Cholesteryl Ester Transfer Protein (CETP) Inhibition as a Strategy to Reduce Cardiovascular Risk

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Running title: CETP inhibition

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Abbreviations:
ABSTRACT

Human and rabbit plasma contain a cholesteryl ester transfer protein (CETP) that promotes net mass transfers of cholesteryl esters from high density lipoproteins (HDLs) 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 pro-atherogenic 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, that 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 anti-atherogenic in humans is still being tested in studies with anacetrapib and evacetrapib, two CETP inhibitors that are much more potent than dalcetrapib and which do not share the off-target adverse effects of torcetrapib.
INTRODUCTION

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 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 as an independent predictor of CV risk (1). This relationship persists even when LDL cholesterol has been decreased to very low levels by treatment with statins (3). Furthermore, as outlined below, HDLs have several properties that have the potential to protect against the development of atherosclerosis (4). While there is still no direct evidence from clinical outcome trials in humans that raising the level of HDL cholesterol will translate into a reduction in clinical CV events, there is a large and compelling body of evidence in animal studies (5) (6); (7) (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 cholesterol as effectively as statins reduce LDL cholesterol levels.
One logical therapeutic approach to raising the concentration of HDL cholesterol is to shift the partitioning of cholesterol between LDLs and HDLs in favour 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 the cholesteryl esters in human plasma originate in HDLs where they are formed in the reaction catalysed by lecithin:cholesterol acyltransferase (LCAT) and (iii) 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) (12) (13) (14), it follows that inhibition of CETP has the potential to retain cholesterol in the HDL fraction and thus increase the concentration of HDL cholesterol while decreasing its concentration in potentially atherogenic, non-HDL particles.

**BIOLOGICAL EFFECTS OF CETP**

CETP is a hydrophobic glycoprotein (15) that is synthesised 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 triglyceride 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 (Figure 1) (12, 18) (19).

(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 (Figure 2)(20-22).

**Shuttle Mechanism**

As shown schematically in Figure 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, and 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 facilitates further exchange of cholesteryl esters and triglyceride between the lipoprotein particle and CETP. In this way, CETP promotes an equilibration of both cholesteryl esters and triglycerides between all lipoprotein particles.

Since most of the cholesteryl esters in plasma are generated in HDLs by the LCAT reaction, while the majority of the triglyceride enters plasma as a
component of chylomicrons and VLDLs (known collectively as triglyceride rich lipoproteins or TRLs), 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 (Figure 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 radiolabelled 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 were to be inhibited, a point will be reached where CETP activity becomes rate limiting and will impact on 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 (Figure 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).

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

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

The first evidence that CETP activity impacts on 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 > 100 mg/dL have mutations of the CETP gene. In addition, 37% of Japanese with levels HDL-C between 75-100 mg/dL have mutations of the CETP gene (24) (25) (26) (27) (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) (31) (32) (33) increases the concentration of both HDL cholesterol and apoA-I (the major apolipoprotein in HDLs) and in some cases also decreases the concentration of LDL cholesterol 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 anti-atherogenic.

The increased concentration of HDLs that occurs with CETP inhibition has the potential to be atheroprotective by a number of mechanisms (Figure 4). The best know 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 anti-atherogenic 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) (46) (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 pro-atherogenic (48) (49) (50) while others suggest that it is anti-atherogenic (51) (52) (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 pro-atherogenic, while in others it has been concluded that CETP is anti-atherogenic. 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 cholesterol 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 in the Women’s Genome Health Study (59).

In the Honolulu Heart Study 7-year prospective data, there was no statistically significant relation between heterozygous mutations of CETP and either CHD or stroke. However, it was concluded from this and another study that a deficiency of CETP is athero-protective, so long as it is accompanied by an HDL-C level > 60 mg/dL (60, 61).

