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REVIEW ARTICLE

HIGH-DENSITY LIPOPROTEIN MODULATION TARGETS

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SUMMARY

Given the strong genetic determinants of favorable high-density lipoprotein cholesterol (HDL-C) levels, the ability to achieve the cardiovascular and longevity benefits associated with this mediator of the reverse cholesterol transport pathway through pharmaceutical intervention is challenging. Niacin is still the most robust HDL-C-raising pharmaceutical agent on the market and its use leads to elevation of HDL-C by up to 35%. Cholesteryl ester transfer protein (CETP) and endothelial lipase are two components of the reverse cholesterol transport pathway that have become potential therapeutic targets for raising HDL-C. However, the Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE) trial, a clinical trial investigating the CETP inhibitor torcetrapib, was stopped in December 2006 due to excess mortality in the treatment group. Other CETP inhibitors currently under development include anacetrapib (MK-0859), dalcetrapib (JTT-705), TA-8995, DRL-17822 and JTT-302. An interesting alternative approach currently in phase II development is an investigational vaccine, known as CETi-1. CETi-1 is designed to elicit antibodies that bind to and inhibit the activity of CETP, thus potentially blocking the ability of the protein to transfer cholesterol from HDL-C to low-density lipoprotein (LDL), resulting in elevated HDL-C levels.

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INTRODUCTION

Low-density lipoprotein cholesterol (LDL-C) has long been known to be atherogenic. More recently, attention has increasingly focused on the effects of high-density lipoprotein cholesterol (HDL-C), as it has been clearly shown that there is an inverse relationship between HDL-C levels and cardiovascular disease (CVD) risk (1). That being said, the precise effects of HDL-C on health and longevity are still being questioned. This review will highlight several important factors that determine HDL-C levels and different therapeutic strategies in preclinical and clinical stages for the modulation of these factors to increase HDL-C levels (Table I).

HDL-C AND CARDIOVASCULAR HEALTH

A favorable lipid profile is strongly genetically determined. For example, a subset of Ashkenazi Jews with exceptional longevity have been found to have an inherited phenotype of significantly larger HDL-C particle sizes than matched controls (2). In addition, high levels of total HDL-C have been associated with longevity during healthy aging in very old Japanese-American men (3).

Lipoproteins are diverse molecules with a range of sizes and densities. One issue of interest has been whether this heterogeneity correlates with CVD risk. Epidemiological studies show that the levels of LDL-C and HDL-C can predict variable incidences of CVD; however, there is limited evidence relating lipoprotein subfractions and composite measures of subfractions to the risk for CVD in prospective cohort studies. HDL-C subfractions are among the new emerging risk factors for atherosclerosis, and HDL 2b in particular has been linked to cardiovascular risk. Mueller et al. recently examined the utility of HDL 2b for predicting cardiovascular risk (incidence of myocardial infarction [MI]) using a novel microfluidic device to distinguish HDL 2b from total HDL cholesterol (4).

Efforts to characterize the phenotype and genotype associated with exceptional longevity in a genetically homogeneous Ashkenazi Jewish population revealed that specific genes governing HDL particle size underlie the inherited phenotype (2, 5). Since the controls for the

Table I. *Targets and approaches for the modulation of high-density lipoprotein cholesterol (HDL-C) levels.*

Strategy	Targets
Increase Apo-AI production	Apo-AI expression Apo-AI Milano
Promote reverse cholesterol transport	LXR ABC-1 agonists
Niacin receptor	HM74B agonist
Delay catabolism of HDL	Endothelial lipase

Apo-AI, apolipoprotein A-I; LXR, liver X receptor; ABC-1, ATP-binding cassette transporter 1.

centenarians had passed away, the study also examined their offspring and an aged-matched control group that included mostly the spouses of the offspring or their neighbors. Blood tests were conducted to determine lipoprotein and lipoprotein subclass profiles, and particle sizes were determined by proton nuclear magnetic resonance (NMR). Genotyping for an isoleucine to valine substitution variant at position 405 (I405V) of the gene for cholesteryl ester transfer protein (CETP) was performed. The HDL and LDL particle sizes of the offspring of the centenarians were larger than those of the control subjects. On the basis of gender and specific lipoprotein profile, this characteristic of HDL and LDL particle size was found to be highly heritable (0.4-0.7) (2).

