Increasing High-Density Lipoprotein Cholesterol in Dyslipidemia by Cholesteryl Ester Transfer Protein Inhibition
An Update for Clinicians
James S. Forrester, MD; Rajenda Makkar, MD; P.K. Shah, MD

Abstract—Reduced HDL cholesterol may be a risk factor comparable in importance to increased LDL cholesterol. Interventions that raise HDL are antiatherosclerotic, presumably through acceleration of reverse cholesterol transport and by antioxidant and antiinflammatory effects. In the hypercholesterolemic rabbit, HDL levels can be increased by >50% by inhibition of cholesteryl ester transfer protein (CETP), a molecule that plays a central role in HDL metabolism. This HDL-raising effect is antiatherosclerotic in moderately severe hyperlipidemia but appears to be ineffective in the presence of severe hypertriglyceridemia. In humans, mutations resulting in CETP inhibition have been associated with both reduced and increased risk of atherosclerosis. Proposed explanations for these apparently disparate observations are that the antiatherosclerotic effect of CETP inhibition varies with either the metabolic milieu or the degree of CETP inhibition. We now have pharmacological inhibitors of CETP that are capable of increasing HDL by as much as 50% to 100% in humans. The importance of this development is that reduced HDL is a risk factor independent of LDL and that these new agents alter HDL by a magnitude comparable to that of statins on LDL. Clinical trials, now beginning, will need to identify the patient subsets in which CETP inhibition may be more or less effective. (Circulation. 2005;111:1847-1854.)

Key Words: atherosclerosis ■ cholesterol ■ lipids ■ metabolism ■ statins

Although lipid-lowering therapies now have the capability of reducing LDL cholesterol to levels recommended by the National Cholesterol Education Program (NCEP) guidelines in as many as 90% of treated patients,1 the rate of cardiovascular events is reduced by only 20% to 35% in large, randomized trials. These data suggest that the LDL target set by NCEP guidelines may be too high.1-5 Indeed, among patients with acute myocardial infarction, ≈15% have LDL levels <100 mg/dL on presentation.

A complementary hypothesis is that there is a finite limit to the benefit of LDL lowering and that other non-LDL lipid risk factors must be managed for optimal outcomes. Indeed, epidemiological studies suggest that low HDL cholesterol may be a risk factor comparable in importance to high LDL and that the 2 risk factors are independent (Figure 1).6,7 Although the mechanism by which HDL reduces the development of atherosclerosis has not been defined with certainty, acceleration of reverse cholesterol transport, antioxidant activity, and antiinflammatory action are likely to play central roles.8-10

In contrast to the ability of potent statins to reduce the level of LDL by >50%, however, no currently approved therapy increases HDL by a comparable magnitude. The most potent, currently available HDL-raising therapy is nicotinic acid. This therapy also has a multitude of other antiatherosclerotic effects on the levels of LDL and triglycerides, lipid oxidation, and endothelial function.11 Combination therapy with a statin has been reported to increase HDL levels by ≈30%.12 and to decrease cardiac events.13

Recent studies in the animal laboratory have demonstrated that inhibition of cholesteryl ester transfer protein (CETP), a molecule that plays a central role in HDL metabolism, can raise HDL levels by as much as 50%. Initial testing of pharmacological CETP inhibitors in the hypercholesterolemic rabbit model of atherosclerosis has suggested that this effect could substantially alter the course of atherogenesis. In this article, we will critically analyze these data as clinical trials of this potentially important therapeutic strategy for prevention of atherosclerosis begin.

