

The end of the road for CETP inhibitors after torcetrapib?

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Purpose of review

Because high-density lipoprotein cholesterol (HDL-C) levels are inversely related to cardiovascular disease (CVD), raising HDL-C levels would seem intuitively valuable. However, the recent failure of the cholesteryl ester transfer protein (CETP) inhibitor torcetrapib to decrease CVD has raised doubts regarding HDL-C raising in general and CETP inhibition in particular for CVD prevention. We briefly discuss the complexity of HDL metabolism, caveats of CETP inhibition, possible mechanisms for torcetrapib's failure, and the potential utility of other CETP inhibitors.

Recent findings

Torcetrapib likely failed because of off-target effects, since other CETP inhibitors, such as dalcetrapib (JTT-705/R1658) or anacetrapib (MK-0859), do not increase blood pressure, a specific pressor effect of torcetrapib that appears to be CETP-independent. In small human trials of short duration, anacetrapib and dalcetrapib appear to improve the lipoprotein profile without obvious adverse effects, so far.

Summary

The relationship between HDL metabolism, pharmacologic CETP inhibition, and atherosclerosis requires further elucidation. There seems to be sufficient evidence that evaluation of CETP inhibitors such as dalcetrapib and anacetrapib should proceed, if cautiously, since it remains uncertain whether the increased CVD risk with torcetrapib was related to agent-specific off-target effects or more generally to CETP inhibition as a mechanism to raise HDL.

Keywords

anacetrapib (MK-0859), CETP inhibitor, dalcetrapib (JTT-705/R1658), HDL, torcetrapib

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Introduction

Statins reduce major cardiovascular disease (CVD) events and all-cause mortality by 21 and 12%, respectively, for every 1 mmol/l (39 mg/dl) of low-density lipoprotein cholesterol (LDL-C) lowering [1]. However, significant residual risk remains even in statin-treated patients. Because of the inverse relation between high-density lipoprotein cholesterol (HDL-C) levels and coronary heart disease (CHD) [2], there has been intense interest in developing pharmaceuticals to raise HDL-C levels, including agents that inhibit cholesteryl ester transfer protein (CETP) activity. However, patients treated with the CETP inhibitor torcetrapib, which raised HDL-C levels by approximately 70%, had a paradoxical increase in CVD outcomes [3]. Concurrent trials demonstrated a lack of benefit for torcetrapib in the surrogate endpoints of carotid [4,5] and coronary [6] atherosclerosis. This convergence of negative findings has led to a critical re-evaluation of the role of CETP inhibition as an appropriate mechanism to raise HDL-C and also of the role of HDL-C raising itself as an appropriate antiatherogenic strategy [7]. We briefly discuss the complexity of HDL

metabolism, caveats for CETP inhibition, possible reasons for the clinical failure of torcetrapib, and the potential utility of additional CETP inhibitors.

High-density lipoprotein and its role in atherosclerosis

High-density lipoprotein particles are heterogeneous in size, shape, density, and antiatherogenic properties. HDL inhibits LDL oxidation, a key initiator of atherosclerosis, and also hydrolyzes oxidized lipids, demonstrating its antioxidant and anti-inflammatory effects [8]. Furthermore, HDL can stimulate nitric oxide and prostacyclin production, inhibit monocyte adhesion to endothelial cells, and reduce platelet aggregation [9–12]. HDL is antithrombotic through several complementary mechanisms [13–17]. However, the primary antiatherogenic role of HDL is postulated to occur through reverse cholesterol transport (RCT).

Reverse cholesterol transport involves the movement of cholesterol from the periphery, such as arterial wall foam cells, to the liver and ultimately bile for excretion. The

multiplex RCT pathway includes: mobilization of cholesterol from cells to form early HDL particles; remodeling of HDL particles, including the activity of CETP; and disposal of HDL lipid content. It is yet to be determined which mechanism is most critical for ameliorating clinical CVD endpoints.

Laboratory determination of HDL-C simply quantifies the amount of cholesterol contained within the HDL fraction, an attribute that does not automatically correlate either with the number of HDL particles or with their net antiatherogenic properties. Furthermore, HDL can also become proatherogenic, particularly in the context of inflammation, which can cause either oxidation or glycosylation of apo A-I residues or alteration of the core lipid content [18]. Oxidation of HDL can further inhibit endothelial nitric oxide synthase [19] and promote expression of plasminogen activation inhibitor-1, which suppresses fibrinolysis [20]. Dysfunction or loss of HDL-associated enzymes occurs not only with inflammation, but also with type 2 diabetes and metabolic syndrome [18]. Thus, HDL-C levels alone might not reflect the functionality and net antiatherogenic properties of the diverse population of circulating HDL particles.

