Cholesteryl Ester Transfer Protein Inhibitors: A Hope Remains

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Cholesteryl ester transfer protein inhibitors: a hope remained

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Running title: CETP and CETP inhibitors

Abstract

Naturally CETP-deficient animals and genetic CETP deficiency caused by TaqIB polymorphism in human are relatively resistant to atherosclerosis including coronary artery disease (CAD). Cholesteryl ester transfer protein (CETP) inhibitors were developed for new therapeutic measure against atherosclerotic vascular disease through increasing HDL-cholesterol and decreasing LDL-cholesterol. Although a clinical trials with torcetrapib was terminated due to hypertension related side effects, two other compounds of anacetrapib and evacetrapib are under clinical trials of the phase III. Although additional failure of dalcetrapib suggested that hypertension-related adverse effect is not only the cause of failure of torcetrapib, but also validity of CETP inhibitor itself is questionable, this review summarized a hope remained in CETP inhibitors as a potential agent reducing residual CAD risk in some clinical setting. Rationale for the CETP inhibitor development is discussed from clinical and experimental insights of lipoprotein phenotype, functional activity on LDL and HDL, and role of CETP activity in relation to inflammation. Structure and function relationship between the N-terminal hydrophobic tunnel of CETP and CE/TG with/without a CETP inhibitor is discussed. CETP antibody may have a differential potential on directional selectivity of neutral lipid transfer in plasma lipoproteins.

Key words

ApoE-rich high density lipoprotein
Cholesteryl ester transfer protein (CETP)
Coronary artery disease (CAD)
Lecithin:cholesterol acyltransferase (LCAT)
Platelet activating factor acetylhydrolase (PAF-AH)
Phospholipid transfer protein (PLTP)
Preβ1-HDL
Reverse cholesterol transport (RCT)
TaqIB polymorphism

Introduction

In the first edition of the HDL handbook in 2010, I have written a chapter on plasma cholesteryl ester transfer protein (CETP) in relation to human pathophysiology of genetic CETP deficiency (1). Since then, more reports have been published to fill a gap in the CETP research area. Furthermore, more progress has been made in the development of CETP inhibitors. This chapter includes a recent knowledge on CETP structure and function relationship, and emerging evidence of CETP inhibitors in the last 5 years along with my opinion.

HDL-TG as a key component determining neutral lipid transfer (Figure 1)

CETP is a 74kD glycoprotein consisted of 476 amino acids and N-glycosylation. Its crystal structure reveals a banana-shaped molecule with N- and C-terminal β-barrel domains, a central β-sheet and a ~60 Å-long hydrophobic central cavity. A long tunnel has a space for hydrophobic 2 molecules of cholestery ester (CE) or triacylglycerol (TG) and plugged by an amphiphilic phosphatidylcholine (PC) at each ends. C-terminal amino acids of 433, 443, 457 and 459 appeared to be close to the tunnel neck (2). By an optimized negative-staining electronmicroscope protocol, CETP C-terminal is more globular and N-terminal is more tapered end (3). HDL and CETP form a binary complex, which could be seen as a tadpole-shape: CETP protruding from spherical HDL surface. The banana-shaped CETP has a concave surface protruding ~45 degree angle from the HDL surface. Since PC-binding pores are located at central β-sheet of CETP, the pore is close to HDL surface, which is composed of PL layers. Thus, CETP bridge HDL to LDL or VLDL to form ternary complex. Based on the asymmetric structure of CETP, N-terminal CETP prefers to bind to HDL and C-terminal end does to VLDL or LDL. Furthermore, recent studies suggested that the distal portion flexibility of N-terminal β-barrel domain is considerably greater in solution than in crystal and it remains hydrophobic in solution (4).

It is believed that CETP mediates hetero-exchange of TG and CE by moving between VLDL and HDL like a shuttle. If a shuttle model is correct, CETP adopts a conformation to enable binding to a large lipoprotein like VLDL in addition to HDL. However, in a recent model proposed by Charles and Kane (5), based on a recent experiment data, it appears to depend on the ternary complex between CETP-HDL and VLDL. The process includes sensing, penetration, and docking of CETP. CETP penetrates ~50 Å into HDL with the N-termial β-barrel domain, while penetrating LDL or VLDL only 20-25 Å through its distal C-terminal β-barrel domain since the outer PL shell of lipoproteins is 18-27 Å thick. CETP, phospholipid transfer protein (PLTP) and lipopolysaccaride (LPS) binding protein (LBP) belong to the tubular lipid-binding (TULIP) domain superfamily (6).

HDL metabolism in CETP deficiency (Figure 2)

Lipoprotein phenotype in CETP deficiency has been well investigated; high HDL and low LDL, but fewer consistent finding was found in TG metabolism. Concentation of preβ1-HDL is inconsistent between homo- and heterozygotes with CETP deficiency (7). Mild reduction of CETP activity found in heterozygotes had low levels of preβ1-HDL. However, complete CETP deficiency had oppositely higher levels of preβ1-HDL, suggesting that reduction of CETP activity is not linearly correlated with preβ1-HDL levels. Preβ1-HDL is believed to be an efficient acceptor for ATP-binding cassette transporter A1 (ABCA1)-mediated cholesterol efflux activity. Preβ1-HDL is converted to spherical HDL via lecithin:cholesterol acyltransferase (LCAT)-mediated cholesterol esterification. Therefore, either increased ABC-A1 or phospholipid（PL） transfer activity or decreased LCAT activity would be associated with increased levels of preβ1-HDL. A cause of increased preβ1-HDL found in homozygous CETP deficiency is currently unknown, but it is likely associated with decreased LCAT activity rather than accelerated lipolysis of VLDL/ chylomicron (CM) (8,9).

