Cholesteryl ester transfer protein inhibition as a strategy to reduce cardiovascular risk

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Abstract Human and rabbit plasma contain a cholesteryl ester transfer protein (CETP) that promotes net mass transfers of cholesteryl esters from high density lipoproteins (HDL) to other plasma lipoprotein fractions. As predicted, inhibition of CETP in both humans and rabbits increases the concentration of cholesterol in the potentially protective HDL fraction, while decreasing it in potentially proatherogenic non-HDL fractions. Inhibition of CETP in rabbits also inhibits the development of diet-induced atherosclerosis. However, use of the CETP inhibitor torcetrapib in humans did not reduce atheroma in three imaging trials and caused an excess of deaths and cardiovascular events in a large clinical outcome trial. The precise explanation for the harm caused by torcetrapib is unknown but may relate to documented, potentially harmful effects unrelated to inhibition of CETP. More recently, a trial using the weak CETP inhibitor dalcetrapib, which raises HDL levels less effectively than torcetrapib and does not lower non-HDL lipoprotein levels, was terminated early for reasons of futility. There was no evidence that dalcetrapib caused harm in that trial. Despite these setbacks, the hypothesis that CETP inhibitors will be antiatherogenic in humans is still being tested in studies with anacetrapib and evacetrapib, two CETP inhibitors that are much more potent than dalcetrapib and that do not share the off-target adverse effects of torcetrapib. — Barter, P. J., and K.-A. Rye. Cholesteryl ester transfer protein inhibition as a strategy to reduce cardiovascular risk. J. Lipid Res. 2012. 53: 1755–1766.

Supplementary key words CETP inhibition • atherosclerosis • clinical trials • drug therapy/hypolipidemic drugs

Population studies have identified the concentration of low-density lipoprotein (LDL) cholesterol as a positive predictor of having an atherosclerotic cardiovascular (CV) event (1). Furthermore, intervention studies have shown that reducing the concentration of LDL cholesterol (LDL-C) by treatment with statins decreases the risk of having a CV event (2). However, even aggressive statin therapy does not eliminate CV risk. One factor that may contribute to residual CV risk in statin-treated patients is a low level of high-density lipoprotein (HDL) cholesterol.

Prospective population studies have also identified a low level of HDL cholesterol (HDL-C) as an independent predictor of CV risk (1). This relationship persists even when LDL-C has been decreased to very low levels by treatment with statins (3). Furthermore, as outlined below, HDLs have several properties with the potential to protect against the development of atherosclerosis (4). Although there is still no direct evidence from clinical outcome trials in humans that raising the level of HDL-C will translate into a reduction in clinical CV events, there is a large and compelling body of evidence in animal studies (5–8) and growing evidence in human studies (9, 10) that HDL-raising therapies reduce (6) progression or even promote regression of atheroma. These observations have collectively led to a major research effort to identify therapies with the capacity to raise the concentration of HDL-C as effectively as statins reduce LDL-C levels.

One logical therapeutic approach to raising the concentration of HDL-C is to shift the partitioning of cholesterol between LDLs and HDLs in favor of the protective HDL fraction. Given that i) most of the cholesterol in human plasma exists in the form of cholesteryl esters, ii) most of

Abbreviations: ACS, acute coronary syndrome; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; CV, cardiovascular; ET-1, endothelin-1; eNOS, endothelial nitric oxide synthase; HDL-C, HDL cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, LDL cholesterol; TG, triglyceride; TRL, triglyceride-rich lipoprotein.

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the cholesteryl esters in human plasma originate in HDLs where they are formed in the reaction catalyzed by lecithin:cholesterol acyltransferase (LCAT), and i) human plasma contains a cholesteryl ester transfer protein (CETP) that promotes the transfer of cholesteryl esters from HDLs to other lipoprotein particles (including LDLs) (11–14), it follows that inhibition of CETP has the potential to retain cholesteryl in the HDL fraction and thus increase the concentration of HDL-C while decreasing its concentration in potentially atherogenic, non-HDL particles.

**BIOLOGICAL EFFECTS OF CETP**

CETP is a hydrophobic glycoprotein (15) that is synthesized in several tissues but mainly in the liver (16). Its crystal structure has been reported and reveals a curved molecule with N- and C-terminal cavities that provide access to cholesteryl esters and triglycerides and a tunnel spanning the entire length of the protein (17). CETP promotes bidirectional transfers of cholesteryl esters and triglycerides between all plasma lipoprotein particles (12).

**Mechanism of action of CETP**

Two hypotheses have been proposed for the mechanism by which CETP transfers neutral lipids between plasma lipoproteins: i) a shuttle mechanism that involves CETP collecting cholesteryl esters from one lipoprotein and delivering them through the aqueous phase to another lipoprotein (Fig. 1) (12, 18, 19), and ii) a tunnel mechanism in which CETP bridges two lipoproteins to form a ternary complex, with lipids flowing from the donor to acceptor lipoprotein through the CETP molecule (Fig. 2) (20–22).

