

The Failure of Torcetrapib Was it the Molecule or the Mechanism?

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Hopes have been running high that treatments aimed at raising HDL levels would soon help to reduce the large burden of cardiovascular disease that remains in patients at high risk of CHD who are now treated with statins. The unexpected and premature termination of the ILLUMINATE study has dashed those hopes. This study was designed to determine the clinical outcome of raising HDL by treatment with the CETP inhibitor torcetrapib in combination with atorvastatin. It had enrolled approximately 15 000 patients at high CHD risk who were randomized to treatment with torcetrapib (60 mg) plus atorvastatin versus atorvastatin alone (10 to 80 mg). As reported in the *Wall Street Journal* (Dec 4th), the study was terminated abruptly and unexpectedly after a little more than a year of treatment, because of an excess of deaths in the torcetrapib/atorvastatin versus atorvastatin groups (82 versus 51, respectively). Increases in heart failure, angina, and revascularization procedures were also observed. So is this the awakening from a dream of a highly effective way to raise HDL, or is it simply a nightmare created by unintended pharmacological effects of this particular CETP inhibitor? There is no clear answer to this question yet, but it is of interest to trace the development of CETP inhibitors, to reflect on the potential underlying reasons for the failure, and to ask if there is a way forward.

The idea of inhibiting CETP as a therapy to increase HDL and lower LDL levels emerged from studies that elucidated human genetic deficiency of CETP in the Japanese population.^{1,2} Heterozygotes with about 40% reductions in CETP had mean 30% increases in HDL levels and no changes in LDL-C, whereas homozygotes with complete deficiency had HDL increases of more than 100%, and 40% decreases in LDL-C and apoB levels, as well as decreased VLDL and IDL cholesterol levels, compared with unaffected family members.^{2,3} The HDL accumulating in CETP-deficient subjects was predominantly a larger HDL-2 particle, reflecting the decreased removal of HDL CE and slower catabolism of apoA-1.⁴ Although the lipoprotein profile resulting from genetic CETP deficiency was clear, it proved much more difficult to define the relationship between CETP deficiency and CHD. The existence of CHD in some CETP-deficient individuals with high HDL and multiple risk factors was clearly documented.⁵ A cross-sectional population study of

elderly men (mean age=78) of Japanese ancestry living in Hawaii showed an increased risk of definite CHD (MI, angina and revascularization) among 193 individuals heterozygous for CETP gene mutations, compared with 3276 wild-type controls.⁶ After adjustment for other risk factors the excess overall risk was moderate (RR=1.55, $P=0.024$), and was concentrated among men with mutations and HDL-C <60 mg/dL; men with HDL-C >60 mg/dL enjoyed a similar low prevalence of CHD, whether they had mutations or not. Among men with mutations, plasma CETP levels were reduced by 35% and the mean increase in HDL-C was about 10%, whereas there was no change in LDL-C. After exclusion of men with CHD, a subsequent prospective 10-year analysis of the remaining very elderly men (n=2340, 118 with mutations) showed a nonsignificant trend to lower CHD incidence in men with CETP mutations compared with the wild-type controls.⁷ Although the results of CETP SNP-CHD association studies in Caucasian populations have yielded mixed results, a meta-analysis suggested that the CETP Taq1B polymorphism located in the first intron is associated with higher HDL levels and reduced CHD.⁸ In summary, although the earlier studies on genetic CETP deficiency suggested some concern that deficiency might result in increased CHD,^{5,6} these studies had limited statistical power and were neither clearly confirmed nor refuted in subsequent work.^{7,8}

The relationship of CETP to atherosclerosis in animal studies also provided a mixed picture. Mice normally lack CETP and in CETP transgenic mouse models, CETP activity led to variable atherosclerosis results, while in rabbits CETP inhibition consistently led to reduced atherosclerosis. In the standard LDLR or apoE knock-out mouse atherosclerosis models, introduction of a CETP transgene led to a moderate less than 2-fold increase in atherosclerosis,⁹ whereas in the apoE*3 (Leiden) background, the increase in atherosclerosis was dramatic.¹⁰ However, in mice with hypertriglyceridemia attributable to apoCIII transgene overexpression, CETP activity led to either no change or a reduction in atherosclerosis.¹¹ Moreover, in a "humanized" transgenic mouse with combined hyperlipidemia, containing human apoA-1, apoCIII, and LDLR KO transgenes, CETP expression markedly reduced overall HDL levels, increased small HDL particles, and increased non-HDL cholesterol but did not increase atherosclerosis.¹² These findings are of interest because in the setting of hypertriglyceridemia, CETP activity leads to profound remodeling of HDL particles, especially on a human apoA-1 transgenic background, apparently leading to release of lipid-poor apoA-1.¹³ This is a dynamic in vivo process that depends on a continuous cycle of CETP-mediated exchange of VLDL TG for HDL-CE, followed by activity of hepatic

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(*Arterioscler Thromb Vasc Biol.* 2007;27:257-260.)