As shown in a kinetic study (10), fractional catabolic rate (FCR) of apoA-I is decreased in CETP deficiency, however, cholesterol/CE clearance rate from

HDL is not established in CETP deficiency. Scavenger receptor class B type I (SR-BI) receptor mediates selective uptake of HDL lipids in the liver, as SR-BI deficiency is a cause of increased HDL levels in humans (11).

RCT from peripheral tissues appeared to be pro-atherogenic if FC/CE in HDL would transfer to the VLDL-intermediate density lipoprotein (IDL)- low density lipoprotein (LDL) pathway via CETP activity, and FC/CE could be reutilize in VLDL formed in the liver after lipoprotein uptake of VLDL-IDL-LDL by the liver receptors such as LDL receptors. In contrast, the SR-BI pathway selectively promoted cholesterol secretion from plasma HDL into bile (12), thereby it is anti-atherogenic. Relationship between RCT and LDL receptor activity is discussed later in the section of statin in perspective of CETP inhibitor.

LDL metabolism in CETP deficiency

LDL-C is largely consisted of CE, which is esterified by LCAT in HDL. CETP-mediated CE transferred from HDL to VLDL is a major determinant of LDL-C, because LDL-C tends to be lower in complete CETP deficiency. However, a reciprocal transfer of TG from VLDL to LDL and HDL is diminished in CETP deficiency, consistent with findings of TG-rich VLDL and TG-poor HDL in CETP deficiency (13). The CETP-deficient homozygotes had polydisperse LDL subclasses from IDL-like particles to small and dense LDL on a native polyacrylamide gel, suggesting that complete CETP deficiency would inhibit inter-conversion of lipids between LDL subclasses (14,15). In contrast, partial CETP deficiency increased LDL size. Since heteroexchange of CE and TG between HDL and LDL, leading to formation of TG-rich LDL and it consequently becomes to be small and dense LDL after lipolysis of the core TG. Thus, decreased formation of TG-rich LDL is expected in heterozygous CETP deficiency, resulting in increase in relatively larger LDL subclasses.

VLDL metabolism in CETP deficiency

Postprandial lipemia is the most common risk of cardiovascular disease. Although predominant lipoproteins appeared in the postprandial periods have been discussed, it appears to be VLDL remnants (16). In post-prandial periods challenged by oral fat load, plasma TG response was diminished in the hetero- and homozygous CETP deficiency (17). It is compatible that CETP deficiency induced LDL receptor expression in the liver, which may accelerate TG-rich lipoprotein clearance.

However, relationship between CETP activity and the magnitude of post-prandial lipemia is controversial, as it appears to be dependent on the metabolic context of subjects. In women who have lower hepatic lipase activity than men, it is shown that low CETP activity would deteriorate post-prandial TG response (19). Since CETP mediates the transfer of CE from HDL particles to VLDL in exchange for TG. When VLDL increases post-prandially, VLDL act as CE acceptors for CETP activity, thereby an increased net rate of TG transfer from VLDL to HDL/LDL is expected. In result, TG-rich HDL and LDL are avidly bound to hepatic lipase that effectively hydrolyzed TG, and they become HDL3 and small dense LDL, respectively. Thus, in this step CETP helps to lower TG in plasma via enhancing lipolysis of TG in LDL and HDL fractions.

Antioxidant activity in CETP deficiency

Serum PON1 activity was increased in two cases with homozygous CETP deficiency but PON1 activity / apoA-I level ratio are comparable to controls (20). The oxidized LDL (oxLDL) levels were positively correlated with apoB, PLTP activity, but negatively with CETP activity in the general population (21). Since CETP enhances the ability of HDL to inhibit LDL oxidation in vitro, low CETP activity state may be susceptible to oxidative stress (22). However, CETP-deficient subjects did not reveal elevated levels of oxLDL and 8-isoprostane, nor decreased levels of paraoxonase activity (23).

Plasma PLTP concentration was increased in CETP deficiency by +57%, but PLTP activity was not increased (24). The role of increased PLTP mass is currently unclear in CETP deficiency.

Difference of lipoprotein phenotype between homozygotes and heterozygotes with CETP deficiency (Table 1)

CETP may have dual aspects of its atherogenicity. On one hand of pro-atherogenicity, CETP would increase CE contents in VLDL-IDL; after LPL and hepatic lipase-mediated lipolysis, VLDL-IDL becomes CE-rich LDL. If VLDL is concurrently increased in combined hyperlipidemia (also called as mixed hyperlipidemia) or in post-prandial periods, hetero-exchange of CE and TG between VLDL and LDL results in formation of small, dense LDL via HDL-mediated lipid transfer. Similarly, TG-rich HDL produced by CETP-mediated TG transfer enhances catabolism of HDL. On the other hand, CETP-mediated CE net-transfer from HDL to VLDL-IDL-LDL is beneficial as long as hepatic LDL receptor activity is not saturated. CETP may help lipoprotein conversion among HDL subclasses via recycling from large HDL to small HDL including preβ1-HDL formation.

CETP deficiency increased HDL-C and decreased LDL-C levels in adults. Thus, CETP inhibition may delay cholesterol clearance as plasma HDL-C levels increase. However, heterozygous CETP deficiency in a fetus showed decreased LDL-C without HDL-C changes (25), which may be associated with concurrently decreased LCAT activity.

Source of CETP and cholesteryl ester transfer (CET) determinant

CETP mRNA is abundant in tissues of liver, spleen and adipose. Net CE transfer rate is determined not only by plasma CETP activity but also VLDL levels, which

is a potent CE acceptor for the CETP-mediated lipid transfer. Some studies suggested that increased CET is a stronger risk factor than plasma CETP mass or activity. CET is associated with PAF-AH activity, which is also known as lipoprotein-associated phospholipase A2 (31). Since free fatty acid (FFA) generated by phosholipase activity by PAF-AH may increase binding of CETP to LDL, CET is accelerated.