Overall, the observations in both animal models and human genetic studies support decisions to test the hypothesis that inhibiting CETP in humans will be
anti-atherogenic 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 (62). 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 below 100 mg/dL, treatment with torcetrapib at a daily dose of 60 mg increased the concentration of HDL cholesterol and apoA-I by 70% and 25%, respectively, and decreased levels of LDL cholesterol 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 (63), while the other two, RADIANCE 1 (64) and RADIANCE 2 (65), 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
cholesterol by approximately 60% in all three of these imaging trials. In addition, the LDL cholesterol 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 (63) or on carotid artery intima-media thickness (64, 65).

**Effects of torcetrapib on cardiovascular events**

The ILLUMINATE 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 cholesterol 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 cholesterol concentration and a 25% decrease in the level of LDL cholesterol 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 of terminating the study the hazard ratio for the primary outcome (major cardiovascular events) was 1.25 in the atorvastatin/torcetrapib group compared to the atorvastatin-only group (P < 0.001). The hazard ratio estimates for the individual components of the composite outcome ranged from 1.35 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 group treated with atorvastatin plus torcetrapib vs. 35 in the atorvastatin only group) and non-cardiovascular causes (40 in the group treated with atorvastatin plus torcetrapib vs. 20 in the atorvastatin only group). No single cause of death explained the increased number of cardiovascular deaths. For death from non-cardiovascular 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). It should be noted, however, that there was no difference in the total (fatal plus non-fatal) numbers of neoplasms and infections between the two groups (30).

**Why did torcetrapib cause harm in the ILLUMINATE trial?**

Possible explanations for the harm caused by torcetrapib include:

(i) A decrease in the reverse cholesterol transport pathway in which cholesterol in peripheral cells (including macrophages in the artery wall) is delivered to the liver for excretion from the body in bile. 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 so formed 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 1 (SR-B1) and an indirect pathway where 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 pro-atherogenic. 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).

(ii) Generation of HDLs that do not function normally. However, as outlined below, there is mounting evidence that this is not the case.

(iii) The observed inverse relationship between the concentration of HDL cholesterol and CV risk in population studies reflects an epiphenomenon rather than a direct anti-atherogenic effect of HDLs. This will remain a possibility until tested in human clinical outcome trials using HDL-raising agents. However, while 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.
(iv) The harm caused by torcetrapib was unrelated to CETP inhibition. As outlined 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 post-hoc 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 cholesterol or apolipoprotein A-I was greater than the median compared with those whose increases were below the median level of change (30). In additional post-hoc analyses of the ILLUMINATE trial, the level of HDL cholesterol achieved in the torcetrapib-treated patients was an inverse predictor of events (66). However, it must be emphasized that post-hoc 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 (67).
Additional support for the normal functionality of HDLs in patients treated with torcetrapib has emerged from a post-hoc analysis of the ILLUSTRATE trial that used intravascular ultrasound to assess the effect of torcetrapib on coronary atheromoma burden. In patients treated with torcetrapib in this trial, there was a significant inverse relationship between changes in HDL cholesterol and percentage coronary atheroma volume (68). Moreover, there was significant regression of coronary atheroma in the group of torcetrapib-treated patients who achieved the highest on-treatment HDL cholesterol levels (68).

**Effects of torcetrapib unrelated to CETP inhibition**

Twelve months of treatment with torcetrapib in the ILLUMINATE trial was associated with a 5 mm Hg 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 coronary heart disease (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 (69). Torcetrapib has been shown in tissue culture studies to increase the synthesis of both aldosterone and cortisol in adrenal cortical cells.
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 (70), while other CETP inhibitors currently in development, including dalcetrapib (71) and anacetrapib (69) (31), 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 (69). 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 (72). Torcetrapib also impairs endothelial function in a process that is independent of either CETP inhibition or changes in HDL cholesterol levels (73) (74). However, while 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 post-hoc and preclinical studies cannot be regarded as definitive.

