High-Density Lipoproteins: A New Potential Therapeutic Target for the Prevention of Cardiovascular Disease

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Epidemiological studies have identified low-density lipoproteins (LDL) and high-density lipoproteins (HDL) as independent risk factors that modulate cardiovascular disease (CVD) risk.1,2 Over the past decade, clinical trials of LDL-lowering drugs have clearly established that reductions in LDL are associated with a 30% to 45% reduction in clinical events.3–7 However, despite lowered LDL, many patients continue to have cardiac events. Low HDL is often present in high-risk patients with CVD.8 As a result, a great deal of research interest recently has been focused on raising plasma HDL levels by dietary, pharmacological, or genetic manipulations as a potential strategy for the treatment of CVD. In addition to epidemiologic studies, other lines of evidence suggest that raising HDL would reduce the risk of CVD. Infusion of HDL in the form of apoA-I/phospholipids complexes was associated with regression of atherosclerosis in cholesterol-fed rabbits.9 Moreover, increased plasma HDL concentrations achieved by overexpressing human apoA-I in transgenic animals protects against the development of diet-induced10 and genetically determined atherosclerosis.11 Recently, 5 weekly infusions of apoA-I Milano/phospholipid complexes were shown to regress total atheroma volume by 4.2% in 36 patients compared with 11 controls after an acute coronary event using intravascular ultrasound to quantitate coronary atheroma.12 These combined results provide support for the concept that raising HDL may represent an additional therapeutic target for prevention of CVD.

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Although our understanding of how HDL protects against CVD is still incomplete, there is evidence that supports at least 3 major atheroprotective mechanisms of HDL. HDL-mediated efflux of cholesterol from cholesterol-loaded macrophages is a well-established anti-atherogenic function of HDL. Cholesterol efflux from macrophages to HDL can occur by passive diffusion,13 by interaction with the SR-BI receptor,14 or by binding to the ABCA1 transporter15–18 (Figure 1, left). The preferred acceptor for the ABCA1 transporter-mediated cholesterol efflux is poorly lipidated apoA-I10 (Figure 1, left) which is converted to spherical α-HDL after esterification of free cholesterol (FC) to cholesteryl esters (CE) by lecithin/cholesterol acyltransferase (LCAT). Both the SR-BI20 and ABCA1 transporter21–23 pathways are modulated by the cellular content of oxysterols, which regulate the LXR pathway and expression of the SR-BI and ABCA1 transporter genes (Figure 1, right). After accepting excess cellular cholesterol from arterial macrophages and other peripheral tissues, HDL transports the excess cholesterol to the liver for disposal. HDL is thus an integral component of the atheroprotective reverse cholesterol transport process, functioning as a carrier of excess cellular cholesterol from peripheral tissues to the liver, where it is excreted from the body as bile acids and cholesterol.24

A second major mechanism by which HDL decreases atherosclerosis is to protect LDL from oxidation. Oxidized or modified LDL, unlike normal LDL, is readily taken up by the scavenger receptor SR-A or CD36 on macrophages, resulting in CE accumulation with foam cell formation. The cholester-ol-loaded macrophage produces a number of inflammatory cytokines and stimulates MCP-1 as well as endothelial cell adhesion molecules. Oxidized lipids are transferred to HDL from LDL and are hydrolyzed by HDL paraoxonase and PAF acetylhydrolase.25,26 Finally, a third mechanism by which HDL may protect against CVD is the selective decrease of endothelial cell adhesion molecules, which facilitate the binding of mononuclear cells to the vessel wall and promote lesion development.27 Each of these 3 mechanisms may play an important role in HDL-mediated protection against CVD.

In clinical practice, several drugs presently approved for the treatment of patients with high LDL cholesterol (LDL-C) and/or triglycerides also raise plasma HDL cholesterol (HDL-C) levels. Statins increase plasma HDL-C concentrations by 10% to 15%,28 and fibrates and niacin raise HDL-C by up to 25% to 30%.29 Recently, a great deal of interest has focused on a new class of drugs, cholesterol ester transfer protein (CETP) inhibitors, which significantly raise HDL-C and lower LDL-C. The role of CETP in lipoprotein metabolism is illustrated in the updated schematic model of lipoprotein metabolism shown in Figure 2. Cholesterol in HDL is returned to the liver by 2 pathways: transfer of CE by CETP to the VLDL-IDL-LDL lipoproteins with uptake by the liver via the LDL receptor (LDLr)30 and selective uptake of CE by the hepatic SR-BI receptor.31 In humans, the majority of FC is transported to the liver by the HDL-SR-BI pathway, whereas CE is transported mainly by the IDL-LDL-LDLr pathway after transfer by CETP.32–35