Cardiovascular disease risk in CETP deficiency and single nucleotide polymorphisms (SNPs) in the CETP gene

The low CETP genotype of TaqIB2 was associated with decreased prevalence of coronary disease as well as increased HDL-C levels and large LDL size in men of the Framingham Heart Study (32). However, a recent study measuring plasma CETP activity suggested that lower plasma CETP activity was unexpectedly associated with greater cardiovascular risk (myocardial infarction, stroke, or heart failure) with relative risk 1.4 (33). The reason for this apparent discrepancy is unknown. As such, it is important to define whether the cause of the decrease in plasma CETP activity is genetic or environmental, since acute phase reaction and inflammation would decrease CETP expression. It is possible that lower CETP levels may be just a surrogate marker for inflammation rather than the genetic effect in the latter study as discussed below.

Anti-atherogenic effect of genetically lower CETP levels caused by the TaqI B2 allele has been reproduced in a recent meta-analysis (34). In heterozygous CETP deficiency, the Honolulu Heart Study showed that heterozygotes are anti-atherogenic at least when they have increased HDL-C > 60 mg/dL (35). Homozygous CETP deficiency has been mainly found in Japan. Only very few cases are found in the other populations including European descendants. Thus, epidemiological studies that have been made in a relatively small number in Japan have suggested mixed results in the coronary artery disease (CAD) risk (36,37).

Longevity is expected in some CETP-deficient heterozygous subjects (26,38). A promising effect on longevity has been reported in Ashkenazi Jewish population, where increased homozygosity of I405V was found in offspring of individuals with exceptional longevity (39). Furthermore, the homozygosity of I405V was associated with slower memory decline and lower incident dementia (40).

Meta-analysis of CETP SNPs associating with low CETP activity and high HDL-cholesterol levels are anti-atherogenic such as TaqIB [rs708272] and -629C>A [rs1800775] (34,41). In prospective cohort studies, the Copenhagen City Heart Study and the Women's Genome Health Study showed that genetically low CETP activity is anti-atherogenic in men and women (42,43).

However, some recent studies reported inconsistent results. Hiura et al reported that the minor allele of rs3764261, located in the CETP promoter, is associated with elevated HDL levels and unexpectedly increased myocardial infarction (MI) risk in Japanese population (44). Similarly, SNPs located between intron 8 and exon 9, which were associated with exon 9 skipping, manifested an increased MI risk in men (45).

Opposite trends that TaqIB2 is associated with an increased vascular risk were found in statin-treated cardiovascular patients and very high-risk population who had increased HDL and C-reactive protein (CRP) levels and low PAF-AH activity (46,47). These opposite results need to be fully investigated whether or not that finding is related with reverse causality.

CETP in relation to inflammation and adiposity

In a prospective observational study of patients with stable CAD in Germany (KAROLA study), low CETP was associated with increased risk for death with an adjusted hazard ratio 1.84 (48). In the Ludwigshafen Risk and Cardiovascular Health (LURIC) study, CETP levels are lower in smokers, diabetics and unstable CAD. CETP showed a negative correlation with CRP and IL-6 and a positive

correlation with homocysteine and adiponectin. Low CETP is associated with increased hazard ratios for death after multivariate adjustment (49). During experimental endotoxemia, decreased activity of CETP and LCAT were found, in contrast PLTP activity was increased in human subjects (50).

The common allele of TaqIB, i.e. TaqIB1, increased CETP activity and decreased HDL levels, was associated with insulin resistance and metabolic syndrome (51). Hyperalphalipoproteinemic subjects tend to have decreased CRP levels (52). However, there is no evidence that CETP deficiency had low CRP levels.

Drug designs in clinical trials (Figure 3)

1) Torcetrapib (Pfizer)

Torcetrapib is a compound of tetrahydroquinoline that binds to CETP, forming CETP-HDL complex in plasma. It is a noncompetitive inhibitor that could bind to CETP reversibly. The IC_{50} values for CETP activity are 17-79 nM. The binding site on CETP for torcetrapib is in the lipid-binding pocket near N-terminal CETP (53). Favorable lipoprotein profile of increased HDL2 and large LDL are shown with increasing HDL (+106%) and decreasing LDL (−17%) with 240mg torcetrapib in humans. In December 2006, the phase III trial of the ILLUMINATE Study, a combination study with atorvastatin, was terminated because of excess death (hazard ratio 1.58) and major cardiovascular events (hazard ratio 1.25) in the combination arm (54).

The large part of cause of death appeared to be related to hypertension-related vascular events. Later, torcetrapib is found to be associated with high aldosterone levels, which are associated with increased aldosterone synthase (CYP11B2) (55) and endothelial dysfunction (56). Blood pressure was increased in spontaneously hypertensive rats treated with torcetrapib, but not Wistar-Kyoto rats (57). Torcetrapib induced a sustained impairment of endothelial function, decreased eNOS mRNA, protein as well as NO release, stimulates vascular ROS and endothelin-1 production in addition to aldosterone. Since rat and mice are deficient in CETP activity, these hormonal changes related in artery tonus are independent of CETP activity.

Furthermore, imaging trials of coronary and carotid arteries were negative, but a post hoc analysis of the IVUS study of the ILLUSTRATE Study indicated that the only highest HDL group (HDL-C > 87 mg/dL) showed the regression of coronary atherosclerosis (58). Beneficial effects of increased HDL2 are associated with higher cholesterol efflux via SR-BI or ABCG1

pathways (59). Moreover, torcetrapib attenuates the atherogenicity of postprandial TG-rich lipoproteins in type IIB hyperlipidemia (60).