**Shuttle mechanism.** As shown schematically in Fig. 1, CETP collides randomly with particles in all lipoprotein fractions to form transient lipoprotein-CETP complexes that facilitate exchanges of both cholesteryl esters and triglycerides between lipoprotein particles and CETP. The CETP, along with its associated neutral lipids, subsequently dissociates from the lipoprotein particles and circulates in a free state until it collides with another lipoprotein particle in either the same (23) or in a different (12) lipoprotein fraction to form a new transient complex that further exchanges cholesteryl esters and triglyceride between the lipoprotein particle and CETP. In this way, CETP promotes an equilibration of both cholesteryl esters and triglycerides among all lipoprotein particles.

Because most of the cholesteryl esters in plasma are generated in HDLs by the LCAT reaction and although the majority of the triglyceride enters plasma as a component of chylomicrons and VLDLs [known collectively as triglyceride-rich lipoproteins (TRL)], the net effect of the neutral lipid equilibration promoted by CETP is a mass transfer of cholesteryl esters from the HDL fraction to the LDL-TRL fraction and of triglyceride from TRLs to HDLs (Fig. 3).

Under normal physiological conditions, the rate of CETP-mediated cholesteryl ester transfer is rapid relative to the rate of catabolism of HDLs and LDLs (11, 12), such that the pools of cholesteryl esters in HDLs and LDLs are close to complete equilibrium in vivo. This view is supported by the observation that when HDLs and LDLs are incubated in vitro in the presence of CETP, there is a high rate of bidirectional transfer of radiolabeled cholesteryl esters but no net mass transfer in either direction (11). As a consequence of the pools of cholesteryl esters in HDLs and LDLs already being close to equilibrium in vivo, any increase in the activity of CETP beyond physiological levels...
would be predicted to have little impact on the distribution of cholesteryl esters between these lipoprotein fractions. In contrast, if the activity of CETP was inhibited, a point will be reached where CETP activity becomes rate limiting and will affect the distribution of cholesteryl esters between LDLs and HDLs in vivo.

Tunnel mechanism. The tunnel mechanism involves the initial formation of a binary complex between an HDL particle and a CETP molecule, with the subsequent formation (following a collision between the binary complex and an LDL or VLDL particle) of a ternary complex consisting of two lipoprotein particles bridged by a molecule of CETP (Fig. 2) (20–22).

Molecular forces introduced by the lipoproteins at either end of the CETP molecule cause a twisting of the CETP molecule that results in the formation of a tunnel through which cholesteryl esters are transferred from HDL to LDL or VLDL. The ternary complex then dissociates to form VLDL and LDL particles that are enriched in cholesteryl esters and HDL particles that are depleted of cholesteryl esters and thus reduced in size (22).

Although there is experimental evidence supporting the existence of both the shuttle and tunnel mechanisms, the extent to which either mechanism operates in vivo remains completely unknown.

Evidence that activity of CETP affects plasma lipoprotein concentration, composition, and structure

The first evidence that CETP activity affects plasma lipoproteins was provided by observations in people with genetic deficiencies of CETP. The first CETP mutation was identified in Japan in 1989 as a cause of markedly elevated HDL-C. Ten mutations associated with CETP deficiency have since been identified in Asians and one in Caucasians. It was found in Japan that 57% of subjects with levels of HDL-C greater than 100 mg/dl have mutations of the CETP gene. In addition, 37% of Japanese with levels of HDL-C between 75 and 100 mg/dl have mutations of the CETP gene (24–28). Similar conclusions were drawn from studies in rabbits that were treated with an anti-CETP antibody that resulted in a substantial increase in the concentration of HDL-C (29).

Consistent with these observations in CETP-deficient patients and rabbits treated with an anti-CETP antibody, it has since been found that treatment of humans with CETP inhibitor drugs (30–33) increases the concentration of both HDL-C and apoA-I (the major apolipoprotein in HDLs) and, in some cases, also decreases the concentration of LDL-C and apoB (the main LDL apolipoprotein) over and above the effects achieved by treatment with statins.

In the case of transfers of cholesteryl esters between HDLs and the much more rapidly catabolized TRLs, the amount of CETP in plasma is already rate limiting under most conditions (12). This is apparent from the net mass transfer of cholesteryl esters from HDLs to TRLs that occurs when the two fractions are incubated in vitro in the presence of CETP (34). It is therefore not surprising that inhibiting CETP reduces the cholesteryl ester content of TRLs (35).

Thus, inhibiting CETP in humans affects the concentration and composition of all lipoprotein fractions in ways that are potentially antiatherogenic.

The increased concentration of HDLs that occurs with CETP inhibition has the potential to be atheroprotective by a number of mechanisms (Fig. 4). The best known of these relates to the ability of HDLs to promote the efflux of cholesterol from macrophages in the artery wall (36). However, HDLs have several additional potentially antiatherogenic properties. These include an ability to inhibit oxidation of LDLs (37), as well as the inhibition of vascular inflammation (38, 39) and thrombosis (40). HDLs also enhance endothelial function (41), promote both endothelial repair (42, 43) and angiogenesis (44), and improve diabetic control (45–47).

CETP inhibition and atherosclerosis

Relationship between CETP activity and atherosclerosis in animals. CETP exists in the plasma of a small number of species, including humans and rabbits, but not rodents (14).