Raising HDL: Three Limitations Not Recognized by Clinical HDL Measurements
Before analyzing the potential of any new HDL-raising therapy, we must recognize that the traditional HDL blood

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From the Cardiology Division, Cedars-Sinai Medical Center, Los Angeles, Calif.
Correspondence to James S. Forrester, MD; Burns and Allen Professor, Division of Cardiology, Cedars-Sinai Medical Center, 8700 Beverly Blvd, Los Angeles, CA 90048. E-mail forrester@cshs.org
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level has at least 3 limitations either as an index of risk or as a criterion for clinical decision making. The serum HDL level does not assess its functional properties. HDL has both proinflammatory and antiinflammatory properties, both in vitro and in vivo. Ansell et al studied 2 groups of patients. The first group included high-risk, normolipidemic patients studied before and after statin therapy. Despite normal HDL cholesterol levels, the HDL of these patients was found to be proinflammatory compared with age- and sex-matched healthy controls who had antiinflammatory HDL. Statin therapy induced a significant improvement in the proinflammatory HDL, although it remained proinflammatory. In a second group of patients with documented coronary heart disease selected for high HDL (average HDL 95 mg/dl), proinflammatory HDL was found, compared with healthy age- and sex-matched controls who had antiinflammatory HDL. Even within a given individual, HDL may become transiently pro-oxidant in the presence of systemic infection. A second limitation is that the HDL blood level does not necessarily reveal the kinetics of HDL metabolism. For example, subjects with the apoA1 Milano mutation have very low HDL cholesterol levels. These HDL molecules, however, have very high fractional catabolic rates, which result in the antiatherosclerotic effect of an accelerated return of cholesterol ester to the liver. Conversely, in some CETP mutations with genetically high HDL and low LDL values, the increased HDL is represented by a large, apoE-enriched particle that is a weak promoter of cholesterol efflux, and the LDL particle reacts poorly with the LDL receptor. As a consequence, atherosclerosis is increased despite an apparently “favorable” effect of the mutation on the lipid profile.

Finally, the subfractions of HDL generated by CETP inhibition may have different effects on inhibition of atherosclerosis. For instance, HDL2 is typically a potent antiatherogenic molecule, whereas the smaller, denser HDL3 fraction may have less effect. Similarly, the composition of HDL particles may influence their functional properties. Two major apoA1-containing particles exist: LpA-I, which contains only apoA-I, and LpA-I+A-II, which contains both apoA-I and apoA-II. Because apoA-I is antiatherogenic whereas apoA-II can be proatherogenic (based on studies in transgenic mice), LpA-I particles may be more antiatherogenic than LpA-I+A-II–containing particles. It has been shown that the majority of LpA-I has the same density and charge as HDL2, whereas the majority of LpA-I+A-II has the same density and charge as HDL3. Thus, the apparent benefit that might be inferred from a therapy that increases HDL, eg, CETP inhibition, must be tempered by these potential limitations.

Historical Perspective: The Epidemiology and Biochemistry of CETP

Some years ago, a mutation in the CETP gene was discovered in the Japanese. This mutation was accompanied by a substantial increase in HDL. Because HDL regulates reverse cholesterol transport and has antioxidant, antithrombotic, and antiinflammatory properties and the prevalence of atherosclerotic disease is low in Japanese, epidemiological studies were undertaken to define the prevalence of the mutation. These studies suggested that the mutation occurred in ≈11% of the Japanese population and that affected individuals were resistant to atherosclerosis.

During the ensuing years, at least 12 different mutations in the coding region of the CETP gene have been identified. These mutations remain particularly prevalent in the Japanese. In some regions of Japan, the incidence is strikingly high. For instance, as many as 27% of people in the northernmost region, in the environs of Akita, have a CETP gene mutation. Homozygotes have up to 4-fold increases in HDL, with substantial increases in apoA-I and apoA-II and as much as a 40% reduction in LDL cholesterol and apoB.

These epidemiological data triggered interest in the biochemical characteristics of CETP. Detailed analysis of CETP metabolism, beyond the scope of this article, is the subject of several excellent recent reviews of reverse cholesterol transport. CETP is a plasma glycoprotein manufactured in the liver. It circulates in the blood, bound predominantly to HDL. Two principal actions of CETP have been identified. The primary action of CETP is to mediate the transfer of cholesteryl esters from HDL to VLDL and LDL in exchange for triglyceride (Figure 2). CETP also promotes the transformation of HDL2 to HDL3, an action that could promote reverse cholesterol transport. CETP inhibition, on the other hand, results in an increase in HDL by markedly delaying catabolism of apoA-I and A-II. This action also can increase reverse cholesterol transport. This overlap of the potential effects of CETP and CETP inhibition has served to confound an understanding of potential therapeutic mechanisms in atherosclerosis.