Despite the failure in the clinical trials, torcetrapib is considered to induce RCT efficacy. Increased RCT from peripheral macrophages to feces is considered to be anti-atherogenic. A selective uptake of CE from HDL in the liver was increased by 1.7-fold in the treatment of torcetrapib in CETP-transgenic mice (59). In hamsters, a naturally CETP-expressed species, it was shown that increased cholesterol excretion in the feces was found during CETP inhibition by torcetrapib alone (61).

2) Dalcetrapib (RO4607381, Roche; JTT-705, JT)

This compound, formerly named JTT-705, is structurally different from fluorine-containing structures of torcetrapib, anacetrapib and evacetrapib, because it has an ortho-thio-anilide core and it requires Cys-13 of CETP molecule to form a covalent disulfide bond, thereby dalcetrapib irreversible binds to CETP (62). The SH group of Cys-13 resides at the bottom of the lipid-binding pocket of CETP (63). Inhibiting CETP activity is relatively mild (IC_{50} 0.4-10 μM), accordingly it would increase HDL-C levels modestly.

Indeed, dalcetrapib is not associated with increased aldosterone and high blood pressure (64). Moreover, no clinically relevant changes in lymph nodes, or other safety parameters were found in phase I and phase II trials (65). Clinical outcome study is expected in dal-OUTCOMES using 600 mg dalcetrapib in patients (N=15,600), which has been initiated in 2008. Thus, one might expect that dalcetrapib is more effective because it is a weak CETP inhibitor maintaining inter-conversions of HDL subclasses. Moreover, dal-VESSEL is focused on modulation of vascular function such as endothelial function by CETP inhibition. The dal-PLAQUE has been initiated to assess the impact of dalcetrapib on atherosclerotic plaque development using PET-CT and MRI (66).

However, unfortunately, in May 2012, the dal-OUTCOMES Phase III trials were terminated because of a lack of clinically meaningful efficacy, which is recommended by the independent Data and Safety Monitoring Board. All the studies in the dal-HEART program decided to be terminated. Although it is unclear why benefit by increasing HDL levels are not seen in that study, it may be explained by potential adverse effects as follows: the median CRP levels was 0.2 mg/L higher and mean systolic blood pressure was 0.6 mmHg higher with dalcetrapib as compared with a placebo in patients with a recent acute coronary syndrome (67). It is reasonable to state that dalcetrapib is a weak inhibitor without an effect on reducing LDL-C and TG which may be one of reasons for the failure of the dal-OUTCOMES.

3) Anacetrapib (MK-0859, Merck)

Anacetrapib has a triad of trifluoro-methyl-benzene derivative like torcetrapib but it has a distinct biaryl moiety. Although this compound effectively elevates HDL-C along with lowering LDL-C as well as torcetrapib, anacetrapib is not associated with increased aldosterone and high blood pressure (68). It is a noncompetitive inhibitor binding to CETP reversibly. The IC_{50} values for CETP activity are 10-17 nM (69).

The Determining the Efficacy and Tolerability of CETP Inhibition with anacetrapib (DEFINE) Study was reported in 2010 (70). Patients with CAD or at high risks who were taking a statin are included in a randomized, double-blinded, placebo-controlled trial to receive 100 mg of anacetrapib or placebo. LDL-C was decreased from 81 mg/dL to 45 mg/dL (−40%) and HDL-C was increased from 41 mg/dL to 101 mg/dL (+138%) as compared with a placebo with acceptable side effects. In addition to LDL-C lowering effect, anacetrapib decreased plasma Lp(a) levels by −50% (71).

In a detailed analysis of lipoprotein subfraction by density gradient ultracentrifugation in healthy individuals treated with anacetrapib 20 mg,

medium and small LDL levels were decreased whereas very small and dense LDL levels were increased, which is compatible with LDL subclasses found in severe CETP deficiency, but not in partial deficiency (72).

Large reduction in LDL-C needs to be confirmed by another method than the Friedewald formula (calculated LDL-C = TC – HDL-C – TG/5), since the method would underestimate LDL-C because VLDL-C, which is estimated as TG/5, is lower in CETP deficiency and patients treated with anacetrapib than controls (1,73). However, the direct HDL-C method would underestimate HDL-C levels in plasmas with apoE-rich HDL found in CETP deficiency, therefore the direct HDL-C method would overestimate calculated LDL-C through the formula (1).

As anti-atherogenicity of HDL, HDL after treatment with niacin or anacetrapib exhibits potent ability to suppress macrophage toll-like receptor 4-mediated inflammatory responses. The increased HDL fraction is rich in apoE and LCAT, but not in PAF-AH activity (74).

In May 2011, the REVEAL trial (Randomized Evaluation of the Effects of Anacetrapib Through Lipid Modification) was started with a daily dose of 100 mg anacetrapib in patients with CAD with statin therapy (75). This study will recruit 30,000 CAD patients with >=50 years of age. Their LDL-C levels will be controlled with atorvastation with total cholesterol < 155 mg/dL, then patients will be randomized to have anacetrapib or not. It is expected that the REVEAL trial will provide valuable results by January 2017.

4) Evacetrapib (LY2484595, Eli Lilly)

A novel benzazepine compound is a potent, selective CETP inhibitor (76). It contains a quinoline core like torcetrapib and the 3,5-bis-trifluoromethylbenzyl group but also a methyl tetrazole and cyclohexane carboxylic acid side chain. Evacetrapib inhibited human recombinant CETP (5.5 nM IC50) and CETP activity in human plasma (36 nM IC50) as well as torcetrapib and anacetrapib. Importantly, evacetrapib

did not induce aldosterone and cortisol biosynthesis in a human adrenal cortical carcinoma cell line.

