blood pressure, serum aldosterone levels, or cardiovascular events were observed following anacetrapib treatment at 76 weeks. Supported by these substantial improvements in LDL, HDL, and lipoprotein(a), as well as an apparently benign safety profile, the Randomized Evaluation of the Effects of Anacetrapib Through Lipid-modification (REVEAL) is scheduled to begin in April 2011.⁷⁸ This study will examine major coronary events, defined as coronary death, myocardial infarction and coronary revascularization procedures, in 30,000 patients with coronary heart disease, cerebrovascular atherosclerotic disease, or peripheral artery disease. The estimated study completion date is January 2017.⁷⁸

Niacin receptor agonists

Niacin, the first antidyslipidemic agent identified, remains the most potent drug for increasing levels of HDL-cholesterol and apo A-I. Evidence suggests that HDL mediates the potential atheroprotective effects of niacin,⁷⁹ although the role of beneficial changes in other lipid parameters, including a reduction in the concentration of LDL particles and lipoprotein(a), cannot be excluded. Randomized trials have shown a clinical benefit of niacin among patients with coronary heart disease, with low cardiovascular event rates^{80,81} and improvements in surrogate measures of vascular function, as assessed by imaging,^{79,82} associated with niacin administration. One of these studies, for example, revealed regression of carotid intima-media thickness (IMT), the primary imaging end point, associated with niacin treatment, whereas no change in IMT was observed among patients treated with the LDL-lowering drug ezetimibe.⁸² The niacin group achieved better outcomes in IMT

compared with the ezetimibe group in the setting of 23% higher HDL-cholesterol levels, despite 7% higher LDL-cholesterol levels, lending further credence to an HDL-targeted approach.⁸² Definitive data on the incremental value of niacin when added to optimum medical therapy, which includes aggressive statin use, might emerge from two large trials, the Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides and Impact on Global Health Outcomes (AIM-HIGH) study⁸³ and the Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE) study,⁸⁴ which are anticipated to be communicated in 2012 or 2013.

After 50 years of clinical use,⁸⁵ the mechanisms by which niacin raises the levels of HDL (and other lipoproteins) remain elusive. Studies of cultured hepatocytes suggest that these effects might be mediated by decreased catabolism of apo A-I owing to downregulation of the HDL holoparticle (protein plus lipids) receptor, which is a terminal step in the reverse cholesterol transport pathway.⁸⁶ Kinetic studies in humans, on the other hand, demonstrate either decreased clearance^{87,88} or increased synthesis of apo A-I in patients receiving niacin,⁸⁹ the latter finding being notably absent from analyses *in vitro*. The discovery of the niacin receptor GPR109A⁹⁰ promised to usher in a new era in which the molecular mechanisms underlying the effects of niacin on lipids, and its adverse effects on the skin, such as flushing, could be clearly defined. This discovery might also open the way for the development of new synthetic agonists of GPR109A, which would ideally have the same therapeutic effects as niacin, but not the skin-related adverse effects of this drug, which substantially limit its therapeutic use. Activation of GPR109A on skin immune cells, such as Langerhans cells and keratinocytes, stimulates phospholipase A₂, enhancing synthesis of prostaglandin D₂ (PGD₂) and prostaglandin E₂ (PGE₂), which subsequently induce cutaneous capillary vasodilation by binding to subtypes of the PGD₂ receptor (DP1) and the PGE₂ receptor (EP2 and EP4).⁴ Coadministration of the DP1 antagonist laropiprant reduces the incidence of the skin-related adverse effects of niacin.⁹¹ However, flushing still occurs in over half of the patients, and discontinuation of niacin treatment owing to severe skin-related symptoms remains a problem.⁹¹ A study in mice implicates keratinocyte-produced PGE₂ as a key mediator of niacin-induced skin flushing, suggesting that this molecule might be another potential target to minimize flushing.^{92,93}

Although activation of GPR109A on adipocytes clearly leads to an acute reduction in lipolysis and release of free fatty acids,⁹⁰ the role of this receptor in mediating the effects of niacin on plasma lipoproteins remains uncertain. Partial agonism of GPR109A might differentially activate lipolysis and flushing pathways. A study in mice supports this dissociation and implicates ERK1/2 mitogen-activated protein kinase phosphorylation in vasodilation.⁹⁴ Administration of the partial agonist MK-0354 in humans resulted in a lack of flushing; however, despite effective suppression of lipolysis and free-fatty-acid release, this partial agonist had no effect on HDL-cholesterol or other plasma lipoproteins.⁹⁵ These results have brought into question the role of GPR109A agonism in mediating the effects of niacin therapy on plasma lipoproteins. This question is critical for the field, and will determine the future of GPR109A agonists as a therapeutic strategy.