Mice lack CETP and are resistant to the development of atherosclerosis. The results in transgenic mice engineered to express CETP are conflicting and model dependent. Some of these studies in transgenic mice suggest that CETP is proatherogenic (48–50), whereas others suggest that it is antiatherogenic (51–53).
In contrast to mice, rabbits have a high level of CETP activity (14) and are extremely susceptible to the development of diet-induced atherosclerosis. Furthermore, inhibiting CETP in rabbits by the use of antisense oligodeoxynucleotides (54), an anti-CETP vaccine (55), or by administration of small-molecule CETP inhibitors (56, 57) markedly reduces their susceptibility to atherosclerosis.

**CETP and atherosclerosis in humans.** The relationship of CETP to atherosclerosis in humans is confusing. For example, studies of the relationships between polymorphisms of the CETP gene and human atherosclerotic disease have led in some cases to the conclusion that CETP is proatherogenic, whereas in others it has been concluded that CETP is antiatherogenic. However, in a large meta-analysis of 92 studies involving 113,833 participants (58), it was concluded that those CETP polymorphisms that are associated with decreased CETP activity and mass have an elevated concentration of HDL-C and a decreased risk of having a coronary event (58). A similar conclusion was drawn from an analysis of a cohort of 18,245 healthy American women in the Women’s Genome Health Study (59). This conclusion was further supported by a more recent meta-analysis (60) in which it was concluded that a common genetic variation of the CETP gene reduces the risk of myocardial infarction to the same extent as reported in the earlier meta-analysis. In this analysis, it was found that the apparently protective CETP gene variant was accompanied not only by higher levels of HDL-C but also by lower levels of LDL-C (60). This made it difficult to determine whether the protection afforded by the CETP gene variant related to the higher levels of HDL-C or to the lower levels of LDL-C.

In the Honolulu Heart Study’s seven-year prospective data, there was no statistically significant relation between homozygous mutations of CETP and either coronary heart disease (CHD) or stroke. However, it was concluded from this and another study that a deficiency of CETP is atheroprotective, so long as it is accompanied by an elevated HDL-C level greater than 60 mg/dl (61, 62).

Overall, the observations in both animal models and human genetic studies support decisions to test the hypothesis that inhibiting CETP in humans will be antiatherogenic and thus reduce the risk of having an atherosclerotic cardiovascular event.

**SMALL-MOLECULE CETP INHIBITORS**

**Torcetrapib**

Torcetrapib acts by increasing the affinity of CETP for HDLs (63). This generates a tight complex that reduces the amount of CETP available to promote transfers of cholesteryl esters and triglycerides between different lipoprotein particles. In patients taking effective doses of atorvastatin to reduce the level of LDL-C to less than 100 mg/dl, treatment with torcetrapib at a daily dose of 60 mg increased the concentration of HDL-C and apoA-I by 70% and 25%, respectively, and it decreased levels of LDL-C and apoB, respectively, by 25% and 12.5% (30).

**Effects of torcetrapib on atherosclerosis in humans.** The effect of torcetrapib on atherosclerosis in humans was investigated in three imaging trials. One of these trials (ILLUSTRATE) used intravascular ultrasound (IVUS) to assess the effect of torcetrapib on coronary atheroma burden in patients with demonstrable coronary atheroma (64), whereas the other two trials, RADIANCE 1 (65) and RADIANCE 2 (66), used B-mode ultrasound to assess the effects of torcetrapib on carotid intima-media thickness in patients with familial hypercholesterolemia and mixed hyperlipidemia, respectively. Treatment with torcetrapib at a dose of 60 mg per day increased the concentration of HDL-C by approximately 60% in all three of these imaging trials. In addition, the LDL-C level was reduced by approximately 20% over and above that achieved by atorvastatin. Despite this, treatment with torcetrapib had no effect (either positive or negative) on either the atheroma burden in the coronary arteries (64) or on carotid artery intima-media thickness (65, 66).

**Effects of torcetrapib on cardiovascular events.** The ILLUSTRATE trial (30) was designed to test the hypothesis that inhibiting CETP by treatment with torcetrapib would reduce the risk of having a clinical CV event. The trial was conducted in 15,067 people with manifest CV disease or type 2 diabetes. Participants, all of whom were receiving atorvastatin at a dose necessary to reduce the level of LDL-C to less than 100 mg/dl, were randomized to receive either torcetrapib at a dose of 60 mg per day or matching placebo, with an estimated follow-up of 4.5 years. Despite a 72% increase in HDL-C concentration and a 25% decrease in the level of LDL-C in the group receiving torcetrapib, the trial was terminated early because of a statistically significant excess of deaths associated with treatment with torcetrapib (30).

At the time the study was terminated, the hazard ratio for the primary outcome (major cardiovascular events) was 1.25 in the atorvastatin-torcetrapib group compared with the atorvastatin-only group ($P < 0.001$). The hazard ratio estimates for the individual components of the composite outcome ranged from 1.55 for hospitalization for unstable angina ($P = 0.001$) to 1.08 for stroke ($P = 0.74$). At study termination, there were 93 deaths in the atorvastatin-torcetrapib group and 59 in the atorvastatin-only group, for a hazard ratio of 1.58 in the atorvastatin-torcetrapib group ($P = 0.006$).