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Arterioscler Thromb Vasc Biol. is available at <http://www.atvbaha.org>
DOI: 10.1161/01.ATV.0000256728.60226.77

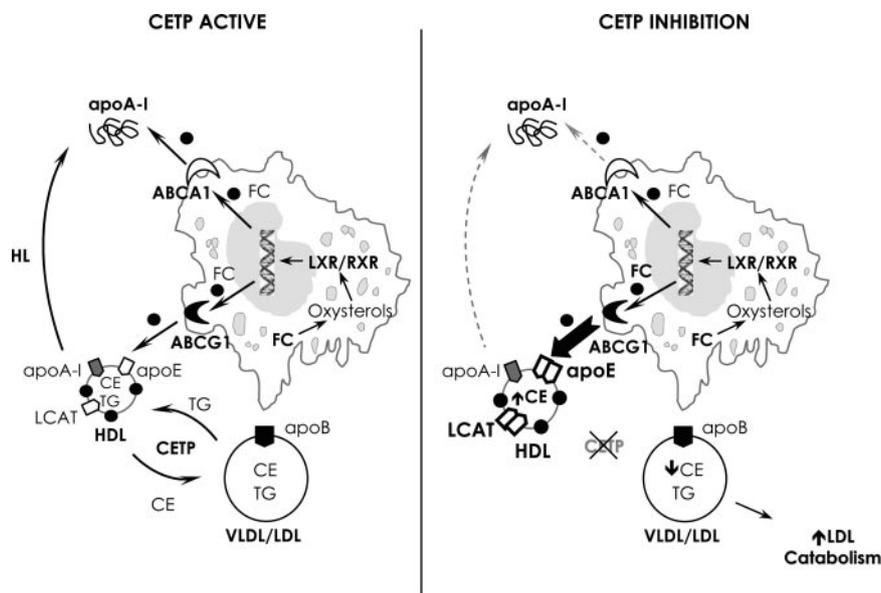


Figure 1. Role of CETP in regulating macrophage cholesterol efflux via ABCA1 and ABCG1. When CETP is active there is exchange of HDL cholesteryl esters (CE) for triglycerides in VLDL, leading to enrichment of HDL with triglyceride; subsequent lipolysis via various lipases may lead to release of lipid-poor apoA-1, providing substrate for ABCA1-mediated cholesterol efflux. Cholesterol efflux to HDL particles occurs via ABCG1 or via passive diffusion processes. Both ABCA1 and ABCG1 are induced in macrophage foam cells by the transcription factors LXR/RXR. When CETP is inhibited, there may be reduced regeneration of lipid-poor apoA-1 via lipid exchange-lipolysis, potentially resulting in less cholesterol efflux via ABCA1. At higher levels of CETP inhibition (probably >50%), large apoE- and LCAT-rich HDL particles accumulate and promote cholesterol efflux via ABCG1 and passive diffusion. We speculate that these particles may have a variety of antiatherogenic properties. In addition, there is reduced cholesterol in the VLDL/LDL fractions, as a result of decreased transfer of CE, increased catabolism of LDL particles, and possibly increased clearance of VLDL remnants by the liver.

lipase on HDL particles and subsequent release of lipid-poor apoA-1 (Figure 1). CETP inhibition in rabbits by a variety of different mechanisms, including different classes of small molecule CETP inhibitors, leads to a reduction in atherosclerosis.¹⁴ Importantly, treatment of cholesterol-fed rabbits with Torcetrapib increased HDL-C levels by about 4-fold without changing VLDL and LDL cholesterol levels, and led to a 60% reduction in atherosclerosis.²³ In contrast to mice, rabbits express CETP naturally, and thus could be considered a more appropriate model in which to examine CETP inhibition. However, unlike mice and humans, rabbits are deficient in hepatic lipase and thus may not rely on the CETP-hepatic lipase interaction to regenerate lipid-poor apoA-1 for interaction with ABCA1 (Figure 1).