Evacetrapib was evaluated in patients with high LDL levels as monotherapy or in combination with statins (77). Evacetrapib 100mg/d increased HDL-C (+54~ +129%) and decreased LDL-C (-14~-36%) in the monotherapy through decreasing CETP activity (-50 ~ -89%) but increasing CETP mass (+64 ~ +137%).

Structural difference of CETP inhibitors in the cavity of CETP

All compounds appeared to be related to increased plasma CETP mass up to 3-fold increase, which is contrast with antisense therapy. The reason for the increase in mass is not fully understood, it may be related to decreased clearance of CETP in plasma. The CETP-CETP inhibitor complex is increased with HDL as seen in the electromicroscope.

The CETP inhibitors are buried deeply within the CETP protein, shifting the bound CE in the N-terminal pocket of the long hydrophobic tunnel and displacing the PL from the pocket. The lipids in the C-terminal pocket of the hydrophobic tunnel remain unchanged. Polar residues of Gln-199, Ser-230 and His-232 are found in the inhibitor-binding site. For example, torcetrapib occupies a volume of ~12 Å x 12 Å x 7 Å within the N-terminal pocket of the CETP tunnel (78).Thus, torcetrapib binding physically interferes with PL binding and forces CE into a position that is presumably unfavorable for lipid transfer by blocking the narrow passage. The trifluoromethyl group of the torcetrapib projects deeply into the N-terminal pocket; sub-pocket formed by Ile-11, Cys-13, Ile-215 and the aromatic faces of His-232 and Phe-263. The binding site of dalcetrapib, Cys-13 is located in between the side chains of His-232 and Phe-263 in the model by Liu et al. Thus, dalcetrapib binding to the CETP is time-dependent in the disulfide bond formation to Cys-13. However, other compounds with trifluoromethyl group are competitive in CETP binding.

Differential effects among CETP inhibitors

1) Differences of levels of preβ1-HDL and HDL2, and cholesterol efflux capacity

Like PLTP, CETP itself is a conversion factor of HDL subclasses. CETP increased size of HDL from HDL3 to HDL2 with giving formation of smaller HDL particles ~ 8 nm. In vitro levels of preβ1-HDL levels are varied after incubation with dalcetrapib or torcetrapib/ anacetrapib. Torcetrapib/ anacetrapib decreased preβ1-HDL levels in the concentration-dependent manner, but dalcetrapib did not decrease them (79). A similar finding is found when neutralizing antibody TP1 was incubated in human plasma (80), complete inhibition of CETP activity would retard preβ1-HDL formation. However, ex vivo analysis of plasmas of CETP deficient human resulted in opposite data (Table 1), partial inhibition would result in low levels of preβ1-HDL, but complete inhibition would increase in the preβ1-HDL levels.

Torcetrapib increased plasma larger HDL2 particles, which are increased post-prandially up to 8 hours and act as active cholesterol acceptor via SR-BI and ABCG1-dependent cholesterol efflux pathway (81).

Cholesterol efflux was also increased to HDL from anacetrapib-treated hamsters via both ABCA1 and ABCG1/ SR-BI pathway. Indeed, anacetrapib induced HDL-C levels rich in cholesteryl linoleate (18:2), which is compatible with findings in CETP deficiency (82).

Khera et al reported that cholesterol efflux capacity was negatively associated with CAD risk independently of HDL-C levels (83). The capacity was determined ex vivo that radiolabeled J774 macrophage cells were incubated with apoB-depleted serum from patients for 4 hours, reflecting HDL capacity for cholesterol efflux activity mediated by ABCA1, ABCG1, and SR-BI pathways as well as aqueous diffusion. Thus, among several HDL functions, acceptor capacity for cholesterol efflux was likely enhanced in patients with CETP inhibitors.

2) Differences of in vivo macrophage-derived reverse cholesterol transport (RCT) among CETP inhibitors

In a method administrating a radioactive cholesterol-labeled macrophage in the peritoneum, Tanigawa et al have measured direct RCT activity from peripheral macrophages to liver, bile and feces (84). In LDL receptor-KO mice, CETP cDNA adeno-associated virus mediated transfection promotes cholesterol to the liver, but not to bile and feces. In contrast, in SR-BI-KO mice, CETP cDNA transfection increased cholesterol loss in the feces, indicating that overall RCT induced by CETP is not via SR-BI, but through LDL receptor in the liver in mice.

In 0.3% cholesterol-diet induced combined hyperlipidemia of hamsters; an increase of aortic cholesterol content is correlated with higher cholesterol/TG ratio in the liver as well as increased plasma levels of non-HDL-cholesterol (3.8 fold) and increased CETP activity (+40%). In the gene expression during cholesterol-fed hamster, mRNA levels of ABCA1 and ABCG5 increased, but those levels of LDL receptor and SR-BI decreased in the liver. In vivo, cholesterol efflux activity from macrophages to plasma and to bile/feces was decreased despite increased HDL-C levels (+90%) in hamsters (85), suggesting that HDL levels do not directly reflect efficacy of macrophage-derived RCT.

Using a hamster macrophage, RCT of radiolabeled cholesterol from the macrophages is maintained in the experiments with dalcetrapib, but it is diminished in studies with torcetrapib and anacetrapib (79). The apparent difference on the induced cholesterol efflux activity appeared to be correlated with levels of preβ1-HDL; namely, dalcetrapib would maintain the levels, but strong inhibitors such as torcetrapib and anacetrapib decreased them. Thus, dalcetrapib may have unique lipoprotein profile such as preserved levels of preβ1-HDL, but it would be interesting to know whether or not it is due to a weaker inhibitor or a compound-specific effect.