In the group treated with torcetrapib, there was an increase in the number of deaths from both cardiovascular causes (49 in the atorvastatin-torcetrapib group vs. 35 in the atorvastatin-only group) and noncardiovascular causes (40 in the atorvastatin-torcetrapib group vs. 20 in the atorvastatin-only group). No single cause of death explained the increased number of cardiovascular deaths. For death from noncardiovascular causes, more patients in the atorvastatin-torcetrapib group than in the atorvastatin-only group died from cancer (24 vs. 14) and infection (9 vs. 0). Note, however, that there was no difference in the total (fatal plus nonfatal) numbers of neoplasms and infections between the two groups (30).
Why did torcetrapib cause harm in the ILLUMINATE trial? Following are possible explanations for the harm caused by torcetrapib.

One explanation is that reverse cholesterol transport, the mechanism by which cholesterol in peripheral cells (including macrophages in the artery wall) is delivered to the liver for excretion from the body in bile, is decreased. The first step of reverse cholesterol transport involves the efflux of cell cholesterol to HDL particles, where it is converted into cholesteryl esters in the LCAT reaction. The cholesteryl esters are then delivered to the liver by either of two pathways: a direct pathway that involves the interaction of HDLs with the hepatic scavenger receptor B type I (SR-B1) or an indirect pathway in which CETP transfers cholesteryl esters from the HDL to the VLDL/LDL fractions, with subsequent delivery to the liver via hepatic uptake of LDL by the LDL receptor. It may therefore be argued that inhibiting CETP will reduce the indirect pathway and potentially compromise reverse cholesterol transport and, thus, be proatherogenic. It is currently not possible to confirm or refute this explanation, although such a suggestion is not supported by the results of inhibiting CETP with torcetrapib in rabbits (57). However, this possibility will remain unanswered until tested in trials using other CETP inhibitors that do not share the adverse effects of torcetrapib (see below).

A second explanation is that HDLs that do not function normally are generated. However, as outlined below, there is mounting evidence that this is not the case.

A third explanation is that the observed inverse relationship between the concentration of HDL-C and CV risk in population studies reflects an epiphenomenon rather than a direct antiatherogenic effect of HDLs. This will remain a possibility until tested in human clinical outcome trials using HDL-raising agents. However, although this could account for the lack of benefit in torcetrapib-treated patients, it cannot account for the observed harm related to treatment with the drug.

A fourth explanation is that the harm caused by torcetrapib was unrelated to CETP inhibition. As discussed below, there is a growing body of evidence consistent with this possibility.

**Torcetrapib and functionality of HDL.** Currently available evidence does not support the proposition that CETP inhibition compromises the function of HDL particles. In a posthoc analysis of the group treated with torcetrapib in the ILLUMINATE trial, coronary death and major CV event rates were lower in those where the increase in HDL-C or apolipoprotein A-I was greater than the median compared with those whose increases were below the median level of change (30). In additional posthoc analyses of the ILLUMINATE trial, the level of HDL-C achieved in the torcetrapib-treated patients was an inverse predictor of events (67). However, it must be emphasized that posthoc observations of this type, while suggesting that the HDLs were apparently functional in torcetrapib-treated patients, do not completely rule out the possibility that the HDLs were dysfunctional or that other unknown adverse effects of CETP inhibition may have contributed to a mechanism-related adverse outcome.

In other studies, HDLs isolated from torcetrapib-treated patients have been investigated in vitro and found to have either a normal or an enhanced ability to promote the efflux of cholesterol from macrophages (68).

Additional support for the normal functionality of HDLs in patients treated with torcetrapib has emerged from a posthoc analysis of the ILLUSTRATE trial that used intravascular ultrasound to assess the effect of torcetrapib on coronary atheroma burden. In patients treated with torcetrapib in this trial, there was a significant inverse relationship between changes in HDL-C and percentage coronary atheroma volume (69). Moreover, there was significant regression of coronary atheroma in the group of torcetrapib-treated patients who achieved the highest on-treatment HDL-C levels (69).

**Effects of torcetrapib unrelated to CETP inhibition.** Twelve months of treatment with torcetrapib in the ILLUMINATE trial was associated with a 5 mmHg increase in systolic blood pressure, an increase in serum aldosterone, a reduction in serum potassium, and an increase in serum concentrations of bicarbonate and sodium (30). The possibility that an increase in aldosterone secretion may have contributed to the clinical harm caused by torcetrapib was supported by the observation of a higher CHD mortality in those whose reduction in serum potassium or increase in bicarbonate (30) was greater than the median.

