Abstract Most of the cholesterol in plasma is in an esterified form that is generated in potentially cardioprotective HDLs. Cholesteryl ester transfer protein (CETP) mediates bidirectional transfers of cholesteryl esters (CEs) and triglycerides (TGs) between plasma lipoproteins. Because CE originates in HDLs and TG enters the plasma as a component of VLDLs, activity of CETP results in a net mass transfer of CE from HDLs to VLDLs and LDLs (Fig. 1). Activity of CETP also results in a net mass transfer of TG from VLDLs to LDLs and HDLs (Fig. 1).

Inhibition of CETP activity reduces these lipid transfers and thus increases the concentration of HDL CE and decreases the concentration of CE in VLDLs and LDLs. The concentration of HDL-cholesterol (HDL-C) is a negative risk factor for atherosclerotic CVD (ASCVD), while the concentration of cholesterol in the non-HDL fractions is a positive risk factor. As humans that are CETP deficient have high plasma HDL-C levels and decreased non-HDL-C levels and are reported to be at decreased risk of developing ASCVD, it follows that inhibiting the activity of CETP may translate into a reduction in cardiovascular risk. Several approaches that inhibit CETP activity and increase plasma HDL-C levels have been proposed and tested. However, as inhibition of CETP activity also decreases apoB and non-HDL-C levels, any reduction in ASCVD risk that is mediated by these agents cannot be attributed to an increase in HDL-C levels alone.

Approaches for inhibiting CETP include anti-CETP neutralizing antibodies (4–8), antisense CETP oligonucleotides (9), and an anti-CETP vaccine (10, 11). The anti-CETP vaccine reduced atherosclerosis in the New Zealand White rabbits (10) and was effective in a phase I clinical trial in humans (11), but has not proceeded to clinical development. The neutralizing antibodies and the antisense oligonucleotide also did not proceed to clinical development.

Several small molecule CETP inhibitors that reduce atherosclerosis in animal models extremely effectively have also been developed (12, 13). Four of these inhibitors (torcetrapib, dalcetrapib, evacetrapib, and anacetrapib) activity of CETP results in a net mass transfer of CE from HDLs to VLDLs and LDLs (Fig. 1). Activity of CETP also results in a net mass transfer of TG from VLDLs to LDLs and HDLs (Fig. 1).

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have been evaluated in large-scale randomized cardiovascular clinical outcome trials. While the trials with torcetrapib, dalcetrapib, and evacetrapib failed to show any cardiovascular benefit of CETP inhibition, treatment with anacetrapib significantly decreased major coronary events (14). However, as the manufacturers of anacetrapib recently decided to suspend development of the drug, the future of CETP inhibition as a potential therapeutic option for reducing major cardiovascular events is currently uncertain.

This review is concerned with the structure, function, and regulation of CETP and its inhibitors. It also outlines functions of CETP that are distinct from its lipid transfer activities, summarizes preclinical studies of CETP inhibition in animal models, and presents details of the outcomes of the randomized clinical outcome trials of the aforementioned small molecule CETP inhibitors.

**STRUCTURE OF CETP**

The structure of CETP has been the focus of numerous investigations. The LTP/LBP gene family, of which CETP is a member, includes several proteins, such as LBP, bactericidal permeability-increasing protein (BPI), and phospholipid transfer protein (PLTP), all of which have a high degree of structural similarity (15). Early structural models of CETP that were based on the crystal structure of BPI (2) identified CETP as a boomerang-shaped molecule with a hydrophobic lipid binding pocket at each end of the concave side (16, 17). CETP is a highly flexible molecule that undergoes a twisting motion when it binds to neutral lipids. This rotating motion enables CETP to bind to the surface of lipoproteins that vary widely in size and surface curvature and is a very important aspect of its mechanism of action.

The assumption of a “boomerang” structure for CETP was confirmed by Qiu et al. (18) who reported the first crystal structure of CETP at 3.5 Å resolution. That study identified the presence of a continuous central tunnel within the CETP molecule, which is unique among members of the LTP/LBP gene family. Two lipid binding pockets in the N- and C-terminal domains, and an amphipathic helix, helix X, located in the C-terminal domain of CETP have also been reported (18, 19). The central CETP tunnel can accommodate two CE molecules, one CE and one TG molecule, or two TG molecules (18). These structural features of CETP have been confirmed in atomistic and coarse-grained simulation studies (20), and by cryo-electron microscopy (21). Evidence that structural integrity of the central CETP tunnel is essential for the transfer activity of CETP was established by mutating selected polar amino acid residues that are located in the tunnel into hydrophobic residues. This altered the tunnel architecture and reduced the transfer activity of CETP (18).