Cholesteryl ester transfer protein inhibition: antiatherogenic or proatherogenic?

Cholesteryl ester transfer protein is a hydrophobic glycoprotein that is secreted primarily from the liver and circulates bound mainly to HDL. During RCT, CETP facilitates the movement of cholesteryl esters from HDL to LDL, intermediate density lipoprotein (IDL), and very-low density lipoprotein (VLDL) in exchange for triglyceride. CETP plays several potential antiatherogenic roles, including: HDL remodeling, leading to the use of lipid-poor apo A-I for cholesterol efflux; shuttling cholesteryl esters to the apo B-containing lipoproteins, namely LDL, IDL, and VLDL, for excretion by the liver; and increasing the total pool of apo A-I and HDL. However, the normal actions of CETP are also potentially proatherogenic, since transfer of cholesteryl ester to apo B-containing lipoproteins could indirectly contribute to atherogenesis. Thus, the normal role of CETP likely reflects a fine balance between potential proatherogenic and antiatherogenic effects.

Cholesteryl ester transfer protein inhibition was recognized as a potential antiatherogenic strategy when rodents lacking plasma CETP activity were observed to have elevated HDL-C levels and resistance to diet-induced atherosclerosis [21]. Subsequently, some patients with naturally occurring germline *CETP* mutations and CETP deficiency demonstrated both elevated HDL-C levels and decreased CHD [22,23]. Japan

Tobacco (JTT-705, now Roche R1658 or dalcetrapib) and Pfizer (torcetrapib) then began development of pharmacologic CETP inhibitors. Importantly, both animal and human evidence that CETP inhibition is a viable and potent antiatherogenic strategy has been conflicting.

In rabbits, CETP inhibition decreased atherosclerosis [24–28]. However, studies involving transgenic CETP expression on different mouse genetic backgrounds have yielded conflicting findings [29–33]. Most recently, compared with atorvastatin, torcetrapib has been demonstrated in CETP transgenic mice to result in atherosclerotic lesions of a more inflammatory phenotype, despite reducing lesion size to a similar degree [34]. These disparate results in mouse models may be attributed in part to mechanisms such as ‘epigenetic’ and ‘position’ effects, or to the presence of ‘passenger’ genes [35,36].

The data linking CETP expression, HDL, and atherosclerosis have been similarly inconclusive in human studies. Whereas some patients with *CETP* mutations were reported to have elevated HDL-C levels and decreased CHD, patients with other *CETP* gene variants – namely I405V, D442G, c.629C→A, and *TaqIB* B2 – appeared to have increased CHD risk [22,23,37–40]. *CETP* haplotype analysis showed that men with the ‘deleterious’ haplotype had almost a six-fold increased risk of myocardial infarction (MI) per additional copy [Odds ratio (OR) = 5.98; 95% confidence interval (CI) 2.4–14.8] [41]. This association was independent of HDL-C levels [41]. Furthermore, a 10-year follow-up of 812 CHD patients treated with statins revealed that, despite elevations in HDL-C levels, *CETP* *TaqIB* B2 allele carriers had increased hazard ratios per allele for atherosclerotic disease death, CHD death, and all-cause mortality [42]. However, a recent meta-analysis of three common *CETP* genotypes and major clinical endpoints was inconclusive [43]. Thus, extrapolating a potential benefit of CETP inhibition from human genetic data is risky at best.

The failure of torcetrapib

In rabbits with diet-induced atherosclerosis, torcetrapib increased plasma HDL-C levels three-fold and reduced atherosclerotic plaque area by 60% [28]. Furthermore, in small early-phase human trials, torcetrapib increased HDL-C by up to 91% and decreased LDL-C by up to 42% at high doses, with no apparent adverse effects [44]. In 18 individuals with type 2B hyperlipidemia, torcetrapib significantly reduced postprandial levels of atherogenic triglyceride-rich lipoproteins and also their core cholesteryl ester content [45]. Yet, torcetrapib fell under closer scrutiny when phase II studies demonstrated increases in systolic blood pressure (SBP) and diastolic blood pressure (DBP) of 1.3–2.2 and 0.9–1.1 mmHg,

respectively, at doses of 60 or 90 mg/day [46,47]. In fact, approximately 4% of individuals experienced a greater than 15 mmHg SBP increase [48]. Consequently, torcetrapib was restricted to a dose of 60 mg/day for subsequent phase III trials.