**Effects of torcetrapib on diabetic control**

One unexpected beneficial effect of torcetrapib observed in a post-hoc analysis of the ILLUMINATE data was evidence of a significant improvement in diabetic control in patients with diabetes (Figure 5) (75). The ILLUMINATE trial included 6661 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/L lower (p<0.0001) and insulin levels 11.7 mU/mL lower (p<0.0001) than those receiving atorvastatin alone (75). 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 (Figure 5). After six-months of treatment HbA1C levels were significantly lower in those taking torcetrapib (7.06%) compared to 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 post-hoc 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 re-examining the hypothesis that inhibition of CETP will be anti-atherogenic. 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, dalcetrapib (previously known as JTT-705), inhibited atherosclerosis in cholesterol-fed rabbits (56) and paved the way for testing the hypothesis that inhibiting CETP in humans may be athero-protective.

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 cholesterol and decreases in LDL-C (32). After 4 weeks of therapy, the 900 mg dose reduced CETP activity by 37%. This was associated with a 34% increase in HDL cholesterol and a 7% reduction in LDL cholesterol. In a subsequent study, dalcetrapib (600 mg per day) given in combination with pravastatin decreased CETP activity by 30% and increased HDL cholesterol by 28% but had little effect on the level of LDL cholesterol relative to those treated with paravastatin alone (76).

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 (77). In studies conducted in hamsters injected with $[^3]H$ cholesterol-labeled macrophages, dalcetrapib significantly increased the fecal elimination of both neutral sterols and bile acids (77), suggesting a positive effect on reverse cholesterol transport.

The effects of dalcetrapib and torcetrapib on haemodynamics and the renin-angiotensin-aldosterone system have been investigated in a rat model (71) (78). In contrast to torcetrapib, dalcetrapib does not increase blood pressure 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 culture in a human adrenocarcinoma cell line (71) (78). 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 multicentre study that used non-invasive multimodality imaging to assess the effect of dalcetrapib on atherosclerosis (79). Patients (aged 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. Endpoints 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 $^{18}$F-fluorodeoxyglucose ($^{18}$F-FDG) PET/CT.

A total of 130 participants were randomly assigned to placebo (n=66) or dalcetrapib (n=64). Of the 5 primary endpoints, all were negative with the exception of the MRI-derived change in total vessel area that was significantly reduced by dalcetrapib compared with placebo. While 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 (79).

**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 cholesterol level was low at baseline but had no effect in those with higher baseline HDL levels (80).

The effects of dalcetrapib on endothelial function have been further investigated in the dal VESSEL study (ClinicalTrials.gov Identifier: NCT00655538). This was a
36-week, multi-centre, 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 cholesterol levels <50 mg/dL.

After 4, 24 and 36 weeks of treatment with dalcetrapib, CETP activity decreased by 51, 53 and 56% while at weeks 4, 12 and 36 HDL-C increased by 25, 27 and 31% (81). 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-VESSEL trial is that there was no evidence that dalcetrapib improved endothelial function (81).

**Effects of dalcetrapib on clinical cardiovascular outcomes in humans**

Dal-OUTCOMES (ClinicalTrials.gov Identifier: 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 syndromes (ACS) (82). More than 15,000 patients, all of whom were treated with
statins to achieve recommended levels of LDL cholesterol, were randomised 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 endpoint that included CHD death, nonfatal acute myocardial infarction, unstable angina requiring hospital admission, resuscitated cardiac arrest or athero-thrombotic stroke). The trial was planned to continue until 1,600 primary end point events have 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, 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 stress that the dal-OUTCOMES trial was not terminated on the basis of safety. As was found in the extensive phase 2 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 cardio-protective 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 stressed that this does not mean that the result would have been positive without the off-target effects. In the case of dalcetrapib where there was no evidence of off-target adverse effects, the explanation for the absence of benefit is completely unknown at the time of writing.

The two most obvious explanations for the failure of dalcetrapib are that:

(i) the increase in HDL cholesterol concentration induced by dalcetrapib is not accompanied by an enhancement of the protective functions of HDL or

(ii) the inverse relationship between HDL cholesterol concentration and cardiovascular risk observed in population studies is an epiphenomenon rather than being 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 cholesterol but also a decrease in concentration of LDL cholesterol (83). 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 cholesterol and a decrease in that of LDL cholesterol.