Torcetrapib increased HDL-C, accelerating a secretion of cholesterol and bile acids in feces in hamsters but not in humans (86). Several regulations in lipid homeostasis in hamsters are different from those in human. In the liver of hamsters, dietary cholesterol-fed increased hepatic expression levels of ABCG5/G8 and PCSK9, but decreased CYP7A, with increasing bile cholesterol secretion. Therefore, decreasing both expression of LDLR and bile acid formation deteriorated magnitude of dyslipidemia in hamsters (87,88). Although dyslipidemic hamsters that are statin-resistant, the LDL-lowering drug berberine upregulates RCT with torcetrapib (89).

Effect of anacetrapib on macrophage-to-feces RCT in hamster models is conflicting (90). Although anacetrapib failed to show induced RCT in normolipidemic hamster in a previous study (79), a recent study showed that dyslipidemic hamster resulted in improved RCT under the condition of strongly inhibited CETP activity by −94% (90).

3) Effects on paraoxonase, PAF-AH (Lp-PLA2), and anti-inflammatory activity (Table 2)

Serum CRP reduction was not reported in any compound, although anti-oxidative enzymes were substantially changed. In an ex-vivo study, anti-inflammatory properties of HDL were maintained in hamsters treated by anacetrapib as in controls (91).

4) Vascular effects

Flow-mediated dilation (FMD) of the brachial artery was increased by 41% in patients with low HDL-C (< 46 mg/dL) treated with dalcetrapib 600 mg, but that effect was not seen in patients with higher HDL-C in the baseline (94).

However, in the dal-VESSEL randomized clinical trial, FMD was not changed during the treatment with dalcetrapib 600 mg (93).

Perspective of CETP inhibitor

1) Glucose tolerance, diabetes incidence during CETPi

High HDL syndrome is often associated with low prevalence of diabetes mellitus (37). In vitro, studies suggested that HDL may offer an anti-diabetic effect by an increased pancreas beta-cell insulin secretion through mediated by ABCA1 and ABCG1 transporters (95). Moreover, HDL may activate AMP-activated protein kinase in skeletal muscle (96), thereby accelerating glucose uptake.

Plasma CETP activity is increased in obesity or metabolic syndrome, but it is decreased in type2 diabetes (97). This may be related to out-of-regulation of SREBP1 and 2 in skeletal muscles and adipose tissues of type2 diabetes (98). However, CETP gene TaqI B2 allele is protective in diabetes, suggesting genetically low CETP activity is beneficial in macroangiopathy development of coronary disease, arteriosclerosis obliterans and cerebral vascular disease (99).

Thus, it would be interesting to know whether or not impact on cardiovascular events by torcetrapib are stronger in diabetic patients involved in the ILLUMINATE trial. Conversely, Barter et al recently reported in the analysis of the ILLUMINATE trial that torcetrapib decreased HOMA-IR in the torcetrapib/atorvastatin arm as compared to the atorvastatin arm, which is associated with increased insulin sensitivity (100). Similarly, torcetrapib induced decrease in HOMA-IR in obese insulin-resistant CETP-apoB100 transgenic mice (101).

2) CETPi in relation to combination therapy with HMG-CoA reductase inhibitor (statin) or other drugs

Statin per se would decrease plasma CETP levels modestly (102), but on-stain CETP is inversely related to coronary outcomes in a large clinical trial based cohort (103). However, Barter et al negated an idea of adverse interaction

between atorvastatin and torcetrapib based on findings of the ILLUMINATE trial. Indeed, higher doses of atorvastatin appeared to protect against the harm effect caused by torcetrapib (104). A recent study suggested that low CETP phenotype linked with genotype of TaqI B2 may predict increased mortality in statin-treated men in contrast with the fact that the genotype is associated with lower coronary risk in a meta-analysis (34,46). Thus, role of low CETP activity is conflicting in the statin-treated population. It should be investigated in a prospective manner.

Plasma CET is not only associated with CETP activity, but also other modulators: VLDL mass and FFA contents of lipoproteins. Thus, either a fibrate or a PAF-AH inhibitor may be good candidates for combination therapy with the CETP inhibitor since fibrate will decrease VLDL levels and PAF-AH inhibitors decrease CET by decreasing LDL-FFA levels.

3) CETPi in relation to apoE-rich HDL levels

Reverse cholesterol transport is enhanced by increase in apoE-rich HDL levels. Xanthohumol, a prenylated chalcone derived from natural products, is a CETP inhibitor. The compound was shown to prevent atherosclerosis in CETP-transgenic mice. Importantly, other factors such as LCAT, apoE, SR-BI and LDLR, which are upregulated in the liver, accelerate RCT along with increased apoE-rich HDL levels (105).

4) Infectious disease risk in CETPi

Torcetrapib-related excess death appeared to be related to non-cardiovascular events such as malignancy and/or infection. Low CETP activity may be associated with high mortality as suggested by a recent prospective study in hospitalized patients (106). In that study, each 1 mg/dL increase in HDL decreased the odds of severe sepsis by 3% during hospitalization, suggesting a role of HDL as LPS scavenger. Similarly, recombinant HDL decreased

LPS-induced inflammatory response in patients with liver cirrhosis (107). Thus, increased HDL would protect from infection.

However, the reduction of plasma CETP was associated with mortality in hospitalized patients (106). It may reflect severe infection reducing CETP expression in hematopoietic cells. Furthermore, in vitro studies it is unlikely that torcetrapib has a direct effect on LBP and bactericidal/permeability increasing protein (BPI) function, nor an inhibitory effect on the interaction with LPS (108).