Then, in December 2006, all trials with torcetrapib were terminated following an interim analysis of the Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events (ILLUMINATE) trial [3], in which 15 067 individuals with high CVD risk were randomized either to atorvastatin (10–80 mg/day) and placebo or atorvastatin and torcetrapib (60 mg/day). After 12 months, despite a 72.1% increase in HDL-C and 24.9% decrease in LDL-C levels, individuals in the torcetrapib arm had a 25% increased risk of CVD events (hazard ratio 1.25; 95% CI 1.09–2.19, $P=0.006$). Furthermore, both CVD and non-CVD deaths were higher in the torcetrapib arm compared with placebo [3]. These disappointing results were corroborated by concurrent trials evaluating the surrogate outcomes of coronary atherosclerosis, namely Investigation of Lipid Level Management Using Coronary Atherosclerosis by CETP Inhibition and HDL Elevation (ILLUSTRATE) [6] and of carotid atherosclerosis, namely Rating Atherosclerosis Disease Change with a New CETP Inhibitor (RADIANCE)-1 [4] and RADIANCE-2 [5].

Do pressor effects explain torcetrapib's failure to reduce cardiovascular disease events?

Explanations for the failure of torcetrapib include specific characteristics of the torcetrapib molecule, particularly a pressor effect that seems to be absent from other CETP inhibitors [49,50,51^{••}]. Importantly, despite restriction of torcetrapib to 60 mg/day, the mean SBP increases in RADIANCE-1, RADIANCE-2, ILLUSTRATE, and ILLUMINATE were 2.8, 5.4, 4.6, and 5.4 mmHg, respectively [3–6]. Five percent of individuals in RADIANCE-2 and 9% of individuals in ILLUSTRATE demonstrated a greater than 15 mmHg SBP increase [5,6]. Because every 10 mmHg SBP increase is associated with an approximately 25% increase in CHD, stroke, and vascular death, the pressor effect of torcetrapib might have neutralized any benefit from HDL-C elevation [52,53]. Torcetrapib's pressor effect was initially postulated to be due to some vascular 'off-target' drug effect, such as calcium-mediated vasospasm or hyperaldosteronism [48]. In fact, post-hoc analysis of the ILLUMINATE individuals revealed increased aldosterone levels in the torcetrapib group, although only approximately 87% of samples were analyzed for aldosterone and more than half of these had aldosterone levels below the lower limit of detection [3]. A potential mineralocorticoid effect was further supported by post-hoc analyses of

ILLUSTRATE, RADIANCE-1, and RADIANCE-2, which all demonstrated increased plasma sodium and decreased plasma potassium levels among individuals who received torcetrapib [54[•],55[•]].

Recently, these 'off-target' effects were studied mechanistically by Forrest *et al.* [51^{••}], who reported that torcetrapib increased BP both in wild-type mice, which normally lack CETP, and also in transgenic mice expressing simian CETP, indicating that the pressor effect is CETP-independent. Importantly, torcetrapib neither exerted a direct contractile effect on vascular smooth muscle nor did it increase BP through neurovascular, angiotensin type 1 or endothelin receptor effects [51^{••}]. Instead, torcetrapib was associated with approximately 3.5 and 2-fold increases in plasma levels of corticosterone and aldosterone, respectively. Furthermore, inhibiting these adrenal steroid metabolites with trilostane, a 3-beta-hydroxysteroid dehydrogenase inhibitor, did not inhibit the BP elevation associated with torcetrapib. In addition, the mineralocorticoid receptor antagonist epleronone did not inhibit torcetrapib's pressor effect. Torcetrapib seemed to increase aldosterone release via a direct action on adrenocortical cells and did not cause a pressor effect in acutely adrenalectomized rodents, indicating that the adrenals are central to torcetrapib's pressor effect [51^{••}]. Thus, the mechanism of the pressor effect seems to be CETP-independent and involves the adrenals, but remains uncharacterized.

Does high-density lipoprotein dysfunction explain torcetrapib's failure to reduce cardiovascular disease events?