**MECHANISM OF ACTION OF CETP**

CETP transfers CE and TG between different lipoproteins by two mechanisms (Fig. 2). The first mechanism is a “shuttle” process (Fig. 2A) that involves random collisions of CETP with HDLs, LDLs, and VLDLs. This leads to the formation of complexes that facilitate bidirectional exchanges of CE and TG between each of the lipoproteins and CETP. The complexes subsequently dissociate from the lipoproteins where they were generated, and remain in the circulation until they randomly collide with another lipoprotein and participate in a further round of CE and TG exchanges. This process is repeated multiple times (3, 4). The crystal structure of CETP supports the shuttle mechanism and is consistent with the interaction of CETP with only one lipoprotein particle at a time (18).

The second mechanism of action of CETP involves the formation of a bridge between CETP and two lipoprotein particles to form a ternary complex (Fig. 2B) (21, 22). Neutral lipids move in both directions between the two lipoproteins through the tunnel in CETP. Evidence consistent with ternary complex formation comes from cryo-electron microscopy studies with anti-CETP polyclonal antibodies and atomistic molecular dynamics simulations (21). The
results of these studies support the penetration of the N-terminal domain of CETP into the surface of an HDL particle together with a concomitant interaction of the C-terminal domain of CETP with an LDL or VLDL particle. Additional analyses have indicated that the transfer of CEs between HDLs and LDLs, or HDLs and VLDLs, by this mechanism is dependent on conformational changes in the N- and the C-terminal domains of CETP that increase tunnel continuity and improve neutral lipid accessibility (19, 21).

However, it should be noted that these observations are not supported by other electron microscopy studies in which HDLs were shown to bind to the N- as well as the C-terminal domain of CETP (23). It should be noted, however, that no interactions of CETP with LDLs, or formation of HDL-CETP-LDL complexes, were observed in that study, and that monoclonal antibodies targeted toward the N- and C-terminal domains of CETP did not prevent the penetration of CETP into the HDL surface or affect CETP activity (23). When taken together, these findings do not support the formation of a ternary complex as a major mechanism of action of CETP. There are, by contrast, multiple reports of anti-CETP antibodies inhibiting CETP-mediated transfers of CE and TG between HDLs and other lipoproteins (5, 24). These discrepant findings highlight a potential dependency of CETP-mediated neutral lipid transfers on the antibodies that are used to target the N- and C-terminal domains of the CETP molecule. For example, Zhang et al. (21) used polyclonal antibodies that recognized a large area of the CETP molecule, whereas the more recent studies of Lauer et al. (23) were undertaken with monoclonal antibodies that recognize specific epitopes within the protein.

REGULATION OF CETP

CETP gene transcription

Transcription of the *CETP* gene is under the control of extrinsic and intrinsic factors. For example, dietary cholesterol upregulates CETP expression in mice transgenic for human CETP (25–27). Plasma cholesterol levels also correlate with CETP mass in human plasma (28). Studies of transgenic mice have established that induction of human *CETP* gene expression in response to cholesterol is a consequence of transactivation of a nuclear receptor binding site in the promoter region of the gene by the transcription factors, liver X receptor (LXR) and retinoid X receptor (29, 30). These results are supported by studies of LXR agonists that increase CETP expression in mice transgenic for human CETP, and in mice with LXRα deficiency in which CETP expression is not increased by administration of an LXR agonist (31). The human *CETP* gene is also regulated by SREBP-1, a transcription factor that transactivates sterol regulatory-like elements in the promoter region of the gene (32).

Lifestyle factors

Light to moderate, but not heavy, alcohol consumption is generally considered to decrease CETP mass and activity, increase HDL-C levels, and decrease CVD risk. However, investigations into this relationship have produced conflicting results. Some investigators have confirmed the association (33), while others have found that the alcohol-mediated increase in HDL-C levels is independent of CETP activity (34, 35) and unrelated to effects on genes that regulate HDL levels (36).