The uncertainty about the potential therapeutic value of CETP inhibition was amplified by subsequent epidemiological studies. Although initially CETP mutations were thought to be atheroprotective, this has not proven to be universally true. Reports of both an increased and a decreased effect on the prevalence of coronary artery disease in patients with CETP deficiency have appeared in the literature.
finding is consistent with that of the Honolulu Heart study.48,49 Thus, Moriyama et al.37 found that in CETP-deficient individuals with HDL >80 mg/dL, the prevalence of coronary disease was reduced to a level comparable to that of individuals with elevated HDL and no CETP deficiency. This finding is consistent with that of the Honolulu Heart Study,38,39 in which CETP-deficient individuals with HDL >60 mg/dL also had a low prevalence of coronary artery disease. On the other hand, individuals with CETP deficiency and an HDL <60 mg/dL or with triglyceride >165 mg/dL appeared to have an increased incidence of coronary heart disease. This pattern of increased cardiac events in CETP-deficient patients without markedly elevated HDL and/or with high triglyceride also has been noted in hyperlipidemic Finns40 and in patients undergoing renal dialysis.41 Thus, some investigators have speculated that although the CETP-deficient genotype may be atheroprotective in some, it can also confer an increase in coronary risk when associated with minimal to modest increases in HDL or elevated triglyceride levels.

Because both biochemical and epidemiological data suggest that reduced circulating CETP could, in principle, have both proatherogenic and antiatherogenic effects, study in animal models has seemed to be an essential step to understanding the potential therapeutic value of CETP inhibition.

Animal Models: Is CETP Proatherogenic?
Analysis of studies in the animal model, however, is potentially confounded by a different set of biological variables. Animal species exhibit major differences in CETP expression and in the mechanisms used for reverse cholesterol transport. Mice lack CETP, and thus, reverse cholesterol transport in mice does not involve this protein. In contrast, rabbits have high CETP levels. Human CETP activity is intermediate among the animal species.42 Thus, the mouse model has been used to study overexpression of CETP, whereas rabbits have been used in the study of CETP inhibition.

Mice transduced with the human CETP gene exhibit a dose-related decrease in HDL, accompanied by an increase in LDL and VLDL.43 Plump et al.44 transduced atherosclerosis-prone, apoE-knockout mice with human CETP and apoA-I transgenes, thereby inducing plasma CETP levels to rise to 5 to 10 times that of healthy normal humans. This increase in CETP was associated with a 76% reduction in HDL cholesterol. In further studies, the atherosclerosis-prone, LDL receptor–knockout mouse was transduced with the human CETP transgene. At 3 months, the mean lesion area was increased by 1.8-fold compared with controls. Other investigators transduced mice with the simian CETP gene in atherosclerosis-prone mice. Similar to the human CETP gene transduction, the formation of fatty streaks increased dramatically.45 Taken together, these transgenic mouse studies suggest a proatherogenic effect of CETP activity when HDL is reduced, LDL clearance is impaired, and cholesteryl esters are redistributed from HDL to VLDL/LDL. The results are consistent with the observation that CETP is expressed by monocyte-derived macrophages and by smooth muscle cells in human atheroma.46 Nonetheless, studies of CETP overexpression in mice are not entirely consistent. In at least 2 transgenic mouse studies, overexpression of CETP decreased atherogenesis. Using apoC-III transgene–induced hypertriglyceridemia, Hayek et al.47 reported that the percentage of mice with atherosclerotic lesions fell from 93% to 38% when they were transduced with both CETP and apoA-I. Although this study suggests that CETP overexpression could be atheroprotective in the presence of hypertriglyceridemia, its clinical relevance is uncer-
tain, because no comparable genotype is found in humans. In the second study, CETP-transgenic mice were crossed with atherosclerosis-prone, lecithin:cholesterol acyltransferase (LCAT)–transgenic mice. Although the mean aortic lesion area was reduced by 41%, this result may reflect species differences, because naturally high-CETP rabbits transduced with the LCAT transgene exhibited an increase in HDL with reduced atherosclerosis.