An alternate explanation for the failure of torcetrapib to reduce CVD events has focused on HDL dysfunction, either through CETP inhibition causing alterations in RCT or through the activity of torcetrapib itself. Post-hoc analyses of the ILLUSTRATE [55[•]] and two RADIANCE trials [54[•]] were undertaken, in part, to characterize the relationships between lipid changes and changes in atheroma burden. In particular, the authors evaluated the relationship between HDL-C levels and atherosclerotic progression. Had disease progression in torcetrapib-treated patients correlated with the increase in HDL-C levels, a simple quantitative increase in HDL-C would not immediately translate into a qualitative antiatherogenic benefit. Conversely, any reduced disease progression associated with increasing HDL-C levels would provide circumstantial evidence that, in the context of CETP inhibition, HDL particles retained antiatherogenic functionality.

In the ILLUSTRATE trial, which assessed the effects of torcetrapib on coronary atherosclerosis using intravascular ultrasound, individuals in the highest quartile of

HDL-C change (>79% increase in HDL-C) demonstrated less coronary atherosclerotic progression compared with those in the lowest quartile (<32% increase in HDL-C): -0.31 ± 0.27 vs. $+0.88 \pm 0.27\%$ change in percentage atheroma volume (PAV) ($P=0.001$) [55[•]]. Furthermore, individuals who had the highest on-treatment HDL-C quartile (>86 mg/dl or >2.20 mmol/l) showed significant regression of PAV ($-0.69 \pm 0.27\%$, $P=0.01$), implying that HDL behaved appropriately and that the overall lack of atherosclerotic benefit in ILLUSTRATE might have been due to adverse effects of the torcetrapib molecule rather than the mechanism of CETP inhibition. Moreover, SBP change did not significantly predict PAV change in ILLUSTRATE [55[•]].

Meanwhile, in the post-hoc pooled analysis of the two RADIANCE trials, individuals with the highest SBP increase showed the greatest progression of carotid intima media thickness (cIMT) [54[•]]. Furthermore, in that analysis, there was no relation between HDL-C increase and decreased cIMT progression, perhaps signifying that CETP-inhibited HDL particles are dysfunctional in terms of their antiatherogenic properties [54[•]]. A major limitation of this analysis was the pooling of two distinct patient groups, namely those with familial hypercholesterolemia and those with mixed dyslipidemia [54[•]].

Conflicting findings have also been seen in other studies examining CETP and markers of RCT. For instance, HDL from CETP-deficient individuals in different experiments both impairs [56] and promotes [56,57] cholesterol efflux from cultured macrophages, although the use of a heparin-affinity column in the former study likely removed apo E-rich HDL [56]. Torcetrapib at a dose of 60 mg/day given to mildly hyperlipidemic patients increased cholesterol efflux efficiency [58], whereas another study of 19 individuals treated with torcetrapib 120 mg once or twice daily for 4 weeks showed no effect on RCT as assessed by fecal sterol clearance [59]. Furthermore, since CVD can be characterized by inflammation, which can in turn transform HDL particles into proatherogenic particles, raising HDL-C by CETP inhibition could preferentially have increased the fraction of proatherogenic HDL particles. Further subgroup analyses – with the caveats applicable to subgroup analysis – could help further define the reasons for the failure of torcetrapib, with implications for other CETP inhibitors (Table 1).

Emerging cholesteryl ester transfer protein inhibitors I: dalcetrapib

In rabbits with diet-induced atherosclerosis, dalcetrapib increased plasma HDL-C levels by approximately two-fold and reduced atherosclerotic plaque area by approximately 70% [27]. Meanwhile, another study in rabbits

with severe diet-induced hyperlipidemia showed that high-dose dalcetrapib did not alter atherosclerosis despite an increase in HDL-C levels of approximately 200% [60]. Furthermore, atheroma size was not correlated with either HDL-C levels or CETP activity, but rather with non-HDL-C levels, suggesting that non-HDL-C levels might be more directly relevant to atheroma formation in the context of severe hyperlipidemia [60].

In a phase II randomized clinical trial (RCT) of 198 individuals with mild hyperlipidemia, 900 mg/day of dalcetrapib reduced CETP activity by 37%, with a resulting 34% increase in HDL-C levels and 7% decrease in LDL-C levels [61]. Dalcetrapib was relatively well tolerated, but no CVD endpoints could be evaluated [61]. In another RCT of 155 individuals with mixed hyperlipidemia, when combined with pravastatin 40 mg/day, dalcetrapib was associated with a 28% increase in HDL-C levels and 5% decrease in LDL-C levels compared with placebo [49]. Dalcetrapib did not demonstrate a torcetrapib-like pressor effect, but again no clinical endpoints were evaluated. Importantly, a recent RCT of 18 individuals treated with dalcetrapib or placebo for 4 weeks demonstrated no improvement in the surrogate CVD marker of flow-mediated dilation (FMD) despite a 26% increase in HDL-C [62]. Subgroup analysis showed a 41% improvement in FMD among individuals with baseline HDL-C levels below the sample median (<1.19 mmol/l) compared with those above the median [62]. In addition to CETP inhibition, dalcetrapib has also been proposed to block cell proliferation and alter angiogenesis [63]. But, whereas dalcetrapib may provide some benefit to the surrogate marker of FMD among those with low HDL-C levels, larger trials of surrogate and clinical endpoints are required.