An important antiatherogenic property of HDL is its ability to promote cholesterol efflux from macrophage foam cells in atherosclerotic lesions. An initial report indicated that HDL accumulating in subjects with homozygous CETP deficiency failed to promote cholesterol mass efflux from cholesterol-loaded human monocyte-macrophages.¹⁵ However, a more recent study of this question suggested the opposite, that HDL particles from subjects with complete CETP deficiency might have improved ability to promote net cholesterol efflux from macrophage foam cells, reflecting a high content of apoE and LCAT in the HDL particles, and enhanced formation of CE in cell media, driving cholesterol efflux from macrophage foam cells.¹⁶ The discrepancy between these reports appears to be explained by the fact that in the earlier study, HDL was passed over a heparin-affinity column, removing the crucial particles containing apoE.¹⁵ Although providing evidence for enhanced function of HDL in CETP deficiency, the cell

culture studies have limitations. Several pathways that mediate net cholesterol efflux from macrophage foam cells to HDL particles have been described.¹⁷ ABCG1 mediates net cholesterol efflux to large spherical HDL particles, and has a central role in supporting the increased cholesterol efflux to CETP deficient HDL particles.¹⁶ ABCA1 mediates cholesterol efflux to lipid-poor apoA-1, but not to large, mature HDL particles such as accumulate in CETP deficiency. However, cell culture studies may fail to capture the in vivo complexity related to continuous in vivo regeneration of lipid-poor apoA-1 as a substrate for ABCA1, dependent on CETP activity (Figure 1). Thus, it is theoretically possible that CETP inhibition results in a switch from an ABCA1 to an ABCG1 dependent pathway of cholesterol efflux in macrophages. Another unknown is the level of ABCA1 and ABCG1 in macrophage foam cells in lesions; these could become rate-limiting for efflux in the presence of higher levels of HDL. Finally, there is a poor understanding of how these lipid efflux pathways relate to potential antiinflammatory effects of HDL. HDL in some settings can acquire proinflammatory properties,¹⁸ and this deserves further evaluation in the setting of CETP deficiency/inhibition. On balance, though, the increase in HDL levels as well as an apparent improvement in HDL functionality reflecting increased amounts of apoE and LCAT in HDL-2 is likely to result in improved functional properties of HDL in CETP deficiency. There is no evidence at all for formation of an HDL particle that actually promotes macrophage foam cell formation or has vascular inflammatory effects.

In phase 2 clinical trials, torcetrapib was found to be effective at raising HDL, and also provided incremental

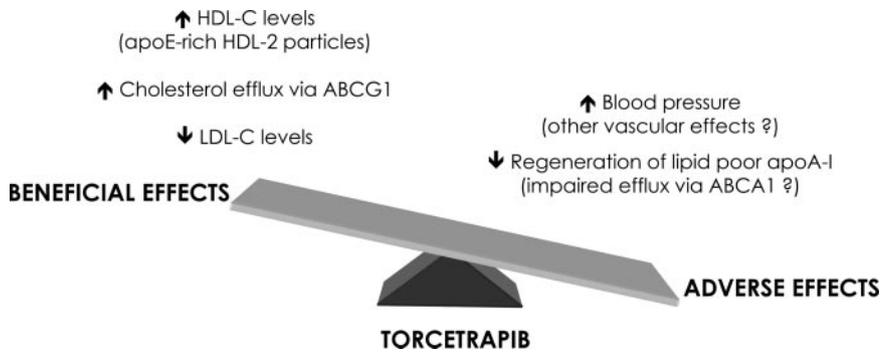


Figure 2. The adverse outcome of the ILLUMINATE study in patients receiving Torcetrapib indicates that potential adverse effects outweighed beneficial effects. Adverse effects may have included hypertension, other non-mechanism related adverse vascular effects, and the putative decrease in ABCA1-mediated cholesterol efflux reflecting diminished lipid exchange-lipolysis of HDL particles. Potential beneficial effects would include decreased levels of VLDL and LDL cholesterol and increases in large apoE-rich HDL particles. However, at the dose of Torcetrapib used (60 mg), the beneficial effects were likely not very pronounced.

lowering of LDL when added to atorvastatin, especially at higher doses.¹⁹ An important adverse side-effect of torcetrapib was the increase in blood pressure (BP). In phase 2 trials, this appeared to be small in magnitude perhaps 1 to 2 mm Hg in systolic BP.²⁰ But from the early phase 3 experience, the increase in BP was reportedly larger, on average 3 to 4 mm Hg. Moreover, about 4% of subjects in phase 2 experienced BP elevations in excess of 15 mm Hg. The hypertensive side-effect does not appear to be mechanism related, as genetic CETP deficiency does not result in hypertension, and some other CETP inhibitors with different chemical structures to torcetrapib do not elevate BP. A mean increase of 3 to 4 mm of BP in itself seems unlikely to completely explain the adverse outcome from ILLUMINATE, but there may have been a sizeable subset of patients with larger BP increases. Moreover, the BP increase could be just the sentinel of a more profound underlying adverse vascular effect of torcetrapib, such as vasospasm or activation of the renin-angiotensin system.