Endothelial lipase inhibitors

Synthesized by and bound to vascular endothelial cells, endothelial lipase exhibits predominant phospholipase activity and affinity for HDL, unique characteristics among the lipoprotein–lipase family.⁹⁶ An association between the expression of endothelial lipase and HDL-cholesterol levels was identified in overexpression and loss-of-function mouse models.^{97–99} Genetic studies indicate a similar association in humans, with rare and low-frequency loss-of-function variants of endothelial lipase, identified through deep sequencing, resulting in elevated HDL-cholesterol levels.¹⁰⁰ How changes in HDL-cholesterol levels attributed to endothelial lipase ultimately affect atherosclerosis remains

uncertain. Some human studies point to an atherogenic role of endothelial lipase, with a positive association between plasma levels of this enzyme and coronary artery calcification, features of the metabolic syndrome (such as impaired fasting glucose, hypertriglyceridemia, low HDL-cholesterol, hypertension, and waist circumference), and inflammation.^{101,102} Carriers of endothelial-lipase variants associated with raised HDL-cholesterol levels have been reported to have a decreased risk of atherothrombotic disease,¹⁰³ although this link has not been observed in other studies.^{104,105} Of note, animal studies have shown that HDL-cholesterol levels do not always correlate with physiological changes associated with decreased atherosclerotic burden, with an endothelial-lipase knockout mouse model failing to demonstrate improved macrophage reverse cholesterol transport with raised HDL-cholesterol levels.¹⁰⁶ More worrisome, in the setting of hepatic-lipase deficiency, is that the absence of endothelial lipase resulted in an accumulation of small dense LDL,¹⁰⁶ a particularly atherogenic subpopulation of LDL. These findings suggest that endothelial-lipase inhibition could potentially exert a detrimental effect on atherosclerosis, even though HDL-cholesterol levels are raised. Despite this uncertainty, endothelial lipase has been the object of substantial interest as a therapeutic target. High-throughput screening identified several compounds sharing a sulfonyl furan urea core as potent and selective endothelial-lipase inhibitors.¹⁰⁷ Further evaluation of these and other small-molecule inhibitors of endothelial lipase has not been performed yet.

Mimicking apo A-I functionality

Another HDL-directed therapeutic approach utilizes small peptides that mimic one or more of the functions of apo A-I.^{108,109} The most well-studied apo A-I mimetic, 4F, consists of 18 amino acids designed to share the lipid-binding properties of apo A-I through a common secondary structure, the class A amphipathic helix.¹¹⁰ Although many studies have employed the parenteral administration of 4F made from L-amino acids (L-4F), the use of D-amino acids (D-4F) enables oral delivery of this compound by conferring resistance to mammalian gastrointestinal proteolytic enzymes.¹¹¹ Studies *in vitro*^{112–116} and in animals^{111,117–119} indicate that 4F recapitulates many of the functions of apo A-I. In respect to reverse cholesterol transport, 4F has been demonstrated to promote cholesterol efflux from macrophages via ABCA1^{109,116} and offloading of cholesterol to hepatocytes via the scavenger receptor class B type I (SR-BI).¹¹⁴ In addition, 4F exhibits apo A-I-like anti-inflammatory properties, inducing a quiescent macrophage phenotype¹¹³ and inhibiting LDL-induced macrophage chemotaxis.¹¹¹ The antioxidant capacity of 4F can surpass that of the native apo A-I, with 4F exhibiting a greater affinity for oxidized phospholipids and fatty acids,¹¹⁵ and retaining the ability to inhibit the production of chemokines from human artery walls despite coinubation with LDL.¹¹² Finally, like apo A-I, 4F has antithrombotic properties, inhibiting platelet aggregation in hyperlipidemic mouse models.¹¹⁷

Animal studies suggest that these functions of 4F translate into atheroprotective effects.^{111,118,119} Several mouse models of accelerated atherosclerosis, including LDL-receptor-knockout and apolipoprotein E (apo E)-knockout mice, developed less atheroma when treated with 4F than controls.^{111,118} One short-term study in LDL receptor-null mice demonstrated no effect of the apo A-I mimetic on established aortic atheroma size and lipid content and a minimal decrease in macrophage content.¹¹¹ Administration of 4F for 6 months in old apo E-null mice with coadministration of statins was associated with regression of established aortic lesions, which is, arguably, a more clinically relevant parameter than the effects on *de novo* plaque development.¹¹⁹

The only reported human study of D-4F to date hints at the possibility of a benefit for this compound in humans.¹²⁰ Compared with HDL isolated from individuals who received placebo, HDL isolated from individuals treated with a single 300 mg or 500 mg dose of

unformulated D-4F increased the inhibition of LDL-induced monocyte chemotaxis in cultures of human aortic endothelial cells. As in animal studies, neither changes in apo A-I or HDL-cholesterol levels nor serious toxicity were observed in treated individuals.