Inhibiting CETP with dalcetrapib, while resulting in a modest increase in the concentration of HDL cholesterol, does not reduce the level of LDL cholesterol. In contrast, the CETP inhibitors, anacetrapib and evacetrapib, 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. (see below).

**ANACETRAPIB**

When given at a daily dose of 100 mg, anacetrapib more than doubles the concentration of HDL cholesterol and reduces LDL cholesterol 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 does not stimulate the synthesis of aldosterone in adrenal cortical cells growing in tissue culture (69). 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 (67). In studies conducted in a dyslipidemic hamster model it has been shown that, like dalcetrapib, anacetrapib 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 immobilises 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=1623) with manifest or at high risk of developing CHD. All participants were taking a statin to achieve optimal levels of LDL cholesterol before being randomised to receive anacetrapib 100 mg per day) or matching placebo (31). By 24 weeks, the LDL cholesterol level had been reduced from 81 mg/dL to 45 mg/dL in the anacetrapib group, as 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 cholesterol level increased from 41 mg/dL at baseline to 101 mg/dL in the anacetrapib group, as 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. Pre-specified adjudicated CV events occurred in 16 patients treated with anacetrapib (2.0%)
and 21 patients receiving placebo (2.6%) \( (P = 0.40) \). The pre-specified 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 \((8 \text{ vs. } 28, P = 0.001)\) \( (31) \). It was concluded that treatment with anacetrapib had robust favourable effects on levels of LDL cholesterol and HDL cholesterol, 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 Figure 6.

**Effects of anacetrapib on clinical cardiovascular outcomes in humans**

REVEAL: Randomized EValuation of the Effects of Anacetrapib through Lipid-modification (ClinicalTrials.gov number, NCT01252953) is a phase III trial designed to determine whether treatment with anacetrapib given at a daily dose of 100 mg reduces the risk of a composite endpoint (coronary death, myocardial infarction or coronary revascularization) in patients with circulatory problems who have their LDL cholesterol optimally treated with a statin. It is planned to randomise 30,000 subjects to anacetrapib 100 mg daily or matching placebo with a predicted follow up of about 5 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 the light of the failure of dalcetrapib.

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 increase blood pressure elevation in rats nor, in a human adrenal cortical carcinoma cell line, does evacetrapib induce aldosterone or cortisol biosynthesis (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 cholesterol or low HDL cholesterol 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 co-primary end points were percentage changes from baseline in HDL cholesterol and LDL cholesterol after 12 weeks of treatment. The mean baseline HDL cholesterol level was 55.1 mg/dL and the mean baseline LDL cholesterol level was 144.3 mg/dL. When given as
monotherapy, evacetrapib produced dose-dependent increases in HDL cholesterol 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 cholesterol 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 cholesterol by 42.1 to 50.5 mg/dL (78.5% to 88.5%) compared with statin monotherapy and decreased LDL cholesterol 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 3 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, but 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.
(i) The first (and most fundamental) relates to suggestions that are frequently made that inhibition of CETP will be pro-atherogenic rather than anti-atherogenic. It should be noted, 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 pro-atherogenic will remain unanswered until the results of ongoing clinical outcome trials are known.

(ii) 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).

(iii) The third area of controversy relates to suggestions that dalcetrapib and anacetrapib inhibit CETP by different mechanisms. While it was suggested that the difference in mechanism may translate into dalcetrapib having superior anti-atherogenic 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 cholesterol and a decrease in concentration of LDL cholesterol. (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 cholesterol and decrease non-HDL cholesterol by about 30% will be anti-atherogenic in humans. This hypothesis is currently being tested in large cardiovascular clinical endpoint trials.
REFERENCES


Legends to figures

Figure 1. Proposed shuttle mechanism for cholesteryl ester transfer by CETP. CETP collides randomly with particles in all lipoprotein fractions to form transient complexes that facilitate an exchange of both cholesteryl esters (CE) and triglycerides (TG) between the lipoprotein particles and CETP. The CETP (and its associated CE and TG) subsequently dissociates from lipoprotein particles to circulate in a free state until it colides with another lipoprotein particle (either in the same or in a different lipoprotein fraction) to form a new transient complex with further exchange of CE and TG between lipoprotein particle and CETP molecule. In this way, CETP promotes an equilibration of both CE and TG between all lipoprotein particles.