The development of agents that inhibit CETP activity as a treatment strategy for CVD has been controversial because CETP appears to have pro-atherogenic and antiatherogenic...
effects. CETP-mediated transfer of CE decreases HDL levels and increases CE in VLDL-IDL-LDL. The decreased HDL levels would reduce the atheroprotective functions of HDL as outlined and would thus be pro-atherogenic. If the increased CE in LDL is taken up by vessel wall macrophages to increase foam cell formation, then this CETP activity would also be pro-atherogenic. However, if the increased CE transfer to VLDL-IDL-LDL instead results in transport of CE back to the liver via the LDLr, then CETP would facilitate reverse cholesterol transport, and this activity would be atheroprotective. Finally, CETP-mediated HDL remodeling during CE transfer results in the production of lipid-poor apoA-I, which can be used for ABCA1-mediated cholesterol efflux, an atheroprotective process. The final result of this combination of CETP activities, in terms of atherosclerosis, is not simple to predict.

Studies in mouse models to evaluate the effect of CETP on atherosclerosis have yielded conflicting results. Expression of CETP in hypertriglyceridemic apoC-III transgenic mice reduced aortic atherosclerosis. Transgenic mice overexpressing LCAT form large apoE-rich and CE-rich HDL, which exhibit abnormal function. The accumulation of these large, dysfunctional HDL particles was associated with significant aortic lesion development; however, expression of CETP reduced the CE-rich and apoE-rich HDL and decreased atherosclerosis in LCAT-transgenic mice. These results led to the concept of dysfunctional HDL as a potential mechanism leading to increased atherosclerosis in the presence of high HDL. In contrast, human CETP expression in apoE knockout mice and simian CETP expression in cholesterol-fed mice were associated with increased atherosclerosis, suggesting that CE transfer into VLDL-IDL-LDL resulted in an increase in atherogenic lipoproteins, which was not compensated by increased reverse cholesterol transport.

The evaluation of patients with a complete absence of CETP activity has provided insight into the potential pro-atherogenic and antiatherogenic roles of CETP in humans. Mutations in the CETP gene have been identified primarily in Japan. The lipoprotein phenotype in homozygous CETP deficiency includes a marked increase in large HDL particles enriched in cholesterol, apoE, apoA-I, and apoA-II; the increase in apoA-I and A-II was found to be caused by delayed catabolism of this abnormal HDL particle. In vitro, the large, apoE-rich HDL present in CETP-deficient subjects had decreased ability to promote cholesterol efflux from cholesterol-loaded macrophages, whereas HDL2 and HDL3 from these patients functioned as effective cholesterol acceptors. In addition, plasma LDL and apoB levels were decreased in CETP-deficient subjects and the LDL was polydisperse. Kinetic studies established that the decrease in LDL was caused primarily by increased catabolism, consistent with upregulation of the LDLr. LDL isolated
Lipoprotein metabolism consists of 2 interconnected cascades: apoB-containing lipoproteins and apoA-I–containing lipoproteins (HDL). The apoB cascade includes the chylomicron/chylomicron remnant pathway that transports dietary lipids from the intestine to peripheral cells and liver, and the VLDL–IDL–LDL pathway, which transports hepatic lipids to peripheral cells and returns cholesterol back to the liver via the LDL receptor (LDLr). The HDL cascade includes synthesis and ABCA1-mediated lipidation of apoA-I in liver and intestine to form nascent/preβ-HDL; formation of preβ-HDL by metabolism of triglyceride-rich lipoproteins; conversion of preβ-HDL to mature α-HDL by LCAT; ABCA1-mediated cholesterol efflux to preβ-HDL; SR-BI–mediated cholesterol efflux to mature α-HDL; mature α-HDL remodeling by hepatic lipase (HL) and phospholipids transfer protein (PLTP) with the generation of lipid-poor apoA-I and preβ-HDL, which can recycle and increase ABCA1-mediated efflux from peripheral cells, including arterial wall macrophages. HDL-C return to the liver by selective uptake of CE by the hepatic SR-BI receptor; and HDL-C return to the liver by transfer of CE by CETP to the VLDL–IDL–LDL lipoproteins with uptake by the liver via the LDLr. The plasma level of preβ-HDL reflects the balance between these various processes. Recently, it has been recognized that the liver regulates the intracellular level of hepatic cholesterol similar to peripheral cells by modulation of cholesterol efflux using the ABCA1 transporter and is a major source of plasma HDL-C. This led to the concept of “reverse reverse cholesterol transport” with the liver as the site of synthesis of poorly lipidated apoA-I and preβ-HDL which can then travel to the periphery and facilitate ABCA1-mediated cholesterol efflux from peripheral cells, with return of the excess cholesterol back to the liver.

from patients with CETP deficiency had delayed catabolism compared with control LDL injected into control subjects, consistent with decreased affinity of the CETP LDL for the LDLr. In vitro, CETP-deficient LDL had decreased binding to normal fibroblasts when compared with control LDL, also consistent with reduced LDLr affinity. These combined results suggest that the plasma lipoproteins that accumulate in patients with complete CETP deficiency have atherogenic properties: the HDL is dysfunctional similar to the HDL in CETP-deficient mice and the LDL is polydisperse with increased LDLr affinity.