Among the centenarians in the study, cognitive capability was tested using the Mini-Mental State Examination (MMSE) to determine whether HDL-C plasma levels were related to cognitive function. Analysis of MMSE distribution in comparison to HDL-C levels showed that subjects with higher HDL-C plasma levels had higher MMSE test scores, suggesting that HDL-C is protective for cognitive function (5). A separate study involving 561 subjects of at least 85 years of age examined the link between serum lipids, cognitive impairment and coronary artery disease (CAD). A low MMSE score was associated with low HDL-C levels, and this association was found even when subjects with CAD and stroke were excluded. These results suggest that the antiaggregatory and anti-inflammatory properties of HDL-C protected against cognitive impairment in this group of centenarians (6).

The role of HDL-C in longevity, and particularly *CETP* as a "longevity gene", is not entirely straightforward. Ashkenazi Jews with exceptional longevity who were homozygous for the I405V substitution variant of *CETP* (the VV genotype) had lower serum CETP levels and larger lipoprotein particle sizes (2). The calculated attributable risk for an individual carrying the VV genotype to become one of the oldest elderly was 18.1. However, another study investigating 256 centenarians and 190 controls for effects of CETP deficiency and the Taq1B polymorphism of the *CETP* gene failed to show an association between either of these genetic variations and longevity (7).

Another study involving 3,469 Japanese-American men in the Honolulu Heart Program was conducted to examine the relationship between longevity phenotypes and two different *CETP* gene variants common in the Japanese population that are associated with decreased levels of CETP: D442G (5.1% of the study population) and intron 14G:A (0.5%), which are associated with decreased CETP activity (–35%) and increased HDL-C levels (+10% for D442G). Among subjects with HDL-C levels > 60 mg/dL, there was a lower prevalence of CVD-related death, independent of mutations, consistent with the inverse relationship between HDL levels and coronary heart disease (CHD). Subjects carrying *CETP* mutations had an increased risk of CVD when their HDL-C values ranged from 41 to 60 mg/dL. The results of this study indicated that CETP deficiency may be an independent risk factor for CHD. Mechanistically, CETP is a vital component of the reverse cholesterol transport pathway, as it traffics atherogenic lipids between HDL-C and LDL-C lipoproteins for subsequent return to the liver and excretion in feces. CETP may also affect HDL-C speciation and lecithin-cholesterol acyltransferase (LCAT) activity. In this manner, the effects of CETP deficiency on reverse cholesterol transport may lead to increased CHD risk and ultimately be more influential than HDL-C levels (8).

LINKS BETWEEN NUTRITIONAL STATUS, INFLAMMATION AND HDL-C IN THE ELDERLY

In addition to genetic determinants, other factors, including nutritional status and inflammation, may affect HDL-C levels. There is a positive relationship between acute-phase reactants such as C-reactive protein (CRP) and interleukin-6 (IL-6) and CVD (9, 10). Several studies have been undertaken to investigate the possible association between these inflammatory markers and HDL-C. In studies involving elderly among the general population and hospitalized elderly patients, HDL-C levels correlated inversely with CRP (6). In centenarians, albumin, prealbumin and transferrin, which are indicative of nutritional status, have been shown to correlate strongly and positively with levels of HDL-C and apolipoprotein A-I (Apo-AI), the apolipoprotein present on the surface of HDL-C particles, while CRP and IL-6 correlate inversely (7). Albumin level was found to be the strongest predictor of HDL-C in centenarians. However, age-related immune alteration or activation is observed in the elderly, particularly the oldest elderly, and CRP and IL-6 levels are slightly increased in centenarians, even those considered healthy. Furthermore, albumin does not always reflect nutritional status. Therefore, in the oldest elderly, it can be difficult to determine the independent effects of a patient's nutritional status or inflammation on HDL-C (6).

DYSFUNCTIONAL HDL-C

Although HDL-C exhibits strong anti-inflammatory properties, HDL-C can become dysfunctional in individuals with chronic inflammatory conditions (10). Such conditions include metabolic syndrome, lupus erythematosus and rheumatoid arthritis. Increased levels of proinflammatory HDL-C have been associated with increased carotid intima–media thickness (CIMT) and an increased risk for adverse clinical outcomes in patients with chronic kidney disease (11). Increased levels of dysfunctional HDL-C occurred in this population without deviations in LDL-C, HDL-C and triglyceride (TG) levels as compared to those without dysfunctional HDL-C. Although dysfunctional HDL-C is not routinely measured outside of research protocols, atorvastatin, LDL apheresis and diet, along with an aerobic exercise program, have been shown to reduce blood concentrations (12, 13).

