The inhibition of cholesteryl ester transfer protein: A long and winding road

by

Kerry-Anne Rye\textsuperscript{1,2,3} and Philip J. Barter\textsuperscript{1,2}

\textsuperscript{1}Lipid Research Group, The Heart Research Institute, Sydney, NSW, 2050, Australia;
\textsuperscript{2}Faculty of Medicine, University of Sydney, NSW, 2006, Australia; \textsuperscript{3}Department of Medicine, University of Melbourne, Victoria, 3010, Australia

Corresponding author: K.-A. Rye, PhD, Lipid Research Group, The Heart Research Institute, 7 Eliza St, Newtown, Sydney, New South Wales, Australia, 2042.
Tel: +61 2 8208 8900; Fax: +61 2 9565 5584;
e-mail: ryek@hri.org.au or karye@ozemail.com.au
The presence in human plasma of a protein that promotes bidirectional transfers of neutral lipids (cholesteryl esters and triglycerides) between all lipoprotein particles was first reported in 1978 (1, 2). This protein was identified as cholesteryl ester transfer protein (CETP) and subsequently cloned in 1987 by Drayna et al. (3). CETP promotes the equilibration of cholesteryl esters and triglycerides between high density lipoproteins (HDL), low density lipoproteins (LDL) and triglyceride-rich lipoproteins (TRL), which include very low density lipoproteins (VLDL) that are produced in the liver, and intestinally-derived chylomicrons and chylomicron remnants. As most of the cholesteryl esters in plasma originate in HDL in the reaction catalysed by lecithin:cholesterol acyltransferase, and triglycerides mostly enter the plasma compartment as a component of TRL, this process of equilibration results in a net mass transfer of cholesteryl esters from potentially anti-atherogenic HDL particles to LDL and TRL, which are known to be atherogenic.

Subsequent studies of Taq1 B polymorphisms of the CETP gene provided the first indication of an inverse relationship between CETP gene expression and activity and plasma HDL-cholesterol levels (4). This raised the possibility that inhibition of CETP activity may increase the concentration of HDL, thereby reducing cardiovascular risk. This relationship is supported by a recent meta-analysis of 92 studies involving 113,833 participants, which concluded that people carrying CETP gene polymorphisms that are associated with decreased CETP activity and mass have elevated HDL-cholesterol levels and are at decreased risk of having a coronary event (5). A similar conclusion was drawn from an analysis of a cohort of 18,245 healthy Americans in the Women’s Genome Health Study (6).

A major advance in the CETP story came in 1990, with the identification of several unrelated families in Japan with CETP deficiency and high plasma HDL levels (7, 8). These
individuals also had reduced apoB and LDL levels, which was attributed to increased catabolism of apoB-containing lipoproteins (9). It was also noteworthy that these people did not have atherosclerosis or other cardiovascular diseases. These observations agree with the results from a recent meta-analysis of several human population studies showing that plasma HDL cholesterol levels are inversely correlated with cardiovascular risk (10). They also provided an incentive for testing the hypothesis that inhibition of CETP in humans is atheroprotective.

Initial preclinical studies were carried out in rabbits and hamsters using neutralising monoclonal antibodies to CETP, which resulted in delayed HDL clearance and increased plasma HDL levels (11, 12). Plasma HDL-cholesterol levels were also increased in cholesterol-fed rabbits treated with antisense oligonucleotides against CETP and vaccine-derived anti-CETP antibodies. More importantly, all of these interventions reduced atherosclerotic lesion development in this animal model (13, 14).

Meanwhile, a number of drug discovery programs were underway, with a view to producing pharmacological inhibitors of CETP for use in humans. The first report of reduced atherosclerosis following pharmacological inhibition of CETP in cholesterol-fed rabbits was published in 2000 (15). The inhibitor used in that study was JTT-705, now known as dalcetrapib. JTT-705 contains a thioester group that inhibits CETP activity by forming a disulphide bond with cysteine 13 of the protein. It also reduces atherosclerotic lesion size by approximately 70% in cholesterol-fed rabbits (15).

Torcetrapib, another CETP inhibitor that was developed at around the same time, was shown to attenuate neutral lipid transfers by a somewhat different mechanism that involved the
formation of a complex with CETP and HDL (16). Preliminary studies of torcetrapib in humans established that it was well tolerated (17). It also inhibited atherosclerosis in cholesterol-fed rabbits (18). In addition to raising HDL-cholesterol levels, torcetrapib when administered to humans either as a monotherapy (17, 19, 20), or in combination with atorvastatin (19-21), had the added benefit of substantially reducing LDL-cholesterol levels, as well as the levels of other apoB-containing lipoproteins.