Preclinical studies conducted since termination of the torcetrapib program have shown that treatment with torcetrapib also increases blood pressure in animals that lack CETP (70). Torcetrapib has been shown in tissue culture studies to increase the synthesis of both aldosterone and cortisol in adrenal cortical cells (71, 72). Furthermore, compounds structurally related to torcetrapib (but lacking CETP inhibitory activity) raise blood pressure in animals and induce synthesis of aldosterone by adrenal cortical cells (71), whereas other CETP inhibitors currently in development, including dalcetrapib (72) and anacetrapib (31, 70), have no effect on blood pressure or serum aldosterone levels in either animals or humans. Nor do they induce synthesis of aldosterone in studies of adrenal cortical cells (70). In a subsequent study investigating the effects of CETP polymorphisms on blood pressure, it was concluded that the hypertensive effects of torcetrapib were unlikely to be due to CETP inhibition or to be shared by chemically dissimilar CETP inhibitors (73). Torcetrapib also impairs endothelial function in a process that is independent of either CETP inhibition or changes in HDL-C levels (74, 75). However, although consistent with a proposition that off-target effects of torcetrapib were responsible for the harm observed in the ILLUMINATE trial and the absence of an effect on atherosclerosis in the imaging trials, these posthoc and preclinical studies cannot be regarded as definitive.

**Effects of torcetrapib on diabetic control.** One unexpected beneficial effect of torcetrapib observed in a posthoc analysis
of the ILLUMINATE data was evidence of a significant improvement in diabetic control in patients with diabetes (Fig. 5) (76). The ILLUMINATE trial included 6,661 patients with type 2 diabetes (30). At baseline, there were no differences between the atorvastatin alone and the atorvastatin plus torcetrapib treatment arms with respect to plasma glucose, insulin, HbA1C, or the homeostasis model assessment of insulin resistance (HOMA-IR). After three months of treatment, the diabetic subjects taking the combination of torcetrapib plus atorvastatin had plasma glucose levels 0.34 mmol/lower \((P < 0.0001)\) and insulin levels 11.7 \(\mu\text{U/mL}\) lower \((P < 0.0001)\) than those receiving atorvastatin alone (76). HOMA-IR values decreased from 49.1 to 47.3 \((P < 0.0001)\) in those taking torcetrapib plus atorvastatin compared with an increase in HOMA-IR in those taking atorvastatin alone (Fig. 5). After six months of treatment, HbA1C levels were significantly lower in those taking torcetrapib \((7.06\%)\) compared with those in the control arm \((7.29\%)\) \((P < 0.0001)\). These effects of torcetrapib remained apparent for up to 12 months. Torcetrapib also lowered both glucose and insulin levels in the participants without diabetes, although the effects were not as great as in those with diabetes. However, it remains to be determined from the results in trials with other CETP inhibitors whether this effect of torcetrapib was the consequence of CETP inhibition, whether it was due to HDL raising, or whether it could be attributed to a (beneficial) off-target effect of torcetrapib unrelated to either a reduction in CETP activity or increased HDL levels.

Future of CETP inhibition after the failure of torcetrapib. The posthoc analyses of the ILLUMINATE and ILLUSTRATE trials, combined with the preclinical data demonstrating adverse off-target effects of torcetrapib unrelated to CETP inhibition, have provided the basis for reexamining the hypothesis that inhibition of CETP will be antiatherogenic.

The hypothesis is currently being tested in large clinical outcome trials with two CETP inhibitors, dalcetrapib and anacetrapib, that do not share the off-target adverse effects of torcetrapib.

Dalcetrapib

The first reported small-molecule CETP inhibitor dalce-
trapib (previously known as JTT-705) inhibited atheroscle-
rosis in cholesterol-fed rabbits (56) and paved the way for
testing the hypothesis that inhibiting CETP in humans
may be atheroprotective.

The first human study of CETP inhibition was conducted
with dalcetrapib at daily doses of 300, 600, and 900 mg
(32). Dalcetrapib was well tolerated and promoted dose-
dependent increases in HDL-C and decreases in LDL-C
(32). After four weeks of therapy, the 900 mg dose reduced
CETP activity by 37%. This was associated with a 34% in-
crease in HDL-C and a 7% reduction in LDL-C. In a subse-
quent study, dalcetrapib \((600 \text{ mg per day})\) given in
combination with pravastatin decreased CETP activity by
30% and increased HDL-C by 28%, but it had little effect
on the level of LDL-C relative to those treated with para-
vastatin alone (77).

The precise mechanism by which dalcetrapib inhibits
CETP activity is still uncertain, although it has been suggested
that it is the consequence of a conformational change in
CETP that decreases the transfer of cholesteryl esters from
HDLs to other lipoprotein fractions while having no effect
on the ability of CETP to remodel HDL (78). In studies con-
ducted in hamsters injected with [3H]cholesterol-labeled
macrophages, dalcetrapib significantly increased the fecal
elimination of both neutral sterols and bile acids (78), sug-
gest a positive effect on reverse cholesterol transport.

The effects of dalcetrapib and torcetrapib on hemody-
namics and the renin-angiotensin-aldosterone system have
been investigated in a rat model (72, 79). In contrast to
torcetrapib, dalcetrapib does not increase blood pressure

Fig. 5. Effects of torcetrapib on diabetic control. The ILLUMINATE trial included 6,661 patients with
type 2 diabetes. At baseline, there were no differences between the two treatment arms with respect to
plasma glucose, insulin, HbA1C, or HOMA-IR. Compared with the patients taking atorvastatin alone (A),
those taking the combination of torcetrapib plus atorvastatin (T/A) had a highly significant improve-
ment in diabetic control.
or renin-angiotensin-aldosterone-related gene expression, providing further evidence that some of the known off-target effects of torcetrapib are not a common feature of all compounds that inhibit CETP. The effects of dalcetrapib and torcetrapib on aldosterone synthesis have also been compared in tissue cultures of a human adrenocarcinoma cell line (72, 79). Again, in contrast to torcetrapib, dalcetrapib had no effect on either aldosterone synthase or aldosterone production in these cells.