CHOLESTEROL METABOLISM

Circulating cholesterol levels are primarily determined by hepatic production through the HMG-CoA reductase enzyme pathway (14).

Cholesterol localizes in cells diffusely, where it functions as a vital nutrient to support the health of phospholipid cell membranes. A limited amount of cholesterol is required for normal cell function; in excess, cholesterol is incorporated into atherogenic lipoproteins such as LDL and causes atherosclerosis. HDL functions as a sophisticated regulator and inhibitor of this process by removing excess cholesterol from tissues. The major apolipoprotein of HDL-C, Apo-AI, exits the liver free of lipids, and acquires lipids to be converted into an HDL particle in the plasma. The membrane transporter ATP-binding cassette transporter 1 (ABC-1) mediates the transfer of phospholipids and some unesterified cholesterol from peripheral cells into nascent, disc-shaped HDL particles (15, 16). These particles can then accept unesterified cholesterol from other plasma lipoproteins and cell membranes. LCAT is activated by Apo-AI and esterifies cholesterol so that it can be incorporated into HDL molecules to create a spheroidal HDL particle containing a core of cholesterol ester (1, 17, 18).

There are two ways in which HDL particles dispose of their cholesterol. The scavenger receptor SRB1 binds to hepatocytes, triggering the excretion of cholesterol into bile or recycling. Alternatively, CETP transfers HDL particles into a very-low-density lipoprotein (VLDL)/LDL fraction. This cholesterol can then be delivered to tissues. Paradoxically, some investigators have argued that CETP-mediated transfer of cholesterol to VLDL/LDL fractions is a proatherogenic process (1, 18, 19). It is important to note here that endothelial lipase is emerging as a key component in HDL-C metabolism. Increased endothelial lipase expression leads to a decrease in HDL-C levels, and as endothelial lipase catalytic capacity increases, HDL-C clearance rates increase. Aside from its role in the intracellular catabolism of reabsorbed HDL particles, endothelial lipase may also facilitate the binding and absorption of HDL particles (20).

ROLE OF HDL-C IN REVERSING ATHEROGENESIS

HDL-C functions in an antiatherogenic manner by removing excess lipids from the vascular wall. HDL-C has also been shown to inhibit cytokine-induced expression of adhesion molecules by vascular endothelial cells, resulting in decreased binding of inflammatory cells and inhibition of atherosclerosis. For example, HDL-C inhibits TNF-α-induced expression of E-selectin and vascular cell adhesion molecule VCAM-1 (21). Clinically, anti-TNF-α treatment of patients with chronic inflammatory arthritis induced a modest but sustained increase in serum HDL-C levels, which could have a favorable effect on reducing cardiovascular risk in these patients (22). Other clinical studies have shown that low HDL-C levels correlate with increased levels of adhesion molecules (1, 18). Modified LDLs (including oxidized LDL) stimulate endothelial cells to express monocyte chemotactic protein (MCP-1), which attracts monocytes to the artery wall (23). Modified LDLs also promote the differentiation of monocytes into macrophages, which express scavenger receptors involved in the uptake of modified LDL-C. The result is the creation of foam cells, a characteristic feature of atherosclerosis (21). Robbesyn et al. (24) investigated the signaling pathways involved in oxidized LDL (oxLDL)-induced nuclear factor NF-kappa-B (NF-κB) activation to determine the mechanism of the anti-inflammatory effects of HDL-C. The data suggested that HDL-C counteracts the proinflammatory effects of oxLDL by inhibiting NF-κB signaling pathways through suppression of the accumulation of reactive oxygen species (ROS) in cells. This antioxidant-like effects of HDL-C may be mediated through antioxidant enzymes such as PAF acetylhydrolase or paraoxonase.