Physical activity in the form of endurance exercise also increases HDL-C levels, decreases plasma CETP levels, and reduces CVD risk in humans (37). However, aerobic exercise has been reported not to affect CETP activity in mice transgenic for the human *CETP* gene (38) or plasma CETP levels in humans (39, 40).

HUMAN GENETIC STUDIES

Loss-of-function mutations in the CETP gene (CETP deficiency)

The first report of a loss-of-function mutation in the *CETP* gene was in a Japanese population with a G-to-A substitution in the 5′-splice donor site of intron 14 (Int 14A) (41). Homozygosity for this mutation is associated with very low or undetectable CETP activity, markedly elevated plasma HDL-C, apoA-I, and apoE levels, a moderate reduction in VLDL-cholesterol, LDL-cholesterol (LDL-C), and apoB levels, a low incidence of atherosclerosis, and increased life span compared with unaffected family members (41, 42). HDLs isolated from people homozygous for this mutation, as well as compound heterozygotes, also have HDLs that are larger than the HDLs in unaffected individuals (41, 43). In addition, people with CETP deficiency
have LDLs that are small and polydisperse relative to people with a normal level of CETP activity (44).

Several other mutations associated with CETP deficiency have been reported (45–47). A missense mutation of Asp to Gly at codon 442 in exon 15 of the CETP gene (Asp442Gly) that is associated with abnormally high levels of HDL-C has been reported in the Japanese population and in Japanese Americans (48, 49). People homozygous for a nonsense mutation in the CETP gene at codon 309 in exon 10 and a G-to-T substitution at codon 181 of exon 6 (G181X) have elevated plasma concentrations of HDL-C and apoA-I (45, 46). A nonsense T-to-G mutation at codon 57 of exon 2 that is associated with high HDL-C levels has also been reported (47).

**Human CETP gene polymorphisms**

Results from small studies of CETP gene polymorphisms in humans have not been conclusive. The results of larger genetic studies are, however, more consistent and have led to the conclusion that CETP is pro-atherogenic and that its inhibition is potentially anti-atherogenic.

In a large meta-analysis of 92 studies involving 113,833 participants, it was concluded that CETP gene polymorphisms that are associated with decreased CETP activity and mass are associated with high HDL-C levels, low LDL-C levels, and a significantly decreased risk of having a coronary event (50). A similar conclusion emerged from a study of 18,245 healthy Americans in the Women’s Genome Health Study, where 20 SNPs in the CETP gene that had genome-wide effects on HDL-C levels were identified (51). In particular, the Taq1B polymorphism at rs708272 in the CETP gene was associated with a per-allele increase in HDL-C levels of 3.1 mg/dl and a 24% lower risk of future myocardial infarction (51). This conclusion was further supported by another meta-analysis in which a common variant in the CETP gene was accompanied by increased HDL-C levels, decreased LDL-C levels, and a reduced risk of myocardial infarction comparable to that reported in the earlier meta-analysis (52).

Perhaps the most compelling genetic evidence in favor of CETP activity being pro-atherogenic comes from the Copenhagen City Heart Study (53) and from a study that examined the effect of protein-truncating variants of the CETP gene (54). In the Copenhagen City Heart Study, 10,261 people were followed for up to 34 years (53). More than 3,000 of these people had a cardiovascular event and 3,807 died. In this study, two common CETP gene polymorphisms known to be associated with low CETP activity were also associated with significant reductions in the risk of ischemic heart disease, myocardial infarction, ischemic cerebrovascular disease, and ischemic stroke. People with these polymorphisms also had increased longevity, with no evidence of adverse effects.

In a study of protein-truncating variants in the CETP gene, it was found that the HDL-C level was 22.6 mg/dl higher and the LDL-C level was 12.2 mg/dl lower than in those without the variants (54). These lipoprotein changes were accompanied by a significant 30% lower risk of having a coronary event (54). Collectively, these human genetic studies support the proposition that CETP inhibition is potentially anti-atherogenic.

