Cholesteryl ester transfer protein. Its role in cardiovascular disease and drug development

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CHAPTER 8

What can we expect from inhibition of CETP activity in the treatment of dyslipidemia?

A review of CETP function and its relation to atherosclerosis.

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Submitted.
Abstract

Purpose of review
Although the atheroprotective role of high-density lipoprotein cholesterol (HDL-c) is well-documented, effective therapeutic drugs to selectively increase plasma HDL-c levels are not yet available. Recent progress in unravelling HDL metabolism in vivo will help the development of strategies to decrease the incidence and progression of coronary artery disease (CAD) by raising HDL-c. In this quest for novel drugs, cholesteryl ester transfer protein (CETP) represents a pivotal target. The role of this plasma protein in HDL metabolism is highlighted by the discovery that genetic CETP deficiency is the main cause of extremely high HDL-c in Asian populations. The potency of using CETP inhibitors to effectively increase HDL-c concentration in man was recently published and data with regard to human atherosclerosis are expected shortly. This review discusses the potential of CETP inhibitors to protect against atherosclerosis in the context of the current knowledge of CETP function in both man and rodents.
Introduction

Coronary artery disease (CAD) continues to be the leading cause of morbidity and mortality in adults in the developed world. Among numerous genetic and lifestyle parameters, dyslipidemia is one of the most important risk factors for CAD. In the past decade, lowering low-density lipoprotein cholesterol (LDL-c) has been the major target in cardiovascular preventive strategies. This approach has proven to be beneficial and effective in both primary and secondary prevention of cardiovascular disease. Randomized studies have unequivocally shown that treatment with HMG-CoA reductase inhibitors (statins) effectively lower LDL-c levels and reduce CAD by approximately 30%. At the same time, this illustrates that a large portion of cardiovascular events cannot be prevented by LDL-c lowering strategies per se, which is not surprising since atherosclerosis is a multifactorial disease. In the search for additional therapeutic targets, attention has recently focused on HDL increasing strategies, since prospective epidemiological studies have clearly shown that a low high-density lipoprotein cholesterol (HDL-c) level is a strong and independent risk factor for the development of CAD.

The attempts to elucidate the precise anti-atherogenic effects of HDL has resulted in the discovery of a multitude of protective properties. First of all, HDL mediates the transport of excess cholesterol from the periphery (including the arterial wall) to the liver. This process of reverse cholesterol transport (RCT) is often invoked to explain the atheroprotective effect of HDL. However, HDL also exerts anti-oxidative, anti-thrombotic (inhibition of platelet activation and platelet aggregation) and anti-inflammatory effects. Moreover, it has also been shown to affect endothelial dysfunction. At present, it is unclear which of these functions contribute most to the anti-atherogenicity of HDL. Taken together, it is clear that HDL metabolism is very complex and development of HDL-specific agents has proven elusive. In terms of novel agents that raise HDL-c, inhibitors of CETP activity hold promise for the near future, and are therefore the focus of this review.

CETP promotes the transfer of cholesteryl esters from HDL to apolipoprotein (apo) B containing particles, i.e. very-low density lipoprotein (VLDL) and LDL, in exchange for triglycerides. With respect to the anti-atherogenicity of this transfer protein, CETP can be seen as a facilitator of cholesterol flux through the RCT system. However, this action also directly relates to decreased HDL-c levels, which can be regarded as atherogenic. Despite the uncertainties surrounding the pro or anti-atherogenic role of CETP, inhibitors of this transfer protein are in an advanced stage of clinical development. To date, at least one CETP inhibitor will be tested in phase III trials shortly.

Since the 1980s, patients with marked hypercholesterolemia due to elevated levels of HDL-c (3.9 - 7.8 mmol/l) have been reported by several investigators, predominantly in Japan. Mutations in the CETP gene
were identified as the molecular defect underlying this increase in serum HDL-c. Subsequent studies of lipids and lipoprotein metabolism in these individuals have provided crucial information on the role of CETP as well as the routes of human cholesterol transport in the circulation. First of all, CETP was shown to affect all lipoprotein classes. In complete absence of CETP function, the failure to transfer cholesteryl esters from HDL to other lipoproteins leads to an accumulation of cholesteryl esters in HDL fraction. To accommodate the increased amounts of core lipids in HDL, other surface components such as apoA-I, phospholipids and unesterified cholesterol are also increased. Interestingly, apoA-I and apoA-II concentrations increase mainly due to a reduced catabolic rate whereas the synthesis of both apolipoproteins is unaltered. In addition to the elevation of HDL-c in homozygotes for CETP gene defects, there is a substantial reduction in the concentration of LDL-c and apoB. These LDL particles are also small and heterogeneous and have a low affinity for the LDL-receptor. In contrast to apoA-I and ApoA-II, however, the catabolism of LDL and apoB is increased substantially which has been proposed to be related to an upregulation of the LDL receptor pathway.

**CETP deficiency and cardiovascular risk**

In all epidemiological studies, an increase in HDL-c combined with a decrease in LDL-c implicates a significant CAD risk reduction. However, the relationship between heterozygous and homozygous CETP deficiency and the susceptibility to atherosclerosis has proven to be complex and somewhat confusing: Both longevity and an increase in CAD risk have been reported. Hirano and co-workers have shown that reduced CETP function (in 201 individuals with HDL-c levels > 2.58 mmol/l) in conjunction with reduced hepatic lipase activity is associated with an increased risk for CAD. This indicates that the metabolic setting of the individual might determine, at least in part, the ultimate effect on atherosclerosis. Furthermore, Hirano et al. reported that in northern Japan the prevalence of CETP deficiency was reduced in individuals over 80 years and, thus, not associated with longevity. In 3,469 men of Japanese ancestry (present in the Honolulu Heart Program) with two different CETP gene mutations the relationship between CETP deficiency and CAD was modified by HDL-c levels. It was shown that males heterozygous for CETP gene defects, with low or moderately increased HDL-c levels (1.0-1.6 mmol/l) had an increased risk for coronary heart disease compared to men with similar HDL levels without CETP gene mutations. By contrast, males with or without CETP gene defects but with very high levels of HDL-c (>1.6mmol/l) had less coronary heart disease. Nonetheless, this ongoing prospective study recently provided further insight (7-year follow-up) indicating that heterozygotes for CETP gene defects are not at increased risk, in line with earlier data on homozygous CETP deficiency. Moreover, Moriyama et al. reported the outcome of a cross-sectional analysis in a population with 19,044 male and 29,487 female Japanese
subjects, in which subjects with very high HDL-c levels as well as subjects with mild-to-moderate HDL-c elevations, appear to be protected against cardiovascular disease, whether or not they had CETP deficiency.\textsuperscript{34} Taken together, the relation between human CETP deficiencies, either in homozygous or heterozygous form, and the risk of cardiovascular disease remains a matter of debate.

CETP gene polymorphisms and cardiovascular risk
Studying common CETP gene variants has, unfortunately, not provided better insight into the relationship between CETP and atherosclerosis. Of these, the TaqI B polymorphism, located in intron 1, is one of the most intensively studied.\textsuperscript{35-41} The associations of TaqI B with various parameters is generally thought to arise from strong linkage disequilibrium between TaqI B and the \textasciitildeC629A polymorphism, which has been shown to directly affect CETP promoter activity.\textsuperscript{42-44} Significant associations of the B1B1 genotype with higher plasma CETP concentration and/or CETP activity, and lower HDL-c levels were found in several studies.\textsuperscript{40,45-47} but this was not consistently observed.\textsuperscript{37,48-50} Furthermore, it has been reported that the effects of TaqI B on the above parameters are gender dependent and also influenced by alcohol use, body mass index and insulin levels.\textsuperscript{37,40,45-51-53} Despite the fact that HDL-c and CETP function are modulated by multiple genetic and environmental factors, and given that HDL only partly determines the risk for atherosclerosis, this single genetic marker has been shown to be associated with the risk of CAD. In the Framingham Offspring Study, the B2 allele was associated with a reduced risk of coronary heart disease in men.\textsuperscript{40} and recently this was confirmed in the Veterans Affair HDL-c Intervention Trial (VA-HIT).\textsuperscript{36} On the contrary, in men and women with a history of myocardial infarction (Coronary and Recurrent Events study)\textsuperscript{54} and in a cohort of healthy US physicians\textsuperscript{39} no association was found between TaqI B genotype and coronary heart disease.

By contrast, other investigators showed that a less frequent CETP polymorphism, resulting in the exchange of an isoleucine to a valine at position 405, is associated with low CETP activity and a higher prevalence of CAD in hypertriglyceridemic men (\textasciitilde1.9 mmol/l).\textsuperscript{55} This again implicates that CETP can be proatherogenic or anti-atherogenic depending on the metabolic setting. In conclusion, the association between CETP genotype and coronary heart disease is as yet unclear, and it is not surprising that a single CETP gene marker is insufficient to predict the progression of a complex chronic disease such as atherosclerosis.

CETP and dyslipidemia, what comes first?
In various human dyslipidemias associated with accelerated atherosclerosis, CETP concentration and/or the rate of net transfer of cholesteryl esters from HDL to apoB-containing lipoproteins is increased. Plasma CETP concentration was found to be increased in individuals with hyper-
cholesterolemia,56-58 combined hyperlipidemia, dysbetalipoproteinemia,59 severe chylomicronemia,57 and nephrotic syndrome.60 Whereas this may imply that CETP is deleterious, it can also be argued that high CETP is the result, rather than the cause of dyslipidemia.61 In this regard, CETP action itself is directed by the composition of various lipoproteins. Lifestyle factors further complicate this issue: it has been described that alcohol abuse and physical exercise, typically associated with increased HDL, are associated with diminished CETP concentration.51:62:63 In line, smoking, associated with low HDL-c, is associated with high CETP activity.64:65 Recent cross-sectional studies have furthermore shown that an inverse relation exists between CETP and HDL-c among hypertriglyceridemic men but not in normotriglyceridemic men.66 These studies clearly indicate that plasma CETP is affected by a variety of metabolic conditions and lifestyle factors that are in themselves associated with changes in CAD risk.

**CETP in animals**

Mice and rats, naturally deficient of CETP, use HDL as the major means of cholesterol transport. In line with the discussed anti-atherogenic potential of this lipoprotein, these animals are relatively resistant to atherosclerosis. On the other hand, rabbits with naturally high CETP levels, transport most of their cholesterol in LDL and by contrast are susceptible to atherosclerosis.67 This brings us to humans who express CETP, transport their cholesterol in mainly LDL, and especially in the Western World are susceptible to atherosclerosis. Off course, human susceptibility to atherosclerosis is also related to deleterious life-style parameters, such as high fat intake, smoking, and lack of physical exercise, but CETP expressing mammals appear to be predisposed to atherosclerosis. Genetically engineered mice have proven to be valid models to further study CETP function and its relation with atherosclerosis. Introduction of the human (h)CETP gene into mice results in a dose-related reduction of HDL-c levels and, as a consequence, have significantly more early atherosclerotic lesions in the proximal aorta compared to control mice.68 Subsequent cross breeding with apoE and LDL receptor knock-out mice, furthermore underlines that high levels of CETP accelerate lesion development.69 In contrast, in the setting of hypertriglyceridemic mice, CETP expression was anti-atherogenic.70 In addition, CETP expression was considered to reduce atherosclerosis in lecithin:cholesterol acyltransferase (LCAT) transgenic mice by correcting the dysfunctional properties of HDL and promoting the hepatic uptake of HDL-cholesteryl esters.71 Summarizing, studies in mice have shown that (over)expression of hCETP in the context of a loss of liver-mediated uptake of atherogenic lipoproteins, is deleterious, but that in the presence of high concentration of triglycerides and dysfunctional HDL, CETP can also have anti-atherogenic effects.
CETP inhibition in animals

Various strategies have been developed to inhibit plasma CETP activity. This paragraph will first discuss the effects on lipids with a subsequent focus on atherosclerosis. In 1989, Whitlock et al. were amongst the first to effectively inhibit CETP in vivo. Intravenous injection of monoclonal antibodies raised against CETP were used to investigate the role of CETP on lipoprotein composition in rabbits. It was concluded that CETP plays an important role in the clearance of cholesteryl ester from plasma. In addition, the use of CETP antibodies in hamsters was also effective in raising HDL-c (33%) and shown to induce effects on lipoproteins that were similar to those reported in human CETP deficiency. In addition to these immunological approaches, several synthetic CETP inhibitors have been developed. One of the first, CGS 25159, induces significant decreases in VLDL-c and VLDL-triglycerides levels on top of an increase in HDL-c (29%) in normal and hyperlipidemic hamsters at 30mg/kg/day dosages. In 2000, JTT-705, another synthetic CETP inhibitor, was described to achieve a 50% inhibition of CETP activity in human plasma in vitro at a concentration of 5 μM and a 95% inhibition of CETP activity in rabbit plasma after administrating this compound at an oral dose of 30mg/kg. In addition, the effects of JTT-705 were compared with the effects of simvastatin in these rabbits. Both JTT-705 and simvastatin caused an increase in HDL-c (90% and 28%, respectively) and a decrease of non-HDL-c (40-50% and 50-70%, respectively). Finally, a recently developed vaccine that elicits antibodies which block CETP function, was also shown to induce a significant increase of HDL-c and a modest decrease of LDL-c concentration.

Beyond lipid modulation, a few promising studies have been published which were primarily conducted in cholesterol-fed rabbits, an excellent model for diet-induced atherosclerosis, that mimics human atherosclerosis. CETP inhibition in this animal model, either achieved through antisense oligonucleotides, vaccine-induced CETP antibodies or the chemical CETP-inhibitor JTT-705, lead to increase of HDL-c levels (35%, 42%, 90%, respectively) with concomitant anti-atherogenic effects. In these studies, atherosclerotic lesions were reduced with 7%, 40% and 70%, respectively, compared to control cholesterol-fed rabbits. In a direct comparison with simvastatin, a typical LDL-c lowering drug, JTT-705 reduced the area of atherosclerotic lesions to a similar extent (-80%). In addition, rabbits with a more severe degree of hypercholesterolemia (total cholesterol 10.5 mmol/l compared to 6.8 mmol/l) were treated with various dosages of this CETP inhibitor, but no effect was seen on aortic cholesterol content in spite of a 70% inhibition of CETP activity and a concomitant 200% increase of HDL-c levels. However, unexpectedly, triglyceride levels increased in these animals, which may explain why there was no effect on atherosclerosis. These animal data may indicate that the scope of JTT-705 to reduce established atherosclerosis may be limited.
CETP inhibitors in humans

To date, there is only one published study on the use of an experimental CETP inhibitor in humans. In phase I, a single-dose (100 to 800 mg) of JTT-705 was well-tolerated and did not result in significant toxicity in healthy Caucasian males. A 2-period crossover bioavailability study revealed that JTT-705 induced more pronounced CETP inhibition in the postprandial phase compared with the fasted state. In a subsequent phase II study, JTT-705 was shown to effectively raise HDL-c up to 34% and apoA-I up to 16%. The rise in HDL-c levels was caused by significant increases in both HDL$_2$ and HDL$_3$ subfractions, representing large and small HDL particles, respectively. In addition to an increase in HDL-c, the high-dose group also had a significant 7% decrease in LDL-c. There were no serious adverse events or clinically relevant changes in safety parameters. Some mild gastrointestinal effects were observed, but no withdrawals occurred for that reason. Although these results hold promise for the future, more studies are needed to investigate whether the observed increase in HDL-c translates into a concomitant reduction in CAD risk.

Discussion

In summary, the literature clearly shows that the relationships between CETP, HDL and atherosclerosis are complex. On the basis of both human and animal studies, it must be concluded that CETP can be both pro-atherogenic and anti-atherogenic, depending on the metabolic setting and perhaps on the species studied. The results of the Honolulu Heart Program indicate that the effects of CETP inhibition may also depend on genetic and environmental factors that influence the concentration of HDL.

Lowering CETP activity may be beneficial in relative hypercholesterolemia in Westernised countries, where high-fat and cholesterol-rich diets increase plasma LDL levels and down-regulate hepatic LDL receptors. In terms of reverse cholesterol transport, CETP inhibition would force delivery of cholesterol through HDL to the liver for subsequent secretion into the bile. In the presence of fully functional HDL-c uptake mechanisms this may be more desirable than uploading atherogenic LDL particles with cholesterol esters from HDL. However, this brings us to the fact that very little is known about the function of scavenger receptor class B type 1 (SR-B1) in humans. The HDL receptor that in mice mediates selective uptake of cholesteryl esters. It remains to be elucidated how the uptake of HDL-associated lipids is controlled in humans. This is of utmost importance when considering that CETP inhibition will result in the accumulation of large cholesteryl ester-enriched HDL particles. However, the role of HDL in human metabolism exceeds that of simply mediating reverse cholesterol transport. The effects of CETP inhibition on inflammation,
thrombosis, endothelial function, and oxidative modification of proteins and lipids also warrant intensive study and in this respect, CETP inhibitors will provide excellent tools to investigate HDL function in these areas.

It is undoubtedly premature to predict the effects of therapeutic CETP inhibition in humans. The therapeutic success of CETP inhibitors in clinical practice will largely depend on successful co-application with evidence-based LDL-c lowering modalities. In addition, various lines of evidence clearly indicate that the scope of CETP inhibition to reduce atherosclerosis and cardiovascular risk may be limited by the metabolic setting, in which triglyceride levels, gender, obesity, alcohol use and smoking play important roles.

Considering the complex relationship between CETP, HDL-c levels, and CAD, it will be important to determine the percentage inhibition of CETP activity that will yield optimal protection against atherosclerosis. The TaqIB polymorphism can be considered a natural experiment in terms of a lower CETP level of approx 20%, with a concomitant 10% elevation of HDL-c levels. Based on the marginal beneficial effects of this variant on cardiovascular risk, one can hypothesize that a greater than 20% inhibition would be desirable. On the other hand, complete inhibition, as observed in human CETP deficiency is probably neither necessary nor advisable although a complete loss of activity does not necessarily lead to adverse outcomes.

Future data will learn whether CETP inhibition is indeed a good target to raise HDL-c in order to reduce CAD in humans. Here, surrogate endpoints of coronary artery disease, such as flow mediated dilation (FMD) and intima-media thickness (IMT), will soon clarify whether CETP inhibition improves vascular function and reduces intima media thickening, while patience is needed for proof of actual reduction in morbidity and mortality. Nonetheless, if a CETP inhibition-related increase in HDL cholesterol will prove to mediate vascular protection, the combination of statins and CETP inhibitors will bring us into a new era of cardiovascular prevention.

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