Effects of dalcetrapib on atherosclerotic plaques in humans. The dal-PLAQUE trial was a multicenter study that used noninvasive multimodality imaging to assess the effect of dalcetrapib on atherosclerosis (80). Patients (ages 18–75 years) with manifest CHD or at high risk of having a coronary event were randomly assigned to receive dalcetrapib 600 mg/day or matching placebo for 24 months. End points included i) total vessel area, wall area, wall thickness, and the normalized carotid artery wall index as assessed by MRI and ii) arterial inflammation within an index vessel (right carotid, left carotid, or ascending thoracic aorta) as assessed by 18F-fluorodeoxyglucose (18F-FDG) PET/CT. A total of 130 participants were randomly assigned to placebo (n = 66) or dalcetrapib (n = 64). Of the five primary end points, all were negative with the exception of the MRI-derived change in total vessel area, which was significantly reduced by dalcetrapib compared with placebo. Although there was no difference between groups in the PET/CT measure of the most-diseased segment, an analysis limited to the carotid artery revealed a significant 7% reduction in the most-diseased segment. Overall, however, it has to be concluded that the effects on most of the primary outcome measures were disappointing. In terms of safety, dalcetrapib had no effect on blood pressure, and the frequency of adverse events was similar in the two groups, although conclusions regarding safety are limited by the very small sample size (80).

Effects of dalcetrapib on endothelial function in humans. In a study of patients with hypercholesterolemia, dalcetrapib improved endothelial function in a subgroup of patients whose HDL-C level was low at baseline, but it had no effect in those with higher baseline HDL levels (81).

The effect of dalcetrapib on endothelial function has been further investigated in the dal VESSEL study (ClinicalTrials.gov ID NCT00655538). This was a 36-week, multicenter, double-blind, placebo-controlled trial designed to assess the effects of dalcetrapib on endothelial function as determined by brachial flow-mediated dilatation. The study included 476 high-risk men and women with HDL-C levels less than 50 mg/dl.

After 4, 24, and 36 weeks of treatment with dalcetrapib, CETP activity decreased by 51, 53, and 56%, whereas at weeks 4, 12, and 36, HDL-C increased by 25, 27, and 31% (82). Dalcetrapib had no effect on LDL-C levels. When compared with the placebo group, dalcetrapib had no effect on FMD after either 12 or 36 weeks of treatment. In terms of safety, dalcetrapib had no effect on ambulatory blood pressure up to 36 weeks of treatment. Biomarkers of inflammation, oxidative stress, and coagulation were unaffected by dalcetrapib up to 36 weeks, although Lp-PLA2 levels were increased by 17% in those taking dalcetrapib.

The major conclusion to be drawn from the dal-VESEL trial is that there was no evidence that dalcetrapib improved endothelial function (82).

Effects of dalcetrapib on clinical cardiovascular outcomes in humans. Dal-OUTCOMES (ClinicalTrials.gov ID NCT00658515) was a phase III, multicenter, randomized, double-blind, placebo-controlled clinical trial designed to test the hypothesis that CETP inhibition with dalcetrapib reduces cardiovascular morbidity and mortality in patients with recent acute coronary syndrome (ACS) (83). More than 15,000 patients, all of whom were treated with statins to achieve recommended levels of LDL-C, were randomized to receive dalcetrapib at a daily dose of 600 mg or matching placebo. The primary outcome was time to first occurrence of a composite cardiovascular end point that included CHD death, nonfatal acute myocardial infarction, unstable angina requiring hospital admission, resuscitated cardiac arrest, or atherothrombotic stroke. The trial was planned to continue until 1,600 primary end-point events had occurred, with an anticipated reporting in 2013.

However, it was announced in early May 2012 that the trial had been terminated on the basis of futility (Roche provides update on Phase III study of dalcetrapib. Roche press release, May 7, 2012; [http://www.roche.com/media/media_releases/med-cor-2012-05-07.htm]).

The decision to terminate the trial was based on the second interim analysis by the Data and Safety Monitoring Committee (an independent committee appointed by Roche), who concluded that further continuation of the study had virtually no chance of yielding a positive result. As a consequence, the decision was made to stop the dal-OUTCOMES trial and to terminate the entire dalcetrapib development program.

It is important to emphasize that the dal-OUTCOMES trial was not terminated on the basis of safety. As was found in the extensive phase II program with dalcetrapib, there was no evidence that dalcetrapib shared any of the off-target adverse effects observed with torcetrapib.

So, what is the future of CETP inhibition as a cardioprotective strategy after the failure of torcetrapib for reasons of safety and the failure of dalcetrapib for reasons of futility?

In the case of torcetrapib, off-target adverse effects provided a plausible explanation for the harm observed in the ILLUMINATE trial, although it must be emphasized that this does not mean that the result would have been positive without the off-target effects. In the case of dalcetrapib, there was no evidence of off-target adverse effects, the explanation for the absence of benefit is completely unknown at the time of this article.