Several other apo A-I mimetics have been developed. Some, such as ETC-642 (also called RLT peptide), were designed to activate lecithin-cholesterol acyltransferase (LCAT; see below);¹²¹ others, such as oxpholipin 11, have antioxidant properties;¹²² and still others, such as ATI-5261, were engineered to maximize cholesterol efflux.¹²³ The latter peptide increased macrophage-specific reverse cholesterol transport and also reduced the development of aortic atherosclerosis by up to 45% over 6 weeks of intravenous treatment in mice.¹²³ As illustrated in a systematic study of 22 apo A-I mimetic peptides, structural elements that enhance one aspect of HDL functionality do not necessarily optimize other atheroprotective qualities that characterize apo A-I.¹²⁴ The clinical development of apo A-I mimetic peptides is in its infancy, but with the current amount of activity in this area of research we can expect to see a growing number of additional peptides being developed. With the exception of D-4F, apo A-I mimetic peptides require parenteral administration and, therefore, are likely to be initially targeted to patients at high risk with acute coronary syndromes.

Enhancing reverse cholesterol transport

As reverse cholesterol transport is the most well-established atheroprotective function of HDL, particularly in its initial steps involving cholesterol efflux from peripheral cells, this process represents a major target of novel HDL-directed therapies.

Agonists of the liver X receptor

Liver X receptors (LXRs), which are members of the nuclear receptor superfamily, have a central role in lipid metabolism. Endogeneously activated by oxysterols, LXRs regulate the transcription of a myriad of target genes by binding to their promoters together with the retinoic acid receptor.¹²⁵ With regard to HDL and reverse cholesterol transport, LXR activation has been demonstrated to promote mobilization of intracellular cholesterol,¹²⁶ increase macrophage cholesterol efflux via macrophage ABCA1 and ABCG1,¹²⁷ and augment intestinal HDL generation.¹²⁸ Two LXR isoforms have been identified—LXR α and LXR β . These isotypes have structural homology, but differ in tissue distribution, with the former being primarily expressed in the liver, macrophages, intestine, kidney, and adipose tissue, whereas the latter is found ubiquitously.¹²⁹

Therapeutic development of LXR agonists has been hindered by hepatic steatosis and increased plasma tri-glyceride concentrations reported in preclinical studies of these drugs.^{130,131} Fortunately, dissociating LXR efficacy and toxicity might be possible owing to the differential effects of LXR agonism by receptor isoform and by tissue-specific effects. For example, gene knockout models in mice implicate LXR α ,¹³² and not LXR β ,¹³³ as the primary stimulus for hepatic lipogenesis and hypertriglyceridemia. Administration of a nonselective LXR agonist to LXR α -deficient mice increased HDL-cholesterol levels and stimulated macrophage ABCA1 expression and cholesterol efflux without inducing fatty liver and with minimal transcription of hepatic sterol regulatory element-binding protein isoform 1c (SREBP1c) mRNA, which upregulates the synthesis of triglycerides in the liver.^{74,134,135} Moreover, in a mouse model susceptible to atherosclerosis that lacked LXR α and apo E, treatment with the LXR α/β agonist GW3965 not only improved cholesterol *in vitro* (decreased total and unesterified cholesterol levels and increased HDL-cholesterol levels) but reduced aortic plaque development.¹³⁶ These results indicate that a selective LXR β agonist might augment reverse cholesterol transport without engendering hypertriglyceridemia or fatty liver.

A second approach to safer LXR development might be to selectively activate intestinal LXR. Fatty liver arises from activation of hepatic LXR, which, through upregulation of SREBP1c, stimulates lipogenesis.^{137,138} Elevation of triglyceride levels occurs via SREBP1c activation¹³⁹ and the subsequent suppression of apolipoprotein A-V (apo A-V),¹⁴⁰ which is an inhibitor of VLDL synthesis (apo A-V can also stimulate VLDL hydrolysis).¹⁴¹ On the other hand, LXR expression on both macrophages and the small intestine contributes to the regulation of reverse cholesterol transport. The absence of macrophage LXRs in a mouse model attenuated, but did not abolish, the increase in macrophage-specific reverse cholesterol transport observed following the administration of the systemic LXR α/β agonist GW3965, which indicates that macrophage LXR expression, but also nonmacrophage LXR expression, contribute to this transport.¹⁴² An intestine-specific LXR α/β agonist, GW6340, promoted macrophage-specific reverse cholesterol transport, augmenting the fecal excretion of radiolabeled sterol by 52% via increased intestinal HDL production and intestinal excretion of HDL-derived cholesterol.¹⁴² Consistent with these findings, a transgenic mouse model of intestine-specific LXR α overexpression demonstrated increased pre β -HDL levels, enhanced macrophage-specific reverse cholesterol transport, and decreased aortic atheroma volume.¹⁴³ Intestine-specific expression of LXR α also protected mice taking a high-cholesterol diet from cholesterol and triglyceride accumulation in the liver.¹⁴³ Intestine-specific LXR agonism, therefore, has potential antiatherogenic effects through the enhancement of reverse cholesterol transport and might avoid the toxicity associated with hepatic LXR activation.

LCAT activators

LCAT esterifies cholesterol, enabling its incorporation into the hydrophobic core of the HDL particle and transforming nascent HDL into spherical mature HDL in the process. Although the importance of LCAT in HDL metabolism is clear, with decreased activity of this enzyme leading to lower HDL-cholesterol levels, its role in reverse cholesterol transport and atherosclerosis in both animals^{144–146} and humans^{147,148} has been increasingly controversial. The evidence further calls into question the relevance of LCAT in the development of atherosclerosis and throws doubt on the therapeutic potential of stimulating LCAT activity. A cross-sectional examination showed that homozygous and heterozygous carriers of mutations associated with LCAT deficiency have no increase in carotid IMT despite low HDL-cholesterol levels (homozygous 9 mg/dl, heterozygous 39 mg/dl).¹⁴⁹ In a case-control analysis of the European Prospective Investigation of Cancer (EPIC)-Norfolk study¹⁵⁰ population, low plasma levels of LCAT were not associated with the development of coronary artery disease, in contrast to results from a prior study by the same group examining families with LCAT gene mutations.¹⁵¹ Perhaps the most striking data on this issue are those from the Prevention of Renal and Vascular Endstage Disease (PREVEND) study,¹⁵² in which high LCAT activity, despite elevated HDL-cholesterol levels, independently predicted an increased risk of cardiovascular events. These findings are consistent with mouse studies of LCAT overexpression, which demonstrated attenuated macrophage-specific reverse cholesterol transport *in vivo* and ABCA1-mediated macrophage cholesterol efflux *ex vivo*.¹⁵³ Only one LCAT activator has reached early clinical development, ETC-642, and little data are available on the outcomes of treatment with this agent.¹²¹ Despite these uncertainties, LCAT activation remains an interesting potential therapeutic target owing to its ability to raise HDL-cholesterol levels and its possible influence on reverse cholesterol transport.

Conclusions

A myriad of novel drugs whose development stems from attempts to leverage the atheroprotective activities of HDL is currently undergoing investigation. Comprehensive

assessment of the effects of these drugs requires the analysis of HDL functionality, including HDL flux through reverse cholesterol transport pathways, and not simply the measurement of static circulating HDL-cholesterol levels. Niacin and the CETP inhibitors dalcetrapib and anacetrapib will provide the earliest wave of critical clinical data of HDL-directed strategies in large contemporary cohorts managed with aggressive medical therapy (Table 2). Time will tell whether the coming decade will witness the rise of the HDL-targeted approach or whether the promise of HDL as a viable therapy for atherosclerotic disease will remain unfulfilled.

Acknowledgments

This work was supported by grants from the National Heart, Lung and Blood Institute (HL22633 and P50 HL70128) and the National Center for Research Resources (UL1-RR-024134). E. M. deGoma's salary is partially funded by a National Heart, Lung and Blood Institute grant (K12 HL083772-01).