Studies in the Omagari region of Japan, where there is an increased incidence of CETP deficiency, revealed a decreased frequency of CETP mutations with age, an increase in ECG changes of ischemia with HDL-C >70 mg/dL, higher plaque score in the carotid artery, and increased pulse wave velocity from aortic root to femoral artery, suggesting that complete CETP deficiency is atherogenic. In contrast, initial studies in the Honolulu Heart Study in Japanese subjects reported an apparent increased CVD risk in heterozygotes but not in homozygotes. A recent analysis of the 7-year prospective data revealed no increased risk of CVD in subjects with CETP mutations. No increased risk of CVD was also reported after analysis of clinical data from another cohort of homozygous patients in Japan. The inability to definitively determine the increased risk of CVD in homozygous CETP patients is caused at least in part by the relatively small number of patients, the presence of other genetic defects in lipoprotein metabolism, and other associated risk and environmental factors in that patient population.

The potential protective effect of CETP inhibition has been further evaluated by studies using antisense oligodeoxynucleotides and anti-CETP antibodies to reduce CETP expression and function. In these studies in cholesterol-fed rabbits, there was an increase in HDL and decreased aortic atherosclerosis. Administration of a chemical CETP inhibitor, JTT-705, to cholesterol-fed rabbits led to a 70% decrease in non-HDL cholesterol, a 70% decrease in atherosclerosis. A second study in which markedly hypercholesterolemic rabbits were fed the JTT-705 inhibitor was not associated with decreased atherosclerosis, presumably because of the marked hyperlipidemia present in these animals. Initial human studies with JTT-705 were performed in a 4-week phase II trial in 198 healthy mildly hyperlipidemic subjects at a dose of 300, 600, or 900 mg/d. At 900 mg/d, CETP activity decreased 37%, HDL-C increased 34%, and LDL-C decreased 7%. Further studies are underway to determine the efficacy of this CETP inhibitor in raising HDL-C and decreasing atherosclerosis.

In the present issue of the Journal, Clark et al provide data on the initial phase 1 multidose study (10, 30, 60, 120 mg/d and 120 mg/bid) of a new CETP inhibitor, torcetrapib, on plasma lipoproteins and apolipoproteins in 40 healthy normolipidemic subjects. Over the dosage range, the CETP activity decreased 12 ± 17, 35 ± 17, 53 ± 8, and 80 ± 6 (30 mg/d to 120 mg/bid) with no change in total plasma cholesterol.
to 120 mg/bid), but an increase in HDL-C from 16% to 91% (10 to 120 mg/bid) and a decrease in LDL-C from 7% to 42% (60 mg/d to 120 mg/bid). At 120 mg/bid, apoA-I and apoE increased 27% and 66%, respectively, and apoB decreased 26%. Of particular importance was the analysis of plasma lipoproteins by FPLC to ascertain whether the lipoproteins were similar to the HDL and LDL present in CETP-deficient patients. Plasma LDL was monodisperse and not polydisperse, as reported in CETP-deficient patients. HDL increased in size; however, the increase was not as large as in CETP-deficient patients and the composition of the HDL was different, with increased CE in CETP-deficient patients and increased FC with the CETP inhibitor. The apparent key reason for the difference in the atherogenic potential of the lipoproteins in the torcetrapib-treated subjects when compared with the lipoproteins in the CETP-deficient patients is the percent reduction in CETP activity. A complete absence of CETP activity, as in the homozygous CETP-deficient patients described, results in potentially atherogenic lipoproteins. In contrast, the CETP inhibitor only partially inhibits CETP; the residual CETP activity prevents the accumulation of very large apoE-rich dysfunctional HDL and abnormal polydisperse LDL.

These combined results indicate that torcetrapib is a well-tolerated new agent that produces a substantial increase in HDL and decrease in LDL. Further studies will be required to evaluate the in vitro functionality of HDL and LDL, the altered kinetics causing increased HDL and reduced LDL levels, and optimization of the dosage for further investigation. Selecting the dosage of torcetrapib for only partial rather than complete inhibition of CETP, as in the CETP-deficient patients, appears to avoid the accumulation of abnormal, dysfunctional lipoproteins reported in CETP deficiency. The ultimate evaluation of the potential protection against atherosclerosis with torcetrapib will require clinical trials using surrogate endpoints, including coronary intravascular ultrasound and carotid IMT as well as hard clinical endpoints. Based on current data, CETP inhibitors hold great promise as a new class of drugs with the potential for major benefit in the treatment of CVD in the high-risk patient.

References
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