The two most obvious explanations for the failure of dalcetrapib are that i) the increase in HDL-C concentration induced by dalcetrapib is not accompanied by an enhancement of the protective functions of HDL or ii) the inverse relationship between HDL-C concentration and cardiovascular risk observed in population studies is an
epiphenomenon rather than reflective of an ability of HDL to protect against cardiovascular disease.

However, a recent study has found that people with genetic variations in the CETP gene associated with a reduction in cardiovascular risk have not only an increase in concentration of HDL-C but also a decrease in concentration of LDL-C (60). It is therefore possible that for CETP inhibition to achieve a reduction in cardiovascular risk, it is necessary to use an inhibitor that results in both an increase in the concentration of HDL-C and a decrease in that of LDL-C.

Inhibiting CETP with dalcetrapib, although resulting in a modest increase in the concentration of HDL-C, does not reduce the level of LDL-C (82). In contrast, the CETP inhibitors anacetrapib and evacetrapib (see discussion below) not only more than double the level of HDL-C but also reduce the level of LDL-C by more than 30%. This provides a powerful motivation for conducting cardiovascular clinical outcome trials with these agents.

**Anacetrapib**

When given at a daily dose of 100 mg, anacetrapib more than doubles the concentration of HDL-C and reduces LDL-C levels by as much as 40% (over and above the reduction achieved with a statin) (31). It has no effect on blood pressure, aldosterone, or serum electrolytes in humans (31), and it does not stimulate the synthesis of aldosterone in adrenal cortical cells growing in tissue culture (70). Furthermore, HDLs isolated from people taking anacetrapib have a normal or enhanced functionality, as assessed by their ability ex vivo to promote efflux of cholesterol from macrophages (68). In studies conducted in a dyslipidemic hamster model, it has been shown that anacetrapib, like dalcetrapib, enhances macrophage-to-feces reverse cholesterol transport as evidenced by an increased fecal excretion of both cholesterol and bile acids (84).

The precise mechanism by which anacetrapib inhibits CETP is not known, although, like torcetrapib, it promotes a tight binding of CETP to HDL particles (85). This immobilizes CETP, making it unavailable to shuttle cholesteryl esters between lipoprotein particles. Anacetrapib, torcetrapib, and dalcetrapib compete with one another for binding CETP (85), although it remains uncertain how this relates to the mechanism of action of any of these agents.

**Safety of anacetrapib in humans.** The DEFINE study was a randomized, double-blind, placebo-controlled 18-month trial designed to assess the lipid efficacy and safety profile of anacetrapib in patients (n = 1,623) with manifest or at high risk of developing CHD. All participants were taking a statin to achieve optimal levels of LDL-C before being randomized to receive anacetrapib 100 mg daily or matching placebo (31). By 24 weeks, the LDL-C level had been reduced from 81 mg/dl to 45 mg/dl in the anacetrapib group compared with a reduction from 82 mg/dl to 77 mg/dl in the placebo group, a 40% reduction with anacetrapib beyond that seen with placebo. The HDL-C level increased from 41 mg/dl at baseline to 101 mg/dl in the anacetrapib group, compared with an increase from 40 mg/dl to 46 mg/dl in the placebo group, an increase of 138% with anacetrapib beyond that seen with placebo. Treatment with anacetrapib had no effect on blood pressure or on electrolyte or aldosterone levels. Prespecified adjudicated CV events occurred in 16 patients treated with anacetrapib (2.0%) and 21 patients receiving placebo (2.6%) (P = 0.40). The prespecified Bayesian analysis indicated that this event distribution provided a predictive probability of 94% that anacetrapib would not be associated with the increase in CV events seen with torcetrapib. Significantly fewer patients in the anacetrapib group than in the placebo group underwent revascularization (8 vs. 28, P = 0.001) (31). It was concluded that treatment with anacetrapib had robust favorable effects on levels of LDL-C and HDL-C, had an acceptable side-effect profile, and within the limits of the power of this study, did not result in the adverse cardiovascular effects observed with torcetrapib. A comparison of the cardiovascular end points in the DEFINE and ILLUMINATE studies is shown in Fig. 6.

Effects of anacetrapib on clinical cardiovascular outcomes in humans. Randomized Evaluation of the Effects of Anacetrapib through Lipid-modification (REVEAL; ClinicalTrials.gov ID NCT01252953) is a phase III trial designed to determine whether treatment with anacetrapib at a daily dose of 100 mg reduces the risk of a composite end point (coronary death, myocardial infarction, or coronary revascularization) in patients with circulatory problems who have their LDL-C optimally treated with a statin. It is planned to randomize 30,000 subjects to anacetrapib 100 mg daily or matching placebo with a predicted follow-up of about five years. This study will include men and women with a history of myocardial infarction, cerebrovascular atherosclerotic disease, peripheral arterial disease, or diabetes mellitus with other evidence of symptomatic CHD. This study is ongoing with no indication that it will terminate in light of the failure of dalcetrapib.

**Fig. 6.** Cardiovascular end points in the DEFINE (31) and ILLUMINATE (3) trials. The hazard ratios and 95% confidence intervals are shown. The primary end point in ILLUMINATE was time to first occurrence of a major cardiovascular event (MCVE), comprising a composite of death from coronary heart disease, nonfatal myocardial infarction, stroke, and hospitalization for unstable angina. A prespecified end point in DEFINE was time to first occurrence of a MCVE, comprising death from cardiovascular causes, nonfatal myocardial infarction, hospitalization for unstable angina, and nonfatal stroke. CETP-I, CETP inhibitor.
OTHER CETP INHIBITORS

Two additional CETP inhibitors are currently in early development.

Evacetrapib

Evacetrapib (LY2484595), a novel benzazepine compound, is a potent and selective inhibitor of CETP. In contrast to torcetrapib, high doses of evacetrapib do not elevate blood pressure in rats, and evacetrapib does not induce aldosterone or cortisol biosynthesis in a human adrenal cortical carcinoma cell line (86).

The biochemical effects, safety, and tolerability of evacetrapib have been assessed in a 12-week randomized, placebo controlled trial that included 398 patients with elevated LDL-C or low HDL-C levels (33). Evacetrapib was given either as monotherapy or in combination with statins. Participants were randomly assigned to receive placebo (n = 38) or evacetrapib monotherapy at doses of 30 mg/d (n = 40), 100 mg/d (n = 39) or 500 mg/d (n = 42). In addition, the effects of evacetrapib at a dose of 100 mg per day were assessed in 239 patients taking statins. The coprimary end points were percentage changes from baseline in HDL-C and LDL-C after 12 weeks of treatment. The mean baseline HDL-C level was 55.1 mg/dl and the mean baseline LDL-C level was 144.3 mg/dl. When given as monotherapy, evacetrapib produced dose-dependent increases in HDL-C of 30 to 66 mg/dl (54% to 129%) compared with a decrease with placebo of −0.7 mg/dl (−3.0%) and decreases in LDL-C of −21 to −51.4 mg/dl (−14% to −36%) compared with an increase with placebo of 7.2 mg/dl (3.9%). When given in combination with a statin, evacetrapib (100 mg per day) increased HDL-C by 42.1 to 50.5 mg/dl (78.5% to 88.5%) compared with statin monotherapy and decreased LDL-C by 67.1 to 75.8 mg/dl (a decrease of 11.2% to 13.9%) compared with statin monotherapy. No adverse effects of evacetrapib were observed in this study (33). There is no indication to date that a planned large phase III clinical outcome study using evacetrapib will be reconsidered in the light of the results of the dal-OUTCOMES trial.

BAY 60-5521

BAY 60-5521 is another CETP inhibitor in early development; to date there is relatively little information of its effects in humans. The early results suggest that the agent is clinically safe and well tolerated, with no effects on heart rate or blood pressure (87) Further results are awaited with interest.

CONTROVERSIES AND UNANSWERED QUESTIONS RELATED TO CETP INHIBITION

There are three main areas of controversy related to the use of CETP inhibitors as agents to reduce cardiovascular risk.

The first (and most fundamental) relates to suggestions that are frequently made that inhibition of CETP will be proatherogenic rather than antiatherogenic. Note, however, that such assertions are not based on hard evidence and cannot be answered by the results of currently available studies. Indeed, the issue of whether CETP inhibition is anti- or proatherogenic will remain unanswered until the results of ongoing clinical outcome trials are known.

A second controversy relates to the effect of CETP inhibition on HDL function. Again, it is often asserted (with no supporting evidence) that inhibition of CETP generates dysfunctional HDL particles. However, this view is not consistent with the observation that HDLs isolated from patients treated with anacetrapib have, if anything, an enhanced ability to promote the efflux of cholesterol from macrophages (88).

The third area of controversy relates to suggestions that dalcetrapib and anacetrapib inhibit CETP by different mechanisms. Although it was suggested that the difference in mechanism may translate into dalcetrapib having superior antiatherogenic properties to those of anacetrapib, the results of the dal-OUTCOMES trial clearly negate such a proposition. However, it remains to be seen whether anacetrapib (or evacetrapib) is any more effective in reducing cardiovascular risk than dalcetrapib.

CONCLUSIONS

Given that i) CETP inhibition in humans increases the concentration of cholesterol in the potentially protective HDL fraction while decreasing it the harmful non-HDL fractions, ii) CETP inhibition in rabbits reduces susceptibility to the development of atherosclerosis, iii) genetic variations in the human CETP gene are associated with a reduction in cardiovascular risk when accompanied by both an increase in concentration of HDL-C and a decrease in concentration of LDL-C, iv) adverse effects of torcetrapib that were unrelated to CETP inhibition may have been responsible for the harm it caused, and v) the absence of benefit in the dal-OUTCOMES trial may have reflected the fact that dalcetrapib is a relatively weak CETP inhibitor that does not decrease levels of atherogenic non-HDL fractions, there is a compelling case for further testing the hypothesis that potent CETP inhibitors that more than double the level HDL-C and decrease non-HDL-C by about 30% will be antiatherogenic in humans. This hypothesis is currently being tested in large cardiovascular clinical end-point trials.

REFERENCES


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