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Key points

- Four HDL-targeted drug approaches exist: directly augmenting apolipoprotein A-I (apo A-I) levels, indirectly augmenting apo A-I and HDL-cholesterol levels, mimicking the effects of apo A-I, and enhancing reverse cholesterol transport
- From a pharmacodynamic standpoint, direct augmentation of lipid-poor apo A-I levels arguably represents the most validated HDL therapeutic approach in terms of antiatherogenic potential
- The clinical efficacy of interventions that raise HDL-cholesterol levels through slowing its metabolism, such as inhibition of cholesteryl ester transfer protein or endothelial lipase, remains to be established
- The discovery of the niacin receptor GPR109A helped to define the mechanisms underlying the effect of niacin on free fatty acids and flushing, although it is unclear how niacin raises HDL-cholesterol levels
- With the exception of D-4F, apo A-I mimetic peptides require parenteral administration and, therefore, are likely to be initially targeted to patients at high risk with acute coronary syndromes
- Intestinal-specific liver X receptor (LXR) agonism or LXR β -specific agonism might enhance reverse cholesterol transport while avoiding toxicity associated with nonselective LXR activation, namely, hepatic lipogenesis and hypertriglyceridemia

Review criteria

A search for original, English-language articles published from 1990 to 2010 was performed in PubMed using the following search terms: “high-density lipoprotein”, “HDL”, “apolipoprotein A-I”, “apoA-I”, “reverse cholesterol transport”, “cholesteryl ester transfer protein”, “CETP”, “niacin”, “nicotinic acid receptor”, “lecithin cholesterol acyltransferase”, “LCAT”, “liver X receptor”, “endothelial lipase”. The reference lists of identified articles were also searched for further references. The NIH website ClinicalTrials.gov was searched using the search terms described above, as well as the following search terms: “CSL-111”, “ETC-216”, “dalcetrapib”, “anacetrapib”.

Figure 1.

HDL metabolism and targets of therapeutic intervention. Synthesized by the liver and the intestine, apo A-I acquires phospholipid to form nascent preβ-HDL. ABCA1 initiates the first step of reverse cholesterol transport, facilitating the efflux of free cholesterol from peripheral cells to nascent preβ-HDL. LCAT esterifies the cholesterol molecules to form cholesteryl esters, which migrate to the core of the HDL particle, resulting in formation of α-HDL. These mature HDL particles can acquire additional lipid via efflux mediated by ABCG1 and SR-BI. CETP mediates exchange of cholesteryl esters for triglycerides with VLDL or LDL, effecting depletion in cholesteryl esters and enrichment in triglycerides of HDL. The resulting HDL3 particles can be either taken up by the liver via SR-BI holoparticle uptake or modified by hepatic lipase and endothelial lipase. Metabolism by the latter releases lipid-poor apo A-I, which can be filtered by the glomeruli and degraded by cubilin/megalin in the proximal renal tubule. Targets of HDL-directed therapeutic interventions are indicated by red arrows and lines. Abbreviations: ABC, ATP-binding cassette transporter; Apo A-I, apolipoprotein A-I; CETP, cholesteryl ester transfer protein; CD36 and LIMPII analogous-1; LCAT, lecithin-cholesterol acyltransferase; LDL-R, LDL receptor; SR-BI, scavenger receptor class B type I.

Table 1Intravenous apo A-I for coronary atherosclerosis in humans^{14–17}

IVUS parameter	ETC-216	CSL-111	Autologous HDL	Atorvastatin 80 mg
Duration of therapy	5 weeks	4 weeks	7 weeks	18 months
Baseline atheroma volume (mm ³)	268.4	151.0	229.3	184.4
Change in total atheroma volume (mm ³ ; %)	−14.1; −1.1	−5.3; −3.4	−12.2; −1.0	−0.4; −0.2

Abbreviation: IVUS, intravascular ultrasonography.

Table 2

Ongoing late-stage clinical trials of HDL-directed therapies

Drug	Trial	Patient population (n)	Anticipated completion date
<i>CETP inhibitors</i>			
Dalcetrapib	DAL-Outcomes ⁷²	Acute coronary syndrome (15,600)	2013
Anacetrapib	REVEAL ⁷⁸	Stable coronary heart disease or risk equivalent (30,000)	2017
<i>Niacin</i>			
Niacin-simvastatin	AIM-HIGH ⁸³	Stable vascular disease with atherogenic dyslipidemia (3,400)	2012
Niacin-laropiprant	HPS2-THRIVE ⁸⁴	Stable vascular disease (25,000)	2013

Abbreviations: AIM-HIGH, Atherothrombosis Intervention in Metabolic Syndrome with Low HDL-C/High Triglycerides and Impact on Global Health Outcomes; CETP, cholesteryl ester transfer protein; DAL, dalcetrapib; HPS2-THRIVE, Heart Protection Study 2—Treatment of HDL to Reduce the Incidence of Vascular Events; REVEAL, Randomized Evaluation of the Effects of Anacetrapib through Lipid-modification.