5) Potential of CETPi against C-termial polypeptide

Vaccine-induced antibodies were tested earlier in rabbits (109). The epitope was consisted of C-terminal CETP (461-476) and the peptide of Tetanus toxin, therefore CETP inhibition was expected in the ternary complex of HDL-CETP-VLDL or HDL-CETP-LDL. The approach results in decrease in CETP activity by -24%, increasing HDL-C levels by +42% with reduced aortic atherosclerosis in cholesterol-fed rabbits. The approach was tested in human clinical trials, but the phase II failed to meet the primary endpoint of increasing HDL-C levels (110). Thus, low concentrations of anti-human CETP antibody need an efficient adjuvant formulation. This approach would be interesting because the antibody inhibits CETP activity through C-terminal CETP, which is involved in the interaction with lipid transfer acceptors such as VLDL or LDL, but not in interconversion among HDL subclasses.

Conclusion

Anti-atherogenicity of low CETP activity appears to be dependent on the cause, whether it is genetic or environmental. Also, it is unclear how much lower CETP activity would be beneficial in human atherosclerosis. Since CETP inhibitors such as anacetrapib and evacetrapib have been tested in the phase III trials, it is expected that those trials will provide results on vascular endpoints by 2017. As structure and function relationship between the hydrophobic tunnel of CETP and CE/TG and PL has been disclosed, different inhibitors targeting the other domains are promising. Also, CETP antibody therapy awaited further investigation.

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Table 1. Summary of anti- and pro-atherogenic aspects in CETP deficiency.

Parameter	Homozygote	Heterozygote	Author, Year (Ref)
HDL-C (mg/dL)	164	66	Inazu, 1990(26)
ApoE-rich HDL1	Very high	High	Koizumi, 1985(27)
			Inazu, 2008(17)
Preβ1-HDL	Increased	Decreased	Asztalos, 2004(7)
Cholesterol esterification rate	Very low	Low	Oliveira, 1997(8)
ABCG1/SR-BI-mediated chol efflux			
	Very high	High	Matsuura, 2006(28)
			Miwa, 2009(29)
LDL-C (mg/dL)	77	111	Inazu, 1990(26)
LDL size	Polydispersed	Large	Yamashita, 1988(14)
			Brown, 1989(15)
			Wang, 2002(30)
Lp(a)	Decreased	Not reported	unpublished*

* Inazu et al (1993)

Table 2. Changes of PON1 and PAF-AH activity in CETP inhibitors

Drug	Subjects	PON1	PAF-AH	Author, Year (Reference)
Dalcetrapib	Low HDL	increased (+41%)	nd	Bisoendial, 2005(92)
Dalcetrapib	CHD	nd	increased (+17%)	Lüsher, 2012(93)
Anacetrapib	Dyslipidemia	nd	no change	Yvan-Charvet, 2010(74)