Figure 2. Proposed tunnel mechanism for cholesteryl ester transfer by CETP. The N-terminal of CETP initially penetrates the HDL surface and forms a binary complex in which the CETP interacts with the cholesteryl ester core of HDL. The binary complex then interacts with LDL or VLDL via the C-terminal domain of CETP to form a ternary complex consisting of HDL, CETP and VLDL/LDL. Molecular forces introduced by the lipoproteins at either end of the CETP molecule cause 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/LDL particles that are enriched in cholesteryl esters and HDL particles that are depleted of cholesteryl esters and reduced in size.
Figure 3. Net effect of CETP on plasma lipoproteins. Since most of the CE in plasma originates in HDLs in the LCAT reaction, while the majority of the triglyceride enters plasma as a component of triglyceride-rich lipoproteins (TRLs), the net effect of the equilibration promoted by CETP as shown in Figure 1 is a mass transfer of CE from HDLs to LDLs and TRLs and of TG from TRLs to HDLs.

Figure 4. Protective properties of HDLs. HDLs have multiple properties with the potential to protect against the development of atherosclerosis.

Figure 5. Effects of torcetrapib on diabetic control. The ILLUMINATE trial included 6661 patients with type 2 diabetes. At baseline there were no differences between the two treatment arms with respect to plasma glucose, insulin, HbA1C or the homeostasis model assessment of insulin resistance (HOMA-IR). Compared to the patients taking atorvastatin alone (A), those taking the combination of torcetrapib plus atorvastatin (T/A) had a highly significant improvement in diabetic control.

Figure 6. Cardiovascular end points in the DEFINE (31) and ILLUMINATE (3) trials. The hazard ratios and 95% confidence intervals are shown. The promary endpoint in ILLUMINATE was time to first occurrence of a major cardiovascular event (MCVE) comprising a composite of death from coronary heart disease,
non-fatal myocardial infarction, stroke and hospitalization for unstable angina. The primary endpoint in DEFINE was time to first occurrence of a MCVE comprising death from cardiovascular causes, non-fatal myocardial infarction, hospitalization for unstable angina and non-fatal stroke. CETP-I denotes CETP inhibitor.
Fig 1
Fig 3

All tissues
Free cholesterol (FC)

Liver, intestine
Triglyceride (TG)

Free cholesterol (FC) → Cholesterol ester (CE) → Triglyceride (TG)

HDL
TRL, LDL

LCAT → CE
CETP

HDL

- Cholesterol efflux from macrophages in the artery wall
- Inhibit vascular inflammation
- Anti-oxidant
- Anti-thrombotic
- Promote endothelial repair
- Promote angiogenesis
- Enhance endothelial function
- Anti-diabetic

Fig 4
Fig 5

A

Glucose

Plasma glucose (mmol/L)

(p<0.0001 for all)

(ns)

A T/A

A T/A

A T/A

A T/A

0 1 3 6

Months of treatment

B

HbA1C

HbA1C (%)

(p<0.0001 for all)

(ns)

A T/A

A T/A

A T/A

A T/A

0 1 3 6

Months of treatment

C

Insulin

Insulin µU/ml

(ns)

(p=0.0007)

A T/A

A T/A

0 3

Months of treatment

D

HOMA-IR

HOMA-IR

(ns)

(p<0.0001)

A T/A

A T/A

0 3

Months of treatment
**ILLUMINATE Trial** (2007)  N=15,067 (torcetrapib)

Primary endpoint: MCVE

Revascularization

**DEFINE trial** (2010)  N = 1,623 (anacetrapib)

Primary endpoint: MCVE

Revascularization

CETP-I Better  CETP-I worse