THE ANTITHROMBOTIC PROPERTIES OF HDL-C

MI or stroke results from the formation of an occlusive intra-arterial thrombus. Reduced blood coagulability may decrease cardiovascular risk for MI. In an observational study in 60 hypercholesterolemic men, levels of fibrinogen and platelet aggregability were significantly associated with reduced levels of antiatherogenic HDL subfraction 2 (HDL_2). Another study in 132 men without a history of CVD found that men in the highest quartile of fibrinogen had significantly lower levels of HDL_2, but not total cholesterol or HDL_3, as compared to the other three quartiles (18).

EFFECTS OF EXERCISE AND DIET ON HDL-C

An exercise level–response relationship exists between exercise and lipoprotein levels, which suggests that the effects of exercise may not result in significant increases in HDL-C until a certain exercise threshold is met. Cross-sectional studies of training volumes indicate that this threshold is between 24 and 32 km/week (15-20 miles/week of brisk walking or jogging) and an expenditure of between 1200 and 2200 kcal/week. This range of energy utilization is associated with a 2-3 mg/dL increase in HDL-C. Results from the National Runner's Health Study involving 8,283 men and 1,837 women showed that in men, HDL-C increases by 0.135 mg/dL/km (0.218 mg/dL/mile) and the total cholesterol (TC):HDL-C ratio decreases by 0.012 per km (25-27). In another study, an extensive lipid profile was evaluated in 40 professional male cross-country skiers, 102 professional male road cyclists and 60 healthy sedentary male controls. HDL-C levels (mmol/L) in sedentary controls, professional skiers and professional cyclists were 1.35 ± 0.27, 1.66 ± 0.28 and 1.74 ± 0.41, respectively (28). HDL-C levels are increased by a diet high in saturated fat and decreased by diets high in polyunsaturated fat (15, 29).

STRATEGIES FOR INCREASING HDL-C

Niacin

Niacin is currently the most effective pharmaceutical agent commercially available for increasing HDL-C. In contrast to statins, which target LDL-C and can robustly lower LDL-C levels by > 50%, niacin can increase HDL-C by up to 35% (1, 30). Niacin is the only pharmaceutical lipid-lowering product that significantly reduces nonesterified fatty acids (NEFA), resulting in a decrease in plasma VLDL/TG levels. Accordingly, there tends to be a strong negative correlation between TG and HDL-C concentration. Niacin can also stimulate cholesterol efflux from macrophages to primary HDL acceptors via the ABC-1 membrane transporter. In APOE*3Leiden mice expressing a human *CETP* transgene (APOE*3Leiden.CETP mice), niacin increased HDL-C through reduced CETP activity, decreased hepatic expression of CETP and reduced plasma CETP levels. Concomitantly, niacin increased HDL-C levels and HDL-C particle size, and decreased the clearance of Apo-AI from plasma (31).

In a double-blind, randomized, placebo-controlled study (ARBITER 2) of patients with CHD and low levels of HDL-C (HDL-C < 45 mg/dL), patients were given 1000 mg of once-daily extended-

release niacin in addition to statin therapy (32). Changes in CIMT were used as a surrogate cardiovascular endpoint to monitor the effects of lipid-lowering therapy (33). CIMT and HDL-C levels were evaluated after 1 year. After 1 year, HDL-C levels increased from 39 ± 7 mg/dL to 47 ± 16 mg/dL (21%) in the niacin group but were unchanged in the placebo group. TG levels were also decreased significantly in the niacin group. Mean CIMT increased significantly in the placebo group (0.044 ± 0.100 mm; $P < 0.001$) and was unchanged in the niacin group (0.014 ± 0.104 mm; $P = 0.23$). The study investigators concluded that adding extended-release niacin to statin therapy counters the progression of atherosclerosis in patients with known CHD and moderately low HDL-C. The patients receiving niacin also likely benefited from the reduction in TG, as well as the increase in HDL-C. There was a nonsignificant trend towards a lower cardiovascular event rate in the niacin group, which was consistent with the results of both the Cholesterol Lowering Atherosclerosis Study (CLAS) and the HDL-Atherosclerosis Treatment Study (HATS), in which slower rates of atherosclerosis progression were associated with lower cardiovascular event rates when niacin was used along with statin. It should be noted that the results of the ARBITER 2 study (lipid and CIMT) can only be generalized to the dose and preparation of niacin studied (extended-release, 1000 mg/day), and that a dose of 2000 mg/day has been shown to have a more robust effect on lipids than lower doses. Also, the 1000-mg niacin dose is much lower than the 2-4 g/day used for the HATS (34).