It was thus quite unexpected to find that torcetrapib treatment in humans did not reduce atherosclerosis in three imaging trials, and caused serious harm in a large clinical endpoint trial (22-24). While these finding resulted in termination of the torcetrapib development program, subsequent investigations revealed that off-target adverse effects that were unrelated to CETP inhibition may have been responsible for the harm caused by the drug (25). This possibility resurrected interest in CETP inhibition as a possible cardioprotective strategy, and the hypothesis is currently being tested with other CETP inhibitors, including dalcetrapib and anacetrapib, that do not share the off-target, adverse effects of torcetrapib (26, 27).

Despite its serious adverse effects, the aforementioned studies with torcetrapib have provided important insights into the underlying mechanisms by which CETP inhibition increases HDL levels and reduces the levels of LDL and other apoB-containing lipoproteins. For example, it was established using primed constant infusions of (5,5,5-2H3)-L-leucine, that the increase in plasma HDL-cholesterol levels in torcetrapib-treated subjects was caused by delayed apoA-I catabolism, irrespective of whether torcetrapib was given as monotherapy or in combination with atorvastatin (28).
The underlying reasons for the torcetrapib-mediated reduction in LDL-cholesterol and apoB-containing lipoprotein levels were, by contrast, more complex. Treatment with torcetrapib alone increased the clearance of apoB-100 in intermediate density lipoproteins (IDL) and in LDL, presumably via increased expression of the LDL receptor (20). VLDL apoB-100 clearance was also increased, possibly because the torcetrapib-mediated reduction in CETP activity inhibited the transfer of triglycerides out of the particles, which converted them into excellent substrates for lipoprotein lipase. Administration of torcetrapib in combination with atorvastatin also increased the clearance of apoB-100 in VLDL. However, in contrast to what was reported for treatment with torcetrapib alone, administration of torcetrapib in combination with atorvastatin reduced apoB-100 levels by increasing VLDL apoB-100 clearance and by reducing the production of apoB-100 in IDL and LDL (20).

ApoB-48 is a C-terminally truncated version of apoB-100. It is encoded by the same gene as apoB-100, but is generated by a premature stop codon in intestinal apoB mRNA that causes apoB translation to terminate prematurely at residue 2152. ApoB-48 is associated with intestinally-derived lipoproteins that also contain apoE, such as chylomicrons and their catabolic products, chylomicron remnants. As the LDL receptor binding domain is missing from apoB-48, the lipoproteins with which it associates are removed from the circulation by receptors that recognise apoE.

Primed constant infusions of stable isotopes in normolipidemic humans have established that apoB-100 and apoB-48 are cleared from the circulation at comparable rates (29). This suggests that the reduction in plasma levels of apoB-48 and apoB-100 in people treated with torcetrapib alone, or in combination with atorvastatin, should also follow similar kinetics. However, an article by Diffenderfer et al. in the current issue of this journal indicates that this
is not the case. Quite unexpectedly, this report shows that torcetrapib monotherapy reduces the level of apoB-48-containing lipoproteins levels by decreasing the rate of apoB-48 production and without changing the fractional catabolic rate of apoB-48. Moreover, this effect was no longer apparent in people that were treated with torcetrapib in combination with atorvastatin. This is the opposite of what the same authors reported previously, where torcetrapib monotherapy lowered the levels of apoB-100-containing lipoproteins by increasing their fractional catabolic rate (20). As the samples that Diffenderer and colleagues used in the present study were obtained from the same subjects in which apoB-100 kinetics were investigated (20), this unexpected observation cannot be attributed to a population effect. While the authors do not describe a definitive mechanism for this unexpected and rather surprising finding, they speculate that it may be a reflection of decreased availability of intestinal lipids and enhanced intracellular degradation of apoB-48 in people treated with torcetrapib. This remains to be demonstrated directly.

Although the present study has some important limitations, including a small number of subjects and an absence of a definitive mechanism for the reduction in apoB-48 production, it is nevertheless extremely thought provoking. It remains to be seen if the discrepancy regarding the effect of treatment with torcetrapib on the kinetics of apoB-100- and apoB-48-containing lipoproteins can be recapitulated in a different, and preferably larger, cohort. It also remains to be seen if this effect is also observed with the other CETP inhibitors that are currently being investigated in large-scale clinical outcome trials. Irrespective of whether or not this turns out to be the case, the present report provides compelling evidence that there are many metabolic and biological aspects of CETP inhibition that are not understood. We anticipate many more surprises will emerge from future studies of CETP inhibitors.
References