### CETP AND ANIMAL MODELS OF ATHEROSCLEROSIS

CETP exists in the plasma of only few species, including humans, rabbits, and hamsters, but not in rodents, which have a low susceptibility to atherosclerotic lesion development (1). As mice are naturally deficient in CETP, rendering them transgenic with the human CETP gene means that studies can be undertaken in the absence of the confounding effects of endogenous CETP activity. However, the results from mice transgenic for CETP are model-dependent, with some studies suggesting that CETP is pro-atherogenic (55–57). Other studies in mice transgenic for human CETP and LCAT, by contrast, have indicated that CETP is anti-atherogenic (58). An anti-atherogenic role for CETP has also been suggested for the db/db mouse model of type 2 diabetes when it is made transgenic for human CETP (59), and for hypertriglyceridemic CETP transgenic mice (60). In contrast to mice, rabbits have approximately twice as much CETP activity in their plasma as humans (1) and they are very susceptible to diet-induced atherosclerosis (61), which is reduced by inhibiting CETP (12).

### NON-LIPID TRANSPORT FUNCTIONS OF CETP

#### CETP and innate immunity

From the available evidence, CETP appears to have a beneficial role in reducing the inflammatory response to bacterial endotoxins through interaction with the innate immune system and the sequestration of pro-inflammatory lipopolysaccharide (LPS). The innate immune system detects highly conserved components of micro-organisms, termed pathogen-associated molecular patterns (PAMPs), by pattern recognition receptors (PRRs), including members of the toll-like receptor (TLR) family. Detection of PAMPs by PRRs is the first line of defense against a nonself pathogen, which leads to activation of the innate immune system and a cascade of inflammatory responses (62, 63). If not effectively regulated, the inflammatory responses that are activated when PRRs detect PAMPs can result in sepsis with shock, end-organ damage, and, ultimately, death of the host. Beyond antimicrobial therapy, treatments for septic shock are limited (64).

LPS is a component of the gram-negative bacteria cell wall and the ligand for TLR4 (63, 65). It potently stimulates the innate immune system and is largely responsible for the septic response to gram-negative bacteremia (66). Effective sequestration and excretion of LPS is required to curtail the inflammatory response. LPS binds to circulating HDLs, LDLs, and VLDLs, making it unavailable for stimulation of the innate immune system (67–69). Following early cessation of the trial of the CETP inhibitor, torcetrapib, in the ILLUMINATE trial, the role of CETP in the immune...
response came under scrutiny because of an excess of deaths related to infection (70, 71). This was not an issue in cardiovascular clinical outcome trials of other CETP inhibitors. Although CETP has an intrinsically weak ability to bind LPS compared with LBP or BPI (72), it is associated with resilience to sepsis. Mice transgenic for human CETP have improved mortality following LPS administration compared with wild-type mice (73, 74). This is likely due, at least in part, to an increase in LPS sequestration by HDLs and LDLs and increased uptake of LPS by the liver (73). Conversely, PTLP knockout mice have increased endotoxin-associated mortality, delayed uptake of LPS by lipoproteins, and decreased LPS clearance (75).

The mechanism of LPS clearance is not well-understood. CETP facilitates the transfer of LPS from HDLs to LDLs (73, 75), and LDL receptor-mediated uptake of LDL-associated LPS by the liver has been reported (76). Hepatic uptake of HDL CEs by scavenger receptor B1 has also been implicated in LPS clearance (77).

In addition to facilitating LPS sequestration and excretion, mice expressing CETP and mice with the cecal ligation/puncture model of polymicrobial sepsis, have reduced production of the pro-inflammatory cytokines, TNF-α and interleukin (IL)-6, in response to LPS administration (73, 74). Furthermore, LPS decreases TNF-α production in macrophages from mice transgenic for human CETP relative to macrophages from wild-type mice (73). Incubation of RAW 264.7 murine macrophages with LPS and human CETP also induces a dose-dependent decrease in TNF-α production (73), possibly due to reduced expression of TLR4. TLR4 and IL-6 secretion are both reduced and survival is improved in mice transgenic for human CETP compared with wild-type mice following LPS administration (74). LPS-stimulated peritoneal macrophages from mice transgenic for human CETP also have reduced TLR4 expression, LPS uptake, nuclear factor (NF)-κB activation, and IL-6 production compared with peritoneal macrophages from wild-type mice (74). The reduced IL-6 production and increased resistance to sepsis in human CETP transgenic mice is consistent with evidence that IL-6 levels are correlated with the risk of death and that this is ameliorated by anti-IL-6 monoclonal antibody treatment in humans (78).

Reverse cholesterol transport, the process whereby excess cholesterol from peripheral tissues is transported to the liver for excretion, is also inhibited in sepsis (79), and CETP protein and mRNA levels are both decreased in hamsters and human CETP transgenic mice in response to LPS (80, 81). This is in line with the outcome of a small study in humans in which an association of increased mortality with the magnitude of CETP reduction in septic hospitalized patients was reported (82).

INHIBITION OF CETP

Targeted inhibition of CETP with neutralizing antibodies

Treatment of New Zealand White rabbits with neutralizing monoclonal antibodies to CETP increases HDL-C levels and reduces atherosclerosis (8). In another study of mice transgenic for human CETP, suppression of plasma CETP activity with an anti-CETP monoclonal antibody increased liver CETP mRNA levels. This unexpected finding was presumably due to increased plasma cholesterol levels in these mice (83). Anti-CETP monoclonal antibodies have also been shown to inhibit CETP activity by 70–80% and increase HDL-C levels by 33% in chow- and cholesterol-fed male Golden Syrian hamsters (84).

Targeted inhibition of CETP with antisense oligonucleotides

Antisense oligonucleotides to CETP that target and degrade CETP mRNA levels and decrease hepatic CETP protein levels have been reported to increase HDL-C levels by 32% and reduce atherosclerosis in cholesterol-fed Japanese White rabbits (9). Similarly, administration of antisense oligonucleotides to LDL receptor-deficient mice transgenic for human CETP inhibits CETP activity by 81% and increases plasma HDL-C levels by 38% (85). Enhanced macrophage reverse cholesterol transport and decreased accumulation of aortic cholesterol have also been reported in these mice relative to mice treated with a control antisense oligonucleotide (85).

Targeted inhibition of CETP with vaccines

Human CETP contains a hydrophobic 26 amino acid residue sequence in the C-terminal domain that is essential for neutral lipid transfer (5, 24). Immunization of cholesterol-fed New Zealand White rabbits with a peptide that targets this region of CETP generates neutralizing antibodies that inhibit CETP activity, increase plasma HDL-C levels, and decrease atherosclerotic lesion area (10). In another study, immunization of high-fat high-cholesterol-fed New Zealand White rabbits with a vaccine in which rabbit IgG-Fc was conjugated to a 26 amino acid C-terminal epitope of CETP increased plasma HDL-C and apoA-I levels. This vaccine also decreased the plasma level of oxidized LDL, as well as atherosclerosis and non-alcoholic hepatic steatosis, in New Zealand White rabbits (86, 87). A similar increase in plasma HDL-C levels and a reduction in atherosclerotic lesion area were observed in New Zealand White rabbits immunized subcutaneously with a heat shock protein-65-CETP vaccine (88). Intranasal administration with the combined heat shock protein-65-CETP vaccine also decreased atherosclerotic lesion area and serum total cholesterol and LDL-C levels, but did not affect TG or HDL-C levels in rabbits (89). Vaccination of cholesterol-fed New Zealand White rabbits with a tetanus toxoid-CETP peptide, by contrast, was associated with only a modest reduction in CETP activity, a modest increase in HDL-C levels, and no effect on atherosclerosis (10, 90). To date only one vaccine, CETi-1, has progressed to a phase I human clinical trial (11). Repeated administration of this vaccine in healthy adults generated variable anti-CETP antibody titers, and did not significantly inhibit CETP activity or increase plasma HDL-C levels (11).
Targeted inhibition of CETP with small molecule inhibitors

Use of small molecule inhibitors of CETP activity mimics the lipid profile changes that occur in humans and animals with CETP deficiency (12, 91). In most cases these inhibitors bind to and inactivate the CETP that is associated with HDLs (92). In doing so they prevent neutral lipid transfers between HDLs and TG-rich lipoproteins, including VLDLs. This results in the retention of CEs in HDLs (93). As discussed in detail below, four small molecule inhibitors that were developed to pharmacologically inhibit CETP activity have been tested in large cardiovascular clinical outcome trials.

SMALL MOLECULE CETP INHIBITORS

Torcetrapib

Torcetrapib is a small lipophilic tetrahydroquinoline derivative (Fig. 3A) that forms a tight complex between HDLs and CETP. This impedes the exchange of CE and TG between HDLs and other lipoproteins (94). Torcetrapib increases plasma HDL-C levels 3-fold and reduces atherosclerosis by 60% in cholesterol-fed rabbits (12). Treatment with torcetrapib also increases plasma HDL-C levels in hamsters, which, in turn, increases macrophage cholesterol efflux (95).

Dalcetrapib

Dalcetrapib (formerly JTT-705) is a thioaniline inhibitor that binds to CETP via cysteine residue-13 (Fig. 3B) (96). It inhibits the transfer of CE between HDLs, VLDLs, and LDLs, but does not inhibit CE transfers between different HDL particles (96). Treatment of mildly hyperlipidemic cholesterol-fed rabbits with dalcetrapib increases HDL-C levels and significantly decreases atherosclerotic lesion progression (13). Dalcetrapib is, however, without effect on atherosclerosis in severely hypercholesterolemic rabbits (97).

Evacetrapib

Evacetrapib is a benzazepine-based CETP inhibitor (Fig. 3C) that dose dependently inhibits CETP activity and increases HDL-C levels by up to 130% in mice transgenic

![Chemical structures for torcetrapib (A), dalcetrapib (B), evacetrapib (C), anacetrapib (D), and TA-8995 (E) are shown.](image-url)
for human CETP and apoA-I (98, 99). It also increases macrophage-to-feces reverse cholesterol transport in CETP transgenic mice and improves the net efflux of cholesterol from macrophages to HDLs (100).

Anacetrapib

Anacetrapib is structurally similar to torcetrapib (Fig. 3D) (99). It also increases HDL-C levels, which leads to enhanced macrophage cholesterol efflux and reverse cholesterol transport in dyslipidemic hamsters (101). In a dose escalation study with APOE*3 Leiden.CETP transgenic mice, anacetrapib, either as a monotherapy or in combination with atorvastatin, promoted a dose-dependent increase in HDL-C levels, improved lesion stability, and reduced atherosclerosis (102).

Anacetrapib has a number of additional cardioprotective functions. Treatment of normocholesterolemic New Zealand White rabbits with endothelial denudation of the abdominal aorta with the anacetrapib analog, des-fluoro-anacetrapib, which has one less fluorine atom than the parent compound, improves endothelial repair and endothelial function (103), increases angiogenesis in New Zealand White rabbits with hind limb ischemia (104), and reduces neointimal hyperplasia in New Zealand White rabbits with endothelial denudation of the iliac artery and stent deployment (105).

RANDOMIZED CLINICAL TRIALS OF CETP INHIBITORS IN HUMANS

ILLUMINATE trial with torcetrapib

The ILLUMINATE trial (Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events) (ClinicalTrials.gov number NCT00134264) was performed in 15,067 high-risk statin-treated people randomized in a double-blind design to receive torcetrapib or placebo (70). The primary endpoint was the time to first occurrence of a major cardiovascular event, a composite that included four components: death from coronary heart disease (defined as fatal myocardial infarction excluding procedure-related events, fatal heart failure, sudden cardiac death, or other cardiac death), nonfatal myocardial infarction (excluding procedure-related events), stroke, and hospitalization for unstable angina. Treatment with torcetrapib increased HDL-C levels by 72% and decreased LDL-C levels by 25%. This trial was terminated after 18 months because of a statistically significant excess of deaths (93 vs. 59) in those treated with torcetrapib. There was also a statistically significant 25% increase in ASCVD events in the participants that received torcetrapib.

The explanation for the harm caused by torcetrapib is not known with certainty, but it may have been the consequence of serious off-target adverse effects of the drug (70), including increased blood pressure, increased synthesis and secretion of aldosterone, and an increase in endothelin-I levels in the artery wall. Given these off-target effects of torcetrapib that are unrelated to CETP inhibition, it was not possible to draw conclusions from the ILLUMINATE trial regarding the potential cardiovascular benefits of CETP inhibition.

The dal-OUTCOMES trial with dalcetrapib

The dal-OUTCOMES trial (ClinicalTrials.gov number NCT00658515) included 15,871 participants recruited soon after an acute coronary syndrome (ACS) event. All patients were treated with a statin and were randomized in a double-blind design to receive either dalcetrapib or placebo (106). The primary endpoint was a composite of death from coronary heart disease, nonfatal myocardial infarction, ischemic stroke, unstable angina, or cardiac arrest with resuscitation. Treatment with dalcetrapib increased the concentration of HDL-C by about 30%, but its effect on LDL-C and apoB levels was minimal. Treatment with dalcetrapib did not reduce ASCVD events (106).

The absence of benefit in the dal-OUTCOMES trial may have been because dalcetrapib did not reduce the level of LDL-C. However, it may also have been because this trial was conducted in patients soon after an ACS event, at a time when HDL function is likely to be compromised. This explanation was supported by the observation in the placebo group in the dal-OUTCOMES trial in which the concentration of HDL-C was unrelated to the risk of having an ASCVD event (106). This is in contrast to what occurs in people with stable ASCVD, where there is an inverse relationship between HDL-C levels and the risk of having an ASCVD event. As was the case in the ILLUMINATE trial, dal-OUTCOMES did not test the hypothesis that CETP inhibition may reduce ASCVD events in people with stable coronary artery disease. As loss of the cardioprotective functions of HDLs after an ACS is likely to be temporary, it is possible that a meaningful reduction of cardiovascular events may have been observed in this trial if the median intervention had been extended beyond 31 months.

ACCELERATE trial with evacetrapib

The ACCELERATE (Assessment of Clinical Effects of Cholesteryl Ester Transfer Protein Inhibition with Evacetrapib in Patients at a High-Risk for Vascular Outcomes) trial (ClinicalTrials.gov number NCT01687998) included approximately 12,500 high-risk statin-treated patients randomized in a double-blind design to receive evacetrapib or placebo (107). The primary endpoint was the first occurrence of any component of the composite endpoint of cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, or coronary revascularization. The planned follow-up was 3 years. Evacetrapib reduced the level of LDL-C by 37% and increased HDL-C levels by 132% compared with placebo. This trial was terminated after just over 2 years when it became apparent that there would not be a positive outcome if it continued to its planned 3 year follow-up. There was no evidence that evacetrapib caused harm. The reason for the failure of evacetrapib to impact on the primary endpoint is not known, but it is possible that the trial was too short to detect benefit. If the REVEAL trial (see below) had stopped at the same time as ACCELERATE, a similar lack of efficacy would have
been found, thus emphasizing the fact that cardiovascular events are unlikely to be decreased in the short term by interventions that increase plasma HDL-C levels and lower plasma LDL-C levels.

**DEFINE trial with anacetrapib**

The DEFINE (Determining the Efficacy and Tolerability of CETP Inhibition with Anacetrapib) trial (ClinicalTrials.gov number NCT00685776) was an 18 month intervention designed to assess the lipid efficacy and safety of anacetrapib. The trial included 1,623 high-risk statin-treated patients who were randomized in a double-blind design to receive anacetrapib or placebo (108). Anacetrapib decreased the concentration of non-HDL-C by 32% and increased HDL-C levels by 138%. Anacetrapib had no effect on blood pressure or on plasma electrolyte or aldosterone levels. DEFINE showed that treatment with anacetrapib had favorable effects on plasma lipid levels by decreasing LDL-C levels and increasing HDL-C levels. It also showed that anacetrapib had an acceptable side-effect profile and, within the limits of the power of the study, did not have any of the adverse effects that were observed with torcetrapib. In a long-term follow-up of participants in the DEFINE trial, it was found that anacetrapib accumulated in adipose tissue and remained detectable in the body for two or more years after the last dose of the drug (109). There was no evidence, however, that retention of anacetrapib was associated with adverse effects. Despite the tendency for anacetrapib to be retained in the body, a decision was made to proceed with the REVEAL trial.

**REVEAL trial with anacetrapib**

The REVEAL (Randomized Evaluation of the Effects of Anacetrapib through Lipid Modification) trial (ClinicalTrials.gov number NCT01252953) included more than 30,000 high-risk statin-treated people who were randomized to receive anacetrapib or placebo. The planned follow-up was 4 years (14). The primary endpoint was the first major coronary event, a composite of coronary death, myocardial infarction, or coronary revascularization. The participants in REVEAL, who were treated intensively with atorvastatin prior to randomization, had a low baseline mean LDL-C level of 61 mg/dl, a mean non-HDL-C level of 92 mg/dl, and a mean HDL-C level of 40 mg/dl. Treatment with anacetrapib increased the level of HDL-C by 104% and decreased non-HDL-C levels by 18%. During the median 4.1 years of follow-up, the primary outcome was reduced from 11.8% in the placebo group to 10.8% in those treated with anacetrapib (rate ratio, 0.91; 95% confidence interval, 0.85–0.97; \( P = 0.004 \)). The magnitude of this benefit was consistent with that observed for comparable reductions in non-HDL-C levels in statin trials (14). Participants with a baseline LDL-C level in the upper tertile (>66 mg/dl) had a statistically significant reduction in the primary endpoint of 13%. In those with a baseline non-HDL-C level in the upper tertile (>101 mg/dl), the reduction in the primary endpoint was statistically significant 17%. There were no significant between-group differences in the risk of death, cancer, or other serious adverse events in this trial.

The reduction in coronary events in those treated with anacetrapib did not become apparent until after 2 years of treatment (14). During the third year, however, the reduction in coronary events was 15%, while coronary events occurring beyond 4 years of treatment were reduced by a statistically significant 17%. This delay in benefit highlights the possibility that the failure of evacetrapib to reduce cardiovascular events in ACCELERATE may have been related to termination of the trial after only 2 years of follow-up.

Treatment with anacetrapib in the REVEAL trial also reduced the risk of developing diabetes from 6.0% in those on statin alone to 5.3% in those treated with a statin plus anacetrapib. A positive effect of CETP inhibition on glycemic control (a reduction in plasma glucose levels and Hba1c) was also observed in participants in the torcetrapib arm of the ILLUMINATE trial (110) and in the evacetrapib arm of the ACCELERATE trial (111).

The mechanism by which CETP inhibition improves glycemic control and reduces the risk of new onset diabetes is uncertain, but may be related to the increased levels of HDL-C and apoA-I. Both HDLs and apoA-I increase the synthesis and secretion of insulin in pancreatic \( \beta \) cells (112, 113). They also enhance glucose uptake by skeletal muscle (114–116) and, thus, improve insulin sensitivity. An increase in either, or both, of these HDL functions in people treated with a CETP inhibitor could explain the improvement in glycemic control and decreased risk of developing diabetes that was observed in these studies.

**NEW CETP INHIBITORS**

TA-8995 is another novel tetrahydroquinoline derivative CETP inhibitor (Fig. 3E) (117). A phase I dose-escalating study of healthy subjects treated with single or multiple doses of TA-8995 (30–150 mg daily) or placebo, confirmed that TA-8995 is well-tolerated and does not adversely affect blood pressure, aldosterone levels, or serum electrolyte concentrations (117). In that study, TA-8995 inhibited CETP activity by 92–99%, increased HDL-C levels by 140%, and decreased LDL-C levels by 53% at the 10 mg/day dose (117).

In the 12 week randomized double-blind parallel-group phase II TULIP (TA-8995 in Patients with Mild Dyslipidemia) trial (ClinicalTrials.gov number NCT01970215), subjects with mild dyslipidemia were randomly assigned to receive placebo, TA-8995 as monotherapy (1–10 mg/day), 10 mg/day TA-8995 with atorvastatin (20 mg) or with rosuvastatin (10 mg), or statin alone (118). TA-8995 dose-dependently inhibited CETP activity, increased HDL-C and apoA-I levels by up to 179% and 63%, respectively, and decreased LDL-C and apoB levels by 45% and 34%, respectively (118). In combination with atorvastatin, TA-8995 increased HDL-C levels by 152% and reduced LDL-C levels by 68%, while in combination with rosuvastatin, HDL-C levels increased by 157% and LDL-C levels decreased by 63% (118). This makes TA-8995 one of the most potent CETP inhibitors available to date (118, 119). Although TA-8995 did not affect plasma TG and total cholesterol levels, it did...
increase the ability of HDLs to promote cholesterol efflux at the 10 mg/day dose (111).

CONCLUSIONS

Our understanding of the structure and function of CETP has progressed rapidly in recent years. There is clear evidence from the REVEAL trial that inhibition of CETP significantly reduces the risk of having a coronary event in statin-treated patients. There is also evidence that CETP inhibition improves glycemic control and reduces new onset diabetes, an effect that has the potential to counteract the increase in new onset diabetes associated with statin treatment. There is thus a compelling case for using the combination of a statin plus a CETP inhibitor in people at high cardiovascular risk that are treated with a statin and are at risk of developing diabetes. This will not only reduce the risk of having a coronary event beyond that achieved by a statin alone, but it will also counteract the statin-induced development of diabetes. Whether new CETP inhibitors, such as TA-8995, will be investigated in outcome trials in development of diabetes. Whether new CETP inhibitors, as awaited with interest.

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