Statins

Statins elevate HDL-C levels by 5-15%. This effect is dependent on the statin and is observed at low doses. Higher doses do not elevate HDL levels more than low-dose therapy with 20 mg/day (35). Atorvastatin and pravastatin increase the levels of large- and medium-sized HDL particles. Evidence from type IIa hyperlipidemic patients treated with atorvastatin has shown that statins exert antioxidant effects through enzymes associated with HDL-C. Patients with CHD treated with simvastatin 40 mg/day for 4 weeks exhibited enhanced HDL functionality leading to enhanced anti-inflammatory characteristics (1). In type 2 diabetic subjects carrying the *CETP* TaqIB polymorphism, increased HDL-C (+7.2%) after atorvastatin treatment correlated with a reduction in CETP mass (–32%).

The relationship between HDL-C levels and CETP has also been studied in animal models. Dietary supplementation with atorvastatin (0.01%) reduced plasma cholesterol in APOE*3Leiden and APOE*3Leiden.CETP mice (–26% and –33%, respectively; $P < 0.05$), whereas HDL-C was increased only in APOE*3Leiden.CETP mice (+52%). In APOE*3Leiden.CETP mice, atorvastatin also decreased hepatic CETP mRNA expression (–57%; $P < 0.01$), total CETP (–29%) and cholesteryl ester (CE) transfer activity (–36%; $P < 0.05$) in plasma. Thus, in APOE*3Leiden.CETP mice, HDL-C is increased by atorvastatin through inhibition of hepatic CETP expression and reduction of CETP-dependent transfer of cholesterol from HDL to VLDL (35).

Fibrates

Fibrates have been shown to increase HDL-C levels, albeit not as much as niacin. Fibrates also decrease plasma levels of TG by as much as 48% and increase CE transfer activity. In type 2 diabetics and hypertriglyceridemic patients, fenofibrate, bezafibrate and gemfibrozil all preferentially increased the number of small- and/or medium-sized HDL particles. In hypertriglyceridemic patients, the increase in small HDL particles was as high as +168%. The Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT) identified a link between plasma levels of small HDL_3-C and cardiovascular risk in insulin-resistant patients treated with gemfibrozil. Thus, fibrates may improve the function of small, dense HDL particles, as well as increase HDL-C levels in plasma (1).

The effects of fenofibrate have also been studied in APOE*3Leiden mice with and without the *CETP* transgene. Dietary supplementation with fenofibrate (0.04%) had no affect on HDL-C in mice that did not carry the transgene, while in APOE*3Leiden.CETP mice, fenofibrate dose-dependently increased HDL-C (as much as +91%), and reduced hepatic CETP mRNA (–72%; $P < 0.01$) and CE transfer activity in plasma (–73%; $P < 0.01$). Similar to statins, fenofibrate increased HDL-C by reducing CETP-dependent transfer of cholesterol from HDL-C to LDL-C (36).

CETP inhibitors

CETP inhibitors represent one of the more compelling therapeutic agents in the modification of CVD risk, as they have been shown to achieve dose-dependent HDL-C increases of up to 100%. Torcetrapib was the first CETP inhibitor studied in a large-scale, prospective, placebo-controlled interventional trial (ILLUMINATE). However, the trial was stopped before completion in December 2006 due to excess mortality in the group of patients treated with torcetrapib (37). In the Rating Atherosclerotic Disease by Imaging with a New CETP Inhibitor (RADIANCE 1) study, CIMT was increased by treatment with torcetrapib plus atorvastatin as compared to atorvastatin alone (0.0076 ± 0.0011 mm/year vs. 0.0025 ± 0.0011 mm/year, respectively; $P = 0.0014$) (38, 39). In the ILLUMINATE trial, patients treated with torcetrapib experienced a 72% increase in HDL-C and a 25% drop in LDL-C. Off-target toxicity led to a 40% increase in cardiovascular mortality, 25% increase in the rate of fatal and nonfatal cardiovascular events, and a 100% increase in noncardiovascular deaths in the group treated with torcetrapib (40).