In summary, the preponderance of mouse data suggests that CETP overexpression is proatherogenic. Like the human epidemiological data, however, they also raise the possibility that CETP could be either proatherogenic or antiatherogenic, depending on the lipid environment.

Is CETP Inhibition Antiatherogenic?

Inhibition of CETP expression has been studied in high-CETP animals, such as the hypercholesterolemic rabbit, a model that more closely models the human condition. Sugano et al used antisense oligodeoxynucleotides against CETP. Total cholesterol and CETP were significantly decreased at 12 and 16 weeks compared with controls. HDL cholesterol, measured by ultracentrifugation and column chromatography, increased significantly. Aortic cholesterol content and the percentage of surface area with atherosclerosis were significantly reduced. Similar effects on CETP and HDL levels have been obtained with anti-CETP antibodies.

Rabbits immunized with a peptide containing a region of the CETP molecule required for neutral lipid transfer function developed antibodies against CETP, with a significant reduction in plasma CETP activity and alterations in the lipoprotein profile. The fraction of plasma cholesterol in HDL was 42% higher and the fraction of plasma cholesterol in LDL was 24% lower in the immunized group than in controls. The extent of aortic surface covered by atherosclerotic plaque was reduced by 39.6%. The vaccine has entered phase I clinical testing. When the vaccine (AVANT Immunotherapeutics, Inc, Needham, Mass) was tested in 15 volunteers, 53% of those who received a second injection of the active vaccine developed anti-CETP antibodies, compared with 1 of 8 placebo controls. HDL levels did not increase significantly, however. The vaccine induced no significant clinical or laboratory abnormalities but also did not significantly increase the level of HDL.

In summary, these rabbit studies suggest that CETP inhibition increases plasma HDL and induces redistribution of cholesterol between HDL and VLDL/LDL, with a fall in both plasma total cholesterol and in aortic tissue cholesterol.

Moving Toward Clinical Application: Pharmacological Inhibition of CETP

In principle, pharmacological inhibition of CETP could accelerate cholesterol transport to the liver by HDL (Figure 3). The first pharmacological CETP inhibitor for potential human use was developed in Japan. JTT-705 (Akros Pharma), a thioester that inhibits CETP by forming disulfide bonds, can reduce CETP activity in rabbits by 90%. The concentration of drug necessary to inhibit 50% of the CETP activity (IC50) is similar in rabbits and humans. HDL extracted from JTT-705–fed rabbits increases cholesterol efflux from cultured macrophages. When given to rabbits with diet-induced atherosclerosis (mean plasma cholesterol 200 mg/dL) for 6 months, JTT-705 inhibited CETP activity by 95%, with a 90% increase in HDL cholesterol and a 40% decrease non-HDL cholesterol. These changes in blood lipids were accompanied by an 80% decrease in aortic atherosclerosis. The relative contribution of increased HDL and decreased LDL to the inhibition of atherosclerosis was, however, not defined.

As with the epidemiological and transgenic mouse data, studies with JTT-705 have not been entirely consistent. A subsequent study in severely hypercholesterolemic rabbits with mean plasma cholesterol values >600 mg/dL compared 2 doses of JTT-705, 100 mg/kg (low dose) and 300 mg/kg (high dose). Both doses failed to reduce aortic atherosclerotic area (60% versus 58%, high dose versus controls) despite a 200% increase in HDL cholesterol. The higher dose induced hypertriglyceridemia. Moreover, the non-HDL cholesterol level was correlated with atherosclerotic area, whereas CETP activity and HDL level were not. The authors speculated that the antiatherogenic mechanism that functioned successfully in the prior moderately hypercholesterolemic rabbit study was overwhelmed in the presence of very severe hyperlipidemia; ie, that in very severe hyperlipidemia, HDL-elevating therapy may be less important than decreasing non-HDL cholesterol.

Extension to Human Investigation

Given the magnitude of HDL increase and atherosclerosis reduction achieved in the animal studies, there is a consensus that strategies that increase HDL and/or augment reverse

Figure 3. Schematic of clinical CETP action and its inhibition. CETP facilitates transfer of cholesteryl esters (CE) and triglyceride (TG) between plasma lipoproteins. Plasma CE is predominantly in HDL, and plasma TG is in VLDL. Consequently, predominant effect of CETP is to exchange CE and TG between HDL and VLDL (left). When CETP is inhibited, (right), one effect is that HDL cholesterol (–C) increases and hepatic uptake of cholesterol via scavenger receptor B-1 (SRB-1) may thus be increased. Net effect of this process is to facilitate movement of cholesterol from peripheral tissues, including blood vessel wall, to liver.

http://circ.ahajournals.org/content/111/13/1850.full.pdf
cholesterol transport should be tested in clinical trials.\textsuperscript{25,26,32} Because reverse cholesterol transport is controlled at several discrete steps by different protein regulators and receptors, CETP inhibition is only one of several potential approaches for augmenting reverse cholesterol transport\textsuperscript{49,59–63} (see the Table).\textsuperscript{59,53,54,58,62,63–67} In fact, the clinical efficacy of pharmacological CETP inhibition could be substantially influenced by both diet and drugs that alter CETP pharmacokinetics. For instance, body weight influences CETP activity. Obese children have substantially increased levels of CETP,\textsuperscript{68} and conversely, weight loss can cause as much as a 37% decrease in CETP activity.\textsuperscript{69} Dietary components also influence CETP. Garlic and red pepper have been reported to inhibit CETP activity.\textsuperscript{70,71} Alcohol consumption may have secondary effects by poorly defined mechanisms in individuals with CETP polymorphisms. For instance, heavy drinkers who are homozygous for one particular CETP allele have a substantially reduced risk of myocardial infarction.\textsuperscript{72}

High CETP concentration has been associated with more rapid progression of coronary disease, and statin therapy is more effective in this subgroup, independent of baseline or on-treatment lipid levels, suggesting that the plasma CETP level may be an important determinant of the response to statins.\textsuperscript{73} Statins reduce plasma CETP activity by \textasciitilde 5% to 10% and cholesteryl ester transfer by \textasciitilde 35%.\textsuperscript{70–78} For instance, atorvastatin (10 mg/d for 6 weeks) reduces CETP activity by 7%, and cholesterol transfer from HDL to non-HDL particles is reduced by 37%.\textsuperscript{77,78} The magnitude of these effects is modified by the CETP genotype.\textsuperscript{30,79} Because statins alone cause a substantial decrease in triglyceride and a small increase in HDL, one might speculate that the combination of a statin with a CETP inhibitor would be more effective than either therapy alone.

JTT-705 has been tested in an initial 4-week, randomized, dose-response trial at 300, 600, and 900 mg/d in 198 healthy mildly hyperlipidemic patients.\textsuperscript{80} The highest dose induced a 37% decrease in CETP activity, a 34% increase in HDL, and a 7% decrease in LDL, with no change in triglyceride. An increase in HDL\textsubscript{2}, HDL\textsubscript{3}, and apoA-I paralleled the increase in total HDL.

No important clinical or laboratory toxicity was noted, and the drug was well tolerated at all doses, although treated patients had significantly more mild gastrointestinal complaints. This small study did not include any surrogate measures of atherosclerotic burden or clinical end points. Phase II studies of JTT-705 in combination with pravastatin are in progress.

A second CETP inhibitor, torcetrapib (Pfizer), has also entered clinical trials. In the first phase I multidose trial, 5 groups of 8 healthy young subjects received 10, 30, 60, and 120 mg daily and 120 torcetrapib mg twice daily for 14 days.\textsuperscript{81} All doses were well tolerated. CETP inhibition increased with escalating dose, leading to elevations of HDL of 16% to 91%. Total plasma cholesterol did not change significantly, however, reflecting a parallel reduction in non-HDL cholesterol. At the highest dose, LDL was decreased by 42%. In a second trial, 19 subjects with HDL \,<\,40 mg/dL received 120 mg torcetrapib, 9 of whom also received 20 mg atorvastatin daily for 4 weeks.\textsuperscript{82} Plasma HDL cholesterol increased by 61% in the atorvastatin group and by 46% in the non-atorvastatin groups. In an additional group, torcetrapib at 120 mg twice daily increased HDL cholesterol by 106%. Torcetrapib also reduced LDL levels by 17% in the atorvastatin group. In all groups, the mean particle size of HDL and LDL increased. These 2 studies suggest that the effect of pharmacological CETP inhibition resembles that observed in partial CETP deficiency and serve as a prelude to trials in patients with atherosclerosis and low HDL or other dyslipemias. The initial phase III trials will test 60 mg torcetrapib. In one such trial, intravascular ultrasound is being used to compare atheroma volume at baseline and after 2 years of therapy with either atorvastatin alone or atorvastatin plus torcetrapib. The results of these randomized trials with JTT-705 and with torcetrapib should serve to clarify the potential value of CETP inhibition for atherosclerotic vascular disease in humans.

\section*{Conclusion}

The most reasonable proposed integration of animal and human data is that the effect of CETP and its inhibition is...
modified by the genetic and metabolic milieu.\textsuperscript{25,32} Using human genetic data as a starting point, we may speculate that CETP deficiency may be antiatherogenic when it is associated with a significant increase in HDL (perhaps >60 mg/dL) but that it is not protective in the presence of substantial hypertriglyceridemia, major increases in LDL cholesterol, or lower attained HDL cholesterol levels. Studies in rabbits are consistent with this idea. In moderate hyperlipidemia, CETP inhibition is antiatherogenic, at least partly through progressive reduction in the rate of transfer of cholesteryl esters from HDL to VLDL and LDL. The lack of effect in the profoundly hyperlipidemic rabbit, despite a major increase in serum HDL, suggests that in this particular metabolic milieu, the HDL itself is insufficient to be antiatherogenic. Thus the effect of CETP and its inhibition seems to be to be dependent on the interaction between the level and distribution of lipoprotein components. Although data are currently insufficient to draw any conclusions, an additional reasonable speculation is that the effect of the metabolic milieu on CETP inhibition may involve alteration in HDL function, catabolism, or particle distribution not detected in the measurement of HDL blood levels.

We now have promising pharmacological inhibitors of CETP that are capable of increasing HDL by a magnitude comparable to that of statins on LDL. From the standpoint of clinical trial design, a reasonable hypothesis is that inhibition of CETP is antiatherogenic in the absence of severe hypertriglyceridemia. Nonetheless, despite our wealth of animal, epidemiological, and genetic data, we cannot predict the antiatherogenic effect of CETP inhibition or identify human subsets in whom the intervention might be more or less effective. Consequently, these randomized clinical trials will exemplify equipoise, the ethical principle that serves as the foundation of clinical trials. The investigator describing the trial will be truly uncertain as to which course of therapy that participation in a clinical trial offers. Thus the effect of CETP and its inhibition seems to be to be dependent on the interaction between the level and distribution of lipoprotein components. Although data are currently insufficient to draw any conclusions, an additional reasonable speculation is that the effect of the metabolic milieu on CETP inhibition may involve alteration in HDL function, catabolism, or particle distribution not detected in the measurement of HDL blood levels.

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