With the outcome of ILLUMINATE, the balance of adverse effects clearly outweighed any potential benefit of torcetrapib treatment (Figure 2). The adverse effects may have included hypertension and a putative reduced function of the ABCA1-cholesterol efflux pathway attributable to decreased *in vivo* regeneration of lipid-poor apoA-I (Figure 1 and 2). The potential beneficial effects likely included increased cholesterol efflux via the ABCG1 pathway and incremental reductions in LDL-C levels beyond the effects of atorvastatin alone. However, these beneficial effects may have been small at the dose used. Our unpublished cell culture studies suggest that at the 60 mg torcetrapib dose there is only a modest increase in net cholesterol efflux properties of HDL, related to increased HDL concentration, whereas at 120 mg torcetrapib, the apoE and LCAT content of HDL is increased and there is considerably increased cholesterol efflux potential of HDL via ABCG1.¹⁶ In addition, the apparent lack of increase of apoE in HDL at the 60 mg dose could be important, as apoE-HDL may compete for the retention of atherogenic lipoproteins on arterial matrix.²¹ In phase II studies with 60 mg Torcetrapib, the incremental LDL-C lowering was only about 8%.²⁰ The first phase 3 study in heterozygous FH however showed a greater benefit: 27% incremental reduction over atorvastatin. In summary, the adverse outcome in ILLUMINATE may have been the result of an adverse off-target drug effect (blood pressure and possible underlying vascular effects) as well as a relatively

low level of CETP inhibition, which may not have lowered non-HDL cholesterol levels by much, nor caused accumulation of antiatherogenic large apoE- and LCAT-rich HDL-2 particles, and may have caused a reduced level of cholesterol efflux via the ABCA1 pathway (Figure 2).

Where do we go from here? There is a strong likelihood that the ongoing intensive analysis of clinical responses and metabolic parameters in the ILLUMINATE study will provide insight into the reasons for the drug's failure. For example, it may turn out that adverse events were concentrated in subjects with a more pronounced hypertensive response and perhaps evidence of renal damage. Alternatively, it could be that subjects with hypertriglyceridemia and low HDL or diabetes had an adverse outcome, perhaps reflecting their reliance on a CETP remodeling-ABCA1 cholesterol efflux pathway, or a role of CETP in adipose tissue. Or something completely unexpected may turn up.

Phase III imaging studies using intravascular ultrasound (IVUS) or carotid ultrasound may also provide additional information. For example, they could show a paradoxical regression of atherosclerosis (for example in the FH heterozygote study where LDL-C lowering appears to be particularly dramatic).²⁴ This would be a clear case for moving forward with other CETP inhibitors. It seems more likely that imaging studies will reflect clinical outcomes and show no marked improvements or even deterioration. However, if adverse clinical and imaging outcomes appear to correlate strongly with hypertensive responses, this should not preclude further studies of CETP inhibitors that do not cause hypertension. A difficult situation will arise if the imaging studies suggest no improvement in atherosclerosis, and if there is also no clear indication that adverse events are related to an off-target effect of torcetrapib. Even though this outcome could still be related to suboptimal dosing and occult off-target effects of torcetrapib, it will be hard to generate the confidence to move forward. Additional insights could come from Roche's phase III studies with the structurally and mechanistically unrelated JTT-705.²² Because of the relatively modest effects of this inhibitor on HDL levels, these studies might show no particular benefit, but also no adverse outcome. In this case, the hypothesis of potential benefit derived from substantial incremental LDL lowering, as well as improved function of HDL at higher levels of CETP inhibition, could still be worth testing. In the setting of the adverse outcome of ILLUMINATE, other approaches to increasing HDL, macrophage cholesterol efflux, and reverse

cholesterol transport will likely receive more attention, including approaches to increasing apoA-I by infusion or synthesis, and induction of a variety of genes involved in macrophage cholesterol efflux, transport and excretion via LXR activation.

Disclosures

Alan Tall is a consultant to Pfizer, Merck, Boehringer-Ingelheim, AstraZeneca, and Amira.

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