After the ILLUMINATE study was terminated, new data indicated that torcetrapib treatment was linked to an increase in aldosterone levels and changes in serum electrolytes, which suggested mineralocorticoid excess, and elevated blood pressure (mean rise in systolic blood pressure of 5.4 mmHg) (37). Mineralcorticoid receptors on the principal cells of the distal tubule of the nephron are activated by aldosterone produced in the adrenal gland. Activation of these receptors induces an increase in sodium and bicarbonate reabsorption, along with an increase in potassium excretion, resulting in increased plasma volume. This mineralocorticoid-based explanation for the adverse effects of torcetrapib is supported by the observation that lower potassium levels correlated with increased systolic blood pressure after torcetrapib administration. Moreover, mineralocorticoid hormones have proatherogenic effects that are not entirely due to effects on blood pressure. For example, aldosterone causes arterial stiffening through collagen deposition in the extracellular matrix, which may explain the increase in CIMT in the torcetrapib group (38, 41).

Another theory to explain the increase in CIMT observed in the torcetrapib group of the ILLUMINATE study is dysfunctional regulation of HDL-C by torcetrapib. Data on the relationship of CETP deficiency due to genetic polymorphisms to atherosclerosis are not clear. Some studies have demonstrated a decrease in atherosclerotic disease, while others have demonstrated an increased propensity. In contrast, partial inhibition should result in functional HDL particles (42).

Anacetrapib, a newer CETP inhibitor, increased HDL-C levels up to 129% in patients with dyslipidemia when administered at a dose of 300 mg/day. In a phase I clinical study, anacetrapib reduced LDL-C levels by up to 38%, without the harmful effects on blood pressure seen with torcetrapib (37).

In a randomized, double-blind, placebo-controlled phase II dose–response trial, the efficacy and safety of daily treatment with 300, 600 and 900 mg of dalcetrapib in 198 patients with mild hyperlipidemia were assessed. Treatment with 900 mg dalcetrapib for 4 weeks resulted in a 37% decrease in CETP activity ($P < 0.0001$), a 34% increase in HDL-C ($P < 0.0001$) and a 7% decrease in LDL-C ($P = 0.017$). For doses up to 900 mg dalcetrapib, there were no changes in body mass index, waist circumference or blood pressure, and no signs of hepatotoxic or nephrotoxic effects. Dalcetrapib may have a mild effect on the gastrointestinal system. Diarrhea occurred in 5, 4, 3 and 2 individuals, respectively, in the 900-mg, 600-mg, 300-mg and placebo groups, and flatulence occurred in 2, 2, 3 and 1 individuals, respectively. After 4 weeks of treatment with dalcetrapib, the 900-mg dose was associated with a higher frequency of gastrointestinal complaints, but the association was not significant (43, 44).

Dalcetrapib in combination with pravastatin has been studied in a randomized, double-blind, placebo-controlled trial involving 152 subjects with LDL-C > 160 mg/dL. Patients were given either pravastatin 40 mg and placebo, pravastatin 40 mg and dalcetrapib 300 mg or pravastatin 40 mg and dalcetrapib 600 mg. After 4 weeks, the group receiving dalcetrapib 600 mg plus pravastatin exhibited a 30% decrease from baseline in CETP activity and a 28% increase from baseline in HDL-C. LDL-C levels were also decreased by 5% from baseline in this study arm. Dalcetrapib 300 mg plus pravastatin resulted in a decrease in CETP activity of about 16% and an increase in HDL-C of about 14% after 4 weeks (45).

The issue of whether CETP inhibition in humans downregulates atherosclerosis and reduces the risk for CVD events is far from settled, and results from phase III clinical trials using dalcetrapib and anacetrapib are greatly anticipated. The mechanism of HDL elevation through CETP, which is a vital component of the reverse cholesterol transport process, is a concern with these therapeutic agents. By comparison, niacin possesses a robust ability to increase HDL-C and has other beneficial effects on fatty acid metabolism as well. As such, it remains a safe and useful agent for individuals with suboptimal HDL-C, particularly those with insulin resistance or at high CVD risk already taking a statin or other lipid-lowering drug (46).

Other CETP inhibitors, including TA-8995 and DRL-17822, are currently in development (see Table II). DRL-17822, a selective inhibitor of CETP, is being evaluated for the treatment of dyslipidemia, atherosclerosis and associated CVD. This compound elicits a strong response in animals, where it elevates HDL-C and reduces atherosclerotic plaques, and has a clean safety profile in preclinical studies (46).