Emerging cholesteryl ester transfer protein inhibitors II: anacetrapib

Anacetrapib is currently the most potent CETP inhibitor under evaluation, with associated increases in HDL-C levels of up to 129% and decreases in LDL-C levels of up to 38% [50]. Two phase I RCTs for anacetrapib were recently published – one focused on dose-dependent lipid efficacy and the other on 24-h ambulatory BP monitoring [50]. After a 2-week diet run-in period, 50 individuals with dyslipidemia were randomized to placebo or one of four doses of anacetrapib (10, 40, 150, or 300 mg) taken daily for 28 days. The 10 mg dose increased HDL-C by 41%, whereas the 300 mg dose increased HDL-C by 129% with an associated 38% decrease in LDL-C. Meanwhile, in a 10-day randomized, double-blind, cross-over trial among 22 healthy individuals, anacetrapib 150 mg/day showed no BP effect, no serious adverse events, and no discontinuations [50]. The absence of a pressor effect was supported by another

phase I trial that evaluated doses up to 800 mg/day for 14 days [65]. The most common adverse effects were headache, taste change, abdominal discomfort, and loose stools [65]. The lack of pressor effect with anacetrapib, although promising, should be interpreted cautiously since the studies were small, of short duration, and excluded individuals with preexisting hypertension [50,65]. However, these results are generally supported by the model-system work of Forrest *et al.* [51^{••}], who demonstrated that anacetrapib had no pressor effect in normal and *CETP*-transgenic mice and did not elevate adrenal steroids, including aldosterone. But, whereas anacetrapib may indeed prove to be a useful CETP inhibitor [3–6,49,50,61], further safety and efficacy evaluation is required.

Emerging cholesteryl ester transfer protein inhibitors III: biological approaches

Cholesteryl ester transfer protein inhibition via antisense oligonucleotides (ASOs) or antibodies is also being actively investigated. Inhibition of hepatic CETP expression using CETP ASOs in HepG2 cells resulted in a 70% reduction in CETP mRNA levels, an increase in HDL formation, and decreased HDL catabolism [66]. Inhibition of human CETP by monoclonal antibodies demonstrated other benefits such as resistance of LDL particles to oxidative modification and decreased uptake of oxidized LDL by macrophages [67]. In rabbits, inhibition of CETP by ASOs [25] or by vaccine-induced antibody [24,26,68,69] formation led to reductions in aortic atherosclerotic lesions. Intranasal immunization in rabbits using antibody nanoparticles resulted in a 59% reduction in aortic atheroma [70]. However, a human phase I trial revealed that inducing immunogenicity might be difficult: only 3% of individuals injected a single time with a CETP vaccine developed antibodies, whereas a second injection resulted in anti-CETP antibody formation in 53% of individuals [71]. Thus, further study of biotherapeutic forms of CETP inhibition and their effect on atherosclerosis is required.

Conclusion

The role of CETP inhibition to reduce atherosclerosis requires further elucidation. Due to the biological complexity of HDL metabolism, merely raising HDL-C levels by inhibiting CETP activity may not be appropriate or sufficient. Perhaps the focus should be on evaluating the effects of CETP on HDL functionality. It remains essential to determine whether the failure of torcetrapib was due to molecule-specific off-target pressor or other adverse effects, or whether CETP inhibition is simply a flawed approach to prevent or reduce atherosclerosis. Answers to these questions will emerge from basic science studies, from deeper evaluation of

patients previously treated with torcetrapib, and from extensive phenotypic or phenomic analysis of patients treated with newer CETP inhibitors such as dalcetrapib and anacetrapib. Indeed, on the basis of the torcetrapib experience, any future HDL-based therapy must be critically evaluated for clear and demonstrable benefit early in development through either validated surrogate or clinical outcomes. Hence, the promising early data for dalcetrapib and anacetrapib need to be interpreted cautiously. Larger and longer-duration clinical trials evaluating validated surrogate or clinical outcomes are awaited. Thus, the road for CETP inhibitors, although bumpy, is not yet closed.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 392).

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