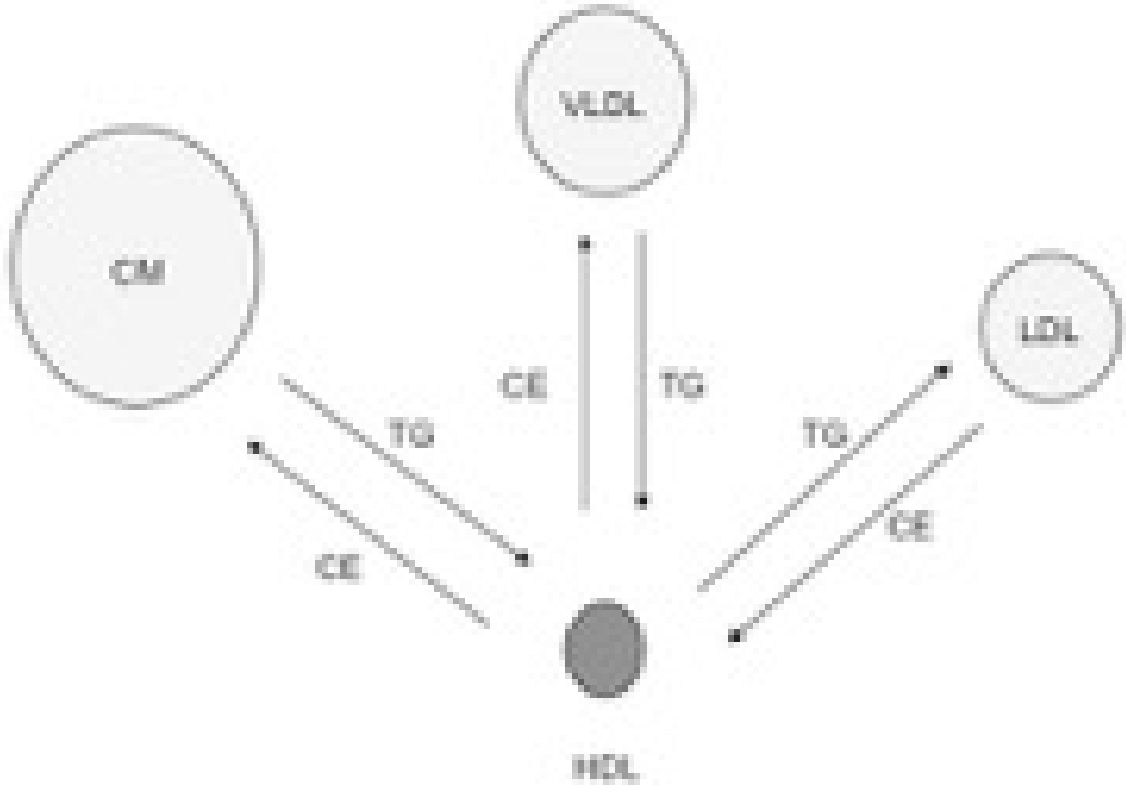
Nd, not determined

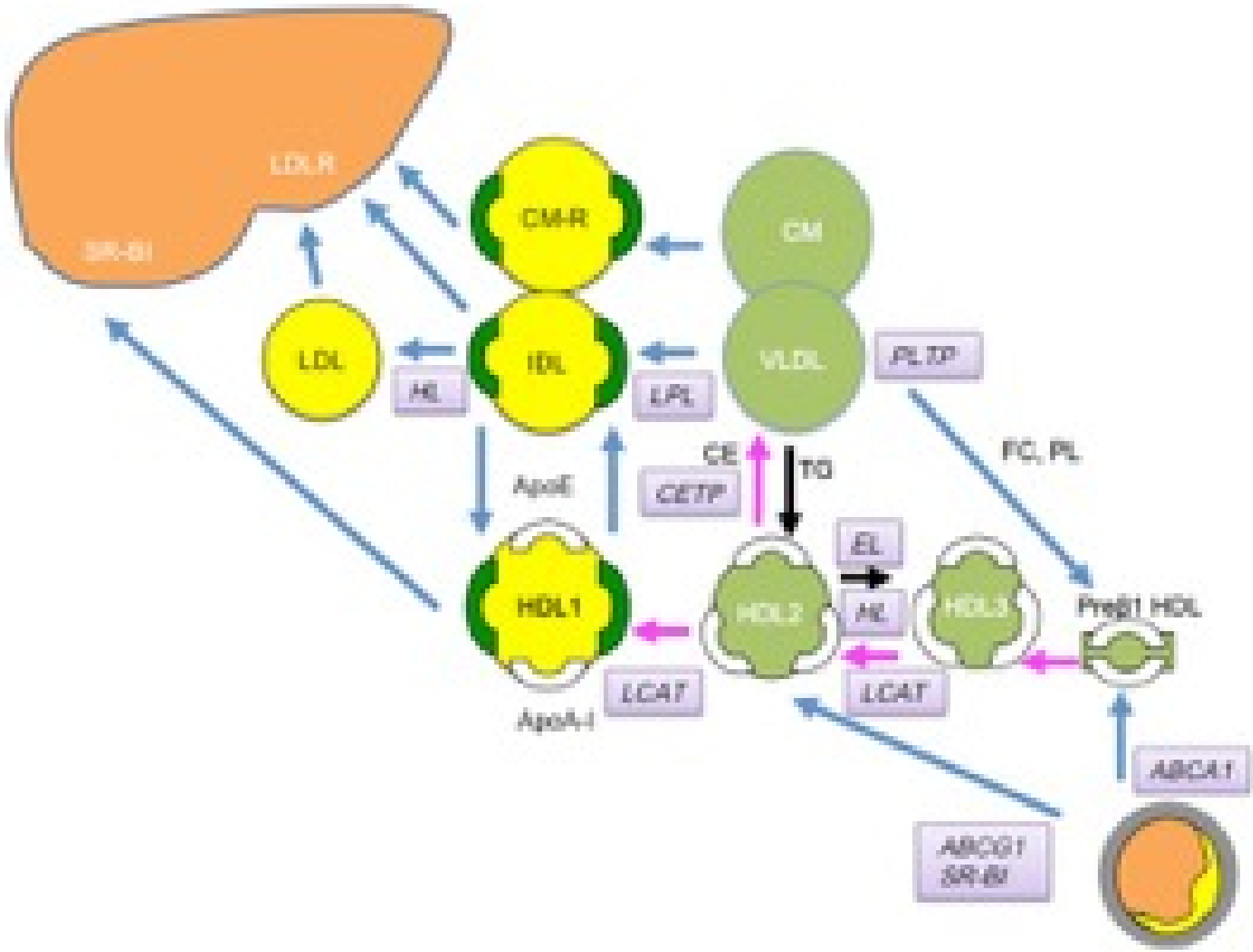
Figure legends

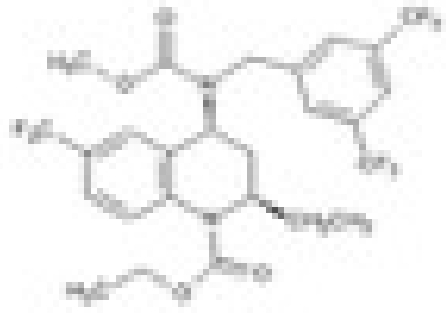
1. HDL as a playmaker for the neutral lipid transfer.
   HDL-TG is a source of TG in LDL by CETP-mediated lipid transfer.

2. HDL as a cholesterol vehicle from atherosclerotic lesions to the liver.
   There are two pathways; one is a direct pathway mediated by apoE-rich HDL and SR-BI receptor in the liver, and the other is an indirect pathway mediated by IDL-LDL or chylomicron remnants and LDLR in the liver.

3. CETP inhibitors

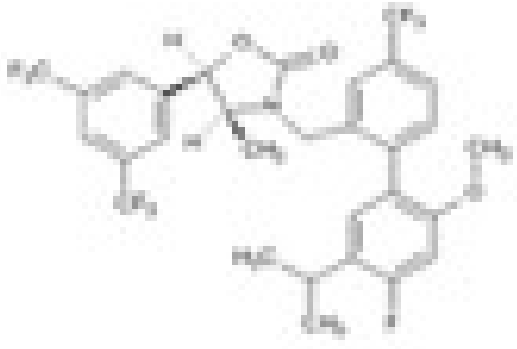

VLDL
CM
LDL
CE
TG
TG
TG
CE
CE
HDL


LDLR
SR-BI
CM-R
CM
LDL
IDL
VLDL
HL
LPL
PLTP
FC, PL
CE
TG
CETP
ApoE
EL
HDL1
HDL2
HDL3
Preβ1 HDL
HL
LCAT
LCAT
ApoA-I
ABCA1
ABCG1
SR-BI



torcetrapib

dalcetrapib



anacetrapib

evacetrapib