Overall, CETP inhibitors seem to be the most promising in terms of magnitude of response. The goal of HDL-C-raising strategies in combination with effective statins is a further reduction in CVD morbidity and mortality as compared to statin monotherapy (46). Torcetrapib substantially increases HDL-C levels (up to 106%) alone or in combination with atorvastatin, although the safety profile did not favor advancing torcetrapib. CETi-1 is an investigational vaccine in phase II development designed to elicit antibodies that bind to and inhibit the activity of CETP (47). This therapeutic approach has been in clinical development for some time.

Endothelial lipase inhibitors

Endothelial lipase is an HDL-degrading enzyme that is activated by inflammation, the expression of which is induced by inflammatory cytokines (20, 48, 49). HDL hydrolysis by endothelial lipase activates peroxisome proliferator-activated receptors (PPARs), suggestive of a potential mechanism by which HDL limits leukocyte adhesion to endothelial and vascular inflammation sites. PPAR activation is somewhat selective for PPARα, and the effects are most pronounced with HDL-C as a substrate, as compared to LDL-C and VLDL-C (48).

Metabolic syndrome can be characterized as a chronic inflammatory condition. Elevated plasma endothelial lipase mass has been found in patients with metabolic syndrome compared to normal controls, and increased plasma concentrations of endothelial lipase were found in individuals with high inflammatory markers (20). Endothelial lipase has also been linked to atherosclerosis. An increase in endothelial lipase mass has been associated with coronary artery calcification, a measure of subclinical atherosclerosis in

Table II. *CETP inhibitors in development.*

Drug	Company	CETP-related	Phase	Comments
Torcetrapib*	Pfizer	CETP inhibitor	III	Discontinued due to prohypertensive side effects seen in phase II, III trials
Atorvastatin	Pfizer	Statin	III	Modest increase in HDL-C levels
Dalcetrapib	Roche	CETP inhibitor	II	Lower potency compared to torcetrapib
CETi-1	Avant	CETP vaccine	II	Biological therapy; lack of potency may limit its market
BAY-60-5521	Bayer	CETP inhibitor	I	Limited information

*Discontinued due to adverse events in phase III. CETP, cholesteryl ester transfer protein; HDL-C, high-density lipoprotein cholesterol.

humans. Pathological sectioning of human coronary arteries has revealed endothelial lipase in atheromatous plaques. Endothelial lipase expression has been observed in macrophages and smooth muscle cells as well. In rats with hypertension, HDL-C levels were greatly reduced as compared to controls in the aorta, heart and lungs. Importantly, all NCEP ATP III-defined metabolic syndrome factors (all of which are risk factors for atherosclerosis) correlate with endothelial lipase concentration in both pre- and post-heparin plasma.

Two studies have examined the role of endothelial lipase in atherosclerosis in mouse models, although the results of these studies are somewhat conflicting. In one study, endothelial lipase/Apo-E double knockout mice were compared to Apo-E knockout mice. Atherosclerotic development was assessed by measuring lesions at the root of the aortic arch. After 12 weeks on a high-fat diet, decreased atherosclerotic lesions were found in endothelial lipase/Apo-E knockout mice as compared to the controls. This difference was only found in the males fed a high-fat diet. Another study examined the effect of endothelial lipase deficiency in an Apo-E-deficient/LDL receptor-deficient background. Mice were fed a high-fat diet for 18 weeks. No significant difference in the level of atherosclerosis between the endothelial lipase knockout mice and the control animals was found (20).

CONCLUSIONS

HDL-C modulates lipid metabolism and the process of atherosclerosis through a variety of pathways. These include reverse cholesterol transport and anti-inflammatory and antioxidant effects. HDL-C was found to be a more important risk predictor for CVD than LDL-C, total cholesterol or TG in the Framingham Heart Study. The ability of CETP inhibitors such as anacetrapib and dalcetrapib, currently in clinical development, to downregulate atherosclerosis and reduce CVD risk with an acceptable safety profile has yet to be demonstrated. These trials follow the failure of the first CETP inhibitor, torcetrapib, administered in combination with statin, due to off-target toxicity linked to mineralocorticoid excess. Exciting progress is being made in the development of endothelial lipase inhibitors and atherosclerosis.

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DISCLOSURES

The authors state no conflicts of interest.

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