Cholesteryl Ester Transfer Protein Inhibition
Effect on Reverse Cholesterol Transport?

Patrick C.N. Rensen, Louis M. Havekes

Statins, inhibitors of the key enzyme in the biosynthesis of cholesterol (ie, 3-hydroxy-3-methylglutaryl [HMG]-coenzyme A [CoA] reductase), are widely used as the prevailing strategy to combat atherosclerosis through reducing LDL cholesterol levels. Large-scale clinical trials have shown that statins markedly reduce coronary events.

A meta-analysis of 22 studies enrolling nearly 70 000 individuals indicated that an effective decrease of LDL cholesterol by 20% to 40% reduces non-fatal myocardial infarction or coronary heart disease mortality by 25%. Although of clear clinical benefit, lowering LDL cholesterol alone using statin monotherapy thus does not prevent 75% of all cardiovascular events, which has led to a considerable interest in targeting other lipid-related risk factors.

Prospective epidemiological studies have shown a strong inverse correlation between HDL cholesterol levels and cardiovascular disease. In fact, a low HDL level, often seen in combination with elevated triglyceride (TG) levels, is the primary lipid abnormality in ~50% of men with coronary heart disease.

Recent studies revealed that high HDL cholesterol levels are indeed protective against plaque progression. It has been shown that pharmacological intervention leading to an increase of HDL cholesterol by 1% is associated with a 2% to 3% decrease in cardiovascular risk.

Although the exact mechanisms through which HDL protects are unclear, HDL has been shown to have antioxidant, antiinflammatory properties, and to mediate the reverse cholesterol transport (RCT).

Based on these beneficial properties of HDL, pharmacological raising of HDL cholesterol levels is thought to hold promise in the treatment of atherosclerotic cardiovascular disease.

Current therapies with statins, fibrates, and nicotinic acid (niacin), which aim at targeting LDL/VLDL metabolism, appear to raise HDL as an advantageous side effect. Meta-analyses of trials with statins, fibrates, and niacin showed an increase of HDL cholesterol of ~5%, 10%, 14%, and 16%, respectively.

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To date, there exists no therapy that is specifically designed to raise HDL cholesterol levels, but CETP inhibition is making strong headway over the last few years with phase III trials ongoing. CETP is mainly produced by the liver and macrophages and plays an important role in HDL metabolism. In plasma, CETP facilitates the transfer of TG and cholesteryl esters (CE) between apoB-containing lipoproteins and HDL, resulting in the net transfer of CE mass from HDL to apoB-containing lipoproteins.

By decreasing HDL cholesterol, CETP may thus represent an atherogenic factor. Genetic association studies in humans showed an association of the C-629A promoter variant of CETP with higher CETP levels, lower HDL cholesterol levels, and an increased progression of CAD. Likewise, increased CETP activity was associated with increased risk for CAD in subjects with elevated TG. Although studies in apoCIII-transgenic mice and LCAT-transgenic mice showed that human CETP expression reduced atherosclerosis, expression of CETP in mice that are hyperlipidemic because of attenuated hepatic uptake of apoE-containing lipoprotein remnants (ie, apoE-deficient and LDL receptor–deficient mice) increased atherosclerosis.

Recent studies from our laboratory have shown that human CETP expression in hyperlipidemic APOE*3-Leiden transgenic mice, with a human-like lipoprotein profile, also causes a tremendous increase in atherosclerosis development (Marit Westerterp, Caroline C. van der Hoort, L.M.H., P.C.N.R., unpublished observations).

Based on these data, CETP inhibition is now widely regarded as a promising novel therapeutic target for raising HDL cholesterol to inhibit atherosclerosis.

Indeed, initial clinical studies with the first two available CETP inhibitors (ie, JTT-705 and torcetrapib) demonstrated a marked increase in HDL cholesterol levels. JTT-705 (900 mg once daily) increased HDL cholesterol (34%) and decreased LDL-cholesterol (7%). Torcetrapib (120 mg once daily) increased HDL cholesterol (46% to 73%), apoAI (16% to 24%), and apoE (47%), as reflected by the accumulation of larger HDL particles, and concomitantly reduced LDL-cholesterol (8% to 21%) and apoB (10% to 12%). Dosing twice daily raised HDL cholesterol even further (91% to 106%). It is, however, still unknown whether these potent effects of CETP inhibition will be pro- or antiatherogenic. For example, CETP
mutations have been described that associate with decreased CETP activity, increased HDL cholesterol, and increased CAD. Second, although one hallmark study in cholesterol-fed rabbits did show that JTT-705 decreased atherosclerosis, no effect was observed in a subsequent study using more severely hypercholesterolemic rabbits despite a 2.5-fold increase in HDL cholesterol.

A major concern with the use of CETP inhibitors as a strategy to raise HDL cholesterol levels is the fact that the serum HDL level does not necessarily reflect its functional benefit, eg, with respect to the role of HDL in RCT. On accepting cholesterol from peripheral cells such as macrophages in the arterial wall, HDL can deliver its CE to the liver by selective uptake via SR-BI and possibly also CETP (ie, direct pathway), or after CETP-mediated transfer of CE to apoB-containing lipoproteins that are subsequently taken up by the liver (ie, indirect pathway) (see Figure). Inhibition of CETP will reduce the indirect pathway and may thus compromise the overall flux of HDL cholesterol to the liver. In fact, a recent kinetic analysis study in humans indicated that not HDL but apoB-containing lipoproteins account for the clearance of CE from the circulation. In addition, a recent study indicated that plasma CETP may directly mediate the hepatic uptake of HDL-CE, independent of the LDL receptor or SR-BI. Therefore, elevation of HDL by inhibition of CETP may reduce the delivery of HDL-derived CE to the liver. This concern is underscored by the observation that CETP inