HDL cholesterol: atherosclerosis and beyond
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The promise of Cholesteryl Ester Transfer Protein (CETP) inhibition in the treatment of cardiovascular disease


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Abstract

There is a strong need to reduce the risk of cardiovascular disease (CVD) beyond the use of statins that lower low-density lipoprotein cholesterol (LDL-c). The inverse relationship of high-density lipoprotein cholesterol (HDL-c) with cardiovascular disease suggests HDL-c raising therapy as a novel target. This review discusses the role of HDL-c in atherogenesis as well as the promise of cholesteryl ester transfer protein (CETP) inhibition in CVD prevention. While genetic studies show conflicting results on correlations between HDL-c and CVD, experimental studies have yielded sufficient encouraging data to proceed with the development of HDL-c raising strategies. CETP inhibition has been shown to successfully increase HDL-c levels in man. However, the first CETP inhibitor tested in phase III trials increased mortality possibly due to torcetrapib-specific vasopressor effects. More recently, dalcetrapib did not show an effect on CVD outcome while raising HDL-c by 30%, thereby refuting the HDL-c hypothesis. Anacetrapib and evacetrapib are currently tested in phase III clinical trials and have not shown adverse effects thus far. Both compounds not only increase HDL-c by 29-51%, they also decrease LDL-c (36-41%) and anacetrapib lowers Lp(a) (17%). Combined, these effects are anticipated to decrease CVD risk and the results will be revealed in 2017.
Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide. Low-density-lipoprotein cholesterol (LDL-c) is a direct contributor to the initiation and progression of atherosclerosis, the underlying chronic disorder of CVD. As a consequence, LDL-c lowering strategies provide the basis for therapeutic efforts aimed at lowering CV-risk; statins reduce major cardiovascular event risk by 25% for every 1 mmol/L LDL-c decrease. However, even when target levels for LDL-c are reached, a substantial residual risk of 65-75% remains which emphasizes the need for additional therapeutic strategies on top of statins.

Large epidemiological surveys have consistently shown that HDL-c is inversely correlated with cardiovascular risk. Every mg/dl increase of HDL-c levels has been reported to be associated with a 2-3% decreased CVD risk. Even when target levels for LDL-c have been reached, HDL-c levels are still found to be predictive of major cardiovascular events. Furthermore, in a post hoc analysis comprising 1455 patients from 4 prospective studies, the HDL-c increase associated with statin therapy was also found to independently predict risk reduction. Moreover, in a meta-analysis of twenty randomized controlled trials (RCTs), statin therapy did not alter the relationship between HDL-c and CVD. These findings have led to the widely accepted idea that raising HDL-c is a promising target to lower CVD risk. By accepting HDL-c increase as a therapeutic target, however, one adheres to the assumption that HDL-c is a causal factor in atherogenesis (for review see ). This assumption has been criticized in the light of recent observations supporting the idea that HDL-c levels merely constitute a biomarker for a pro-atherogenic milieu rather than a causal factor. The profound impact of a wide variety of risk factors such as metabolic syndrome, smoking, inflammation and physical inactivity on HDL-c concentration confounds the relation between HDL-c and CVD. In this scenario, HDL-c can be seen as a sensitive indicator of an adverse CV-risk profile, rather than a protective partaker in the course of atherogenesis. In support, genetically determined high HDL-c was not found to be associated with decreased CVD risk while elevated HDL-c has also been shown to have the capacity to turn into a pro-atherogenic factor. This review will discuss the role of HDL-c in atherogenesis as well as the promise of cholesteryl ester transfer protein (CETP) inhibition in CVD prevention.

Search strategy

We searched for articles on CETP in both animal models and humans, using search terms ‘CETP’, ‘murine’, ‘atherosclerosis’, ‘atherogenesis’, ‘rabbit’, ‘HDL-c’. With regards to the human studies, we searched for articles covering CETP genetics, genome wide association studies (GWAS), studies reporting on CVD risk and articles describing the effects of pharmaceutical CETP inhibition. For this, we used search terms ‘human’, ‘CETP’ ‘genetics’ ‘CETP mutation’ ‘atherosclerosis’, ‘atherogenesis’, ‘cardiovascular’ ‘torcetrapib’, ‘dalcetrapib’, ‘anacetrapib’ and ‘evacetrapib’.
HDL and atherosclerosis

Animal studies
Wild-type mice have no CETP, carry most of their cholesterol in HDL and do not suffer from atherosclerosis even when put on high fat/cholesterol diets. However, after introduction of CETP in mice, HDL-c drops and the mice become prone to atherosclerosis. Already in 1991, raising HDL-c through overexpression of human apolipoprotein AI (apoA-I; an integral part of HDL) in mice was shown to protect against atherosclerosis. Crossing these transgenic mice with atherosclerosis prone strains also resulted in atheroprotection and even plaque regression.

In addition, gene therapeutic approaches using apoA-I have shown clear beneficial effects. However, the relation between HDL-c plasma levels and atherogenesis in experimental models is not straightforward.

Raising HDL-c through overexpression of e.g. lecithin:cholesterol acyltransferase (LCAT) was shown to exert different effects on atherosclerosis dependent on the animal models and mouse strain crosses studied. Also modulation of hepatic lipase activity has provided conflicting results, while loss of scavenger receptor class B1 (SRB1), leading to a stark increase of HDL-c was associated with increased atherosclerosis. Finally, modulation of ATP binding cassette transporter A1 (ABCA1) activity has also provided ambiguous data.

Human studies
While epidemiological evidence that low HDL-c associates with increased CVD risk is irrefutable, evidence from genetic studies is equivocal. Mutations in APOA1 have consistently been reported to be associated with atherosclerosis. However, this is not the case for mutations in other major HDL genes such as ABCA1 and LCAT. In addition, common ABCAI, LCAT and LIPG gene variants were associated with HDL-c levels but not with the incidence of coronary artery disease, while for LIPC increased HDL-c was even associated with an increased risk of ischemic heart disease. Furthermore, GWAS have consistently reported that all major single nucleotide polymorphisms (SNPs) associated with high LDL-c levels correlate with CVD risk while this is not the case for HDL-c. Using an allele scoring system consisting of 14 SNPs that exclusively associate with HDL-c, Voight et al recently also showed no association with CV risk.

Hence, apart from APOAI, other genes in HDL metabolism have failed to provide convincing and consistent evidence in support of HDL-c as a causal factor in atherogenesis.

HDL-c concentration versus HDL function
While HDL is best known for its central role in reverse cholesterol transport (RCT), it has also been found to exert a plethora of anti-atherogenic effects. The latter comprise anti-inflammatory effects, direct stimulation of endothelial nitric oxide availability and hence improvement of endothelial function as well as stimulation of endothelial repair.

A possible explanation for the wide variety of effects associated with HDL-c is provided by the observation that the heterogeneous pool of HDL particles carries large numbers of metabolically active proteins and enzymes with a range of different functions. Thus,
HDL is probably not only a passive carrier of cholesterol. Interestingly, recent studies have demonstrated that in patients with overt CVD, HDL-c has lost its association with eNOS activation, anti-inflammatory action and endothelial regenerative capacity. These findings challenge the concept of simply targeting an increase in HDL-c concentration in CVD prevention strategies. In addition, it has been reported that ‘dynamic’ HDL-c-flux may behave independent from ‘static’ HDL-c concentrations. In fact, an increased HDL-c plasma concentration may even indicate a decreased capacity of the RCT pathway, as can best be exemplified by the situation in SRB1 deficiency, in which the hepatic uptake of HDL cholesterol is compromised. Although there is a strong interest to focus on HDL function, the epidemiological evidence simply points at HDL-c concentration and there is thus far no evidence that HDL function parameters are correlated with CVD in prospective studies.

Collectively, these data imply that raising HDL-c as a target to decrease the CVD risk is a challenging concept, which remains to be established. In view of the complexity of the HDL metabolism, the endpoints need to be validated for each HDL-c raising strategy. This complexity is corroborated by the results of human clinical trials, in which the impact of HDL-c increase on CVD risk reduction has been disappointing. Nicotinic acid and fenofibrate did not offer CVD protection, although design could be related to these failures. Also trials using apoA-I mimetics aimed at improving HDL function have not been uniformly successful thus far. Collectively, these data emphasize the need to provide solid clinical evidence that raising HDL-c actually does decrease CVD risk. However, raising only HDL-c is not easily achieved with the currently used regimens as will be discussed below.

**CETP inhibition and CVD risk**

CETP is a protein that is secreted by the liver, adipose tissue and macrophages. In plasma, CETP accommodates the transfer of cholesterol esters from HDL to (V)LDL in exchange for triglycerides. This reaction drives a decrease of cholesterol in HDL and an increase of cholesterol in LDL, especially when triglyceride levels are elevated. Thus, CETP inhibition retains cholesterol in HDL whilst decreasing the cholesterol content of the atherogenic apoB fraction. The central role of CETP in HDL metabolism was illustrated by Inazu and co workers who reported that genetic CETP deficiency causes very high HDL-c levels (up to 4.24 mmol/L) while a less marked decrease of LDL-c levels was observed. Furthermore, in rabbits, which are naturally characterized by a high level of CETP activity, it has been shown that inhibiting CETP through inhibitors or antisense strategy significantly attenuates atherogenesis.

**CETP genetics**

Whereas the association between functional CETP mutations and plasma HDL-c levels is irrefutable, the association with cardiovascular endpoints is more complex. Curb and co workers did not find decreased coronary heart disease in carriers of deleterious CETP mutations, neither did Moriyama and co workers, while the Honolulu Heart Study even showed increased coronary heart disease in male Japanese-American mutation carriers.
On the other hand, Kakko and co-workers reported that carriers of a mutation resulting in increased CETP activity displayed increased atherosclerosis. In the Multi-Ethnic study of Atherosclerosis, alleles associated with high CETP activity were also associated with increased coronary artery calcium score, a reliable surrogate for CVD risk. CETP was also associated with coronary risk in GWAS. The latter was corroborated in the Copenhagen City Heart Study, showing that CETP gene variation leading to a 14% HDL-c increase was associated with a hazard ratio of 0.74 for ischemic heart disease. Similar findings were reported in the Women’s Genome Health Study. In a meta-analysis comprising more than 100,000 subjects, the relation between decreased CETP and decreased CVD was further corroborated, reporting a 5% CV-risk reduction for 3 commonly reported CETP polymorphisms, all of which were associated with lower CETP activity and higher HDL-c levels. Finally, plasma levels of CETP were also shown to be associated with risk of coronary artery disease, predominantly in individuals with high triglyceride levels. These data on CETP genetics clearly differ from those obtained in studies regarding ABCAI, LCAT and LIPC and LIPG as described above. This apparent discrepancy has been partly attributed to the fact that variation in the CETP gene does not only modulate HDL-c levels but has also marked effects on both LDL-c and triglyceride levels.

In summary, there is sufficient evidence supporting the continuation of programs in humans to test the impact of CETP inhibition on CV-risk.

**Differences between CETP inhibitors**

Torcetrapib, anacetrapib and dalcetrapib all work through promoting the formation of a complex between CETP and HDL. Still, the four CETP inhibitors which are or have been tested in humans do show clear structural differences, resulting in different mechanisms of action. Torcetrapib and anacetrapib give rise to a larger increase in plasma HDL-c than dalcetrapib. Whereas torcetrapib and anacetrapib can be regarded true CETP inhibitors, dalcetrapib has been described to be a CETP modulator, pertaining to the fact that torcetrapib and anacetrapib are 3,5-bis-trifluoromethyl-benzenes while dalcetrapib is a benzenethiol derivative. Whereas torcetrapib and anacetrapib treatment results in larger HDL particles and promote the formation of HDL-cETP complexes, dalcetrapib at lower dosages leads to a smaller increase in HDL particle size. Furthermore, the CETP-lipoprotein complex in case of torcetrapib is unable to efficiently exchange neutral lipids between different lipoprotein particles. In contrast, dalcetrapib decreases CETP activity by facilitating a specific interaction between cystein 13 of CETP and the benzenethiol moiety of dalcetrapib. Still, torcetrapib, anacetrapib and dalcetrapib all induce tight binding of CETP to HDL, indicating that these inhibitors work through promoting the formation of a complex between CETP and HDL. The exact inhibitory effects of evacetrapib (BAY60-5521) have not been fully elucidated.

**Important ‘safety’ lessons learned from torcetrapib**

Torcetrapib was the first CETP inhibitor that was tested in phase III clinical trials. Despite a 70% HDL-c increase and a 25% LDL-c decrease in 15067 patients with overt CV disease,
the drug unexpectedly caused a significant increase in death.\textsuperscript{96} To explain these findings, attention focused on off-target effects. A major worry pertains to the acute vasopressor effect of torcetrapib which also occurred in species that by nature do not have CETP, such as mouse, rat and dog.\textsuperscript{97} Mechanistically, this pressor effect has been attributed to an effect of torcetrapib on the adrenal glands, resulting in increased secretion of aldosterone and corticosterone.\textsuperscript{98,99} Since the latter occurred also in animal models lacking CETP, these effects were independent of the CETP pathway.\textsuperscript{100,101} Moreover, torcetrapib also increased the expression of renin-angiotensin-aldosterone and endothelin-related genes in the vessel wall as well as the adrenal glands of spontaneous hypertensive rats,\textsuperscript{102} possibly also contributing to an enhanced vasopressor response. In patients, torcetrapib has been associated with decreased potassium and increased sodium and bicarbonate levels with a concomitant increase in serum aldosterone level.\textsuperscript{96,103-105} In the ILLUMINATE study, there was an increased risk of death in patients treated with torcetrapib whose reduction in potassium or increase in bicarbonate was greater than the medium change.\textsuperscript{96} Since the use of torcetrapib has also been associated with increased progression of intima media thickness, it likely that the blood pressure is related to the adverse effect of this compound.\textsuperscript{106} Collectively, these observations provide strong evidence that off-target effects of torcetrapib have contributed to its failure. On the other hand, it cannot be ruled out that CETP inhibition results in HDL particles that do not function normally\textsuperscript{95} with slower turnover rates than normal and that the observed inverse relationship between HDL-c and CV-risk reflects an epiphenomenon. Interestingly, the increased mortality in the treatment group cannot be attributed to cardiovascular deaths only. More patients in the treatment group died from cancer and infection.\textsuperscript{96} This finding increased the interest in the role of HDL-c in cancer\textsuperscript{107} and inflammation,\textsuperscript{108,109} however, no definite explanation has been found to date.

**Absence of aldosterone effect of other CETP inhibitors**

The findings from the torcetrapib trial gave rise to an increased interest in blood pressure effects of this class of drugs. Dalcetrapib had no effect on aldosterone synthase or aldosterone production in adrenal cell lines\textsuperscript{97} nor did it increase blood pressure or renin-angiotensin-aldosterone related gene expression in spontaneously hypertensive rats.\textsuperscript{110} In addition, blood pressure profiles did not show clinically relevant changes during 12-week studies with no dose-related trends.\textsuperscript{111} The dal-VESSEL study, assessing effects of dalcetrapib on endothelial function, blood pressure, inflammatory markers and lipids, further established the safety and tolerability of dalcetrapib.\textsuperscript{112} Also anacetrapib had no effect on aldosterone and corticosterone secretion by adrenal cells\textsuperscript{98} and human safety data from this trial have confirmed this.\textsuperscript{98,113} Finally, evacetrapib has no effect on aldosterone and corticosterone secretion by adrenal cells either.\textsuperscript{114}

**Efficacy of dalcetrapib**

In a phase IIb RCT study (the Dal-PLAQUE study), magnetic resonance imaging was used to analyse vessel wall structure and \textsuperscript{18}F-fluoro-deoxyglucose (FDG)-PET-CT to measure vessel wall inflammation. Dalcetrapib, administered for 2 years, increased HDL-c by 27% without
affecting LDL-c and triglycerides in 64 patients. In the active arm, a non-significant reduction in carotid vessel wall inflammation at six months and a reduced total vessel wall area at 24 months were observed. All other primary outcome parameters were not significantly different.\textsuperscript{115}

In the dal-OUTCOMES trial, 15,600 patients who had suffered from an acute coronary event were enrolled, regardless of their HDL-c level, to test the hypothesis that dalcetrapib would reduce CV morbidity and mortality on top of LDL-c lowering therapy.\textsuperscript{116,117} The primary efficacy measure was time to first occurrence of CVD, death, nonfatal acute myocardial infarction, unstable angina requiring hospital admission, resuscitated cardiac arrest or atherothrombotic stroke. On May 7\textsuperscript{th} 2012, Roche announced that the trial had been terminated (http://www.roche.com/media/media_releases/med-cor-2012-05-07.htm). Importantly, whereas the ILLUMINATE trial was discontinued due to excess mortality in the treatment group, the dal-OUTCOMES trial was terminated on the basis of futility. Although there were no adverse safety signals, the Data Safety Monitoring Board concluded that there was virtually no chance of yielding a positive result. Taking into account that the trial was powered to reveal a benefit of an anticipated 30\% HDL-c increase without significant effects on LDL-c and triglycerides, it is fair to state that dalcetrapib does not offer the CVD risk reduction expected on the basis of epidemiological evidence for the HDL hypothesis.\textsuperscript{7,8}

Following the disaster of the torcetrapib program and the disappointment of the dalcetrapib program, the bar for future CETP inhibitors has once again been raised. There is however one crucial, differentiating effect of anacetrapib and evacetrapib versus dalcetrapib. While dalcetrapib had no effect on LDL-c, both anacetrapib and evacetrapib lower LDL-c by 36-41\% in addition to a much larger HDL-c increase (129-151\%) (see table 1). In addition, anacetrapib lowers Lp(a) by 17\%.\textsuperscript{113,114} Irrespective of changes in HDL-c levels, a 40\% decrease of LDL-c (and a 30\% decrease in Lp(a) levels for anacetrapib) would be expected to offer a direct CVD benefit in patients.

**Awaiting evidence for Anacetrapib**

In the DEFINE study, 1623 high risk patients with an HDL-c below 1.6 mmol/L were included and randomized to anacetrapib 100mg per day versus placebo.\textsuperscript{113} After 6 months, a 151\% increase in HDL-c, a 45\% decrease in LDL-c and a 24\% reduction in Lp(a) were observed. Although this study was not powered for endpoints, fewer patients in the anacetrapib group underwent revascularization (8 vs. 21; P=0.001).\textsuperscript{113} Based on the absence of evidence for off-target toxicity and the marked reduction of LDL-c and Lp(a), Merck continued their endpoint study ‘REVEAL’ (Randomized Evaluation of the Effects of Anacetrapib through Lipid-modification; clinicaltrials.gov ID NCT01252953). This study aims to include 30,000 subjects randomized to anacetrapib 100mg daily or placebo on top of LDL-c lowering therapy with a predicted follow up of 5 years. The outcome of this study is due in 2017.

**Emerging evidence for Evacetrapib**

After 3 months, a 129\% increase in HDL-c was accompanied by a 36\% decrease in LDL-c in patients randomized to the 500mg evacetrapib dose.\textsuperscript{118} No data on effect on Lp(a) were provided. To date, no endpoint study has been announced for evacetrapib.
Summary

Raising HDL-c levels as a target in CVD prevention is at present no longer considered to be the
crown prince after statin-induced LDL-c reduction. The intertwinement of HDL-c with a wide
array of pro-atherogenic risk factors, comprising overweight, inactivity, hypertriglyceridemia
and smoking makes it difficult to translate lessons from epidemiology to a potential benefit
of a pharmacological HDL-c-increase. Caution with raising HDL-c as a therapeutic target
has grown following a general lack of genetic evidence as well as the absence of a correlation
between HDL-c increase and CVD benefit in a meta-analysis of lipid-modulating randomized
controlled trials. Although measures of HDL-functionality have been proposed to shed light
on the ‘rocky road’ of HDL-c raising strategies, there is no prospective evidence for the
relevance of HDL-functionality on CVD outcome. Hence, to date we are left with studying the
impact of any agent mediating an increase in HDL-c through CV endpoint studies.

For CETP inhibition, the picture is equally blurred, although recent genetic evidence would
support this intervention. Clinical trials with CETP inhibitors have thus far not been able to
show benefit. Whereas for torcetrapib it can be argued that off target effects have contributed to
increased CV mortality, the absence of a benefit in the dal-OUTCOMES study unambiguously
shows the lack of a significant CV-benefit through an isolated, CETP-inhibitor induced HDL-c
increase. The clearly divergent effects of novel potent CETP-inhibitors, which also significantly
lower LDL-c, Lp(a) and non-HDL-c by 30-40%, however, provide a solid rationale to continue
with clinical trials. However, one should realize that these compounds can not answer the
question whether CETP-inhibition-induced HDL-c increase protects against CVD risk.

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Reference List


23. Rong JX, Li J, Reis ED, et al. Elevating high-density lipoprotein cholesterol in apolipoprotein E-deficient mice remodels advanced atherosclerotic lesions by decreasing...
macrophage and increasing smooth muscle cell content. Circulation 2001;104:2447-2452.


44. Bochem AE, van Wijk DF, Holleboom AG, et al. ABCA1 mutation carriers with low high-density lipoprotein cholesterol are characterized by a larger atherosclerotic burden. Eur Heart J 2012.


100. Vergeer M, Stroes ES. The pharmacology and off-target effects of some cholesteryl ester transfer protein inhibitors. Am J Cardiol 2009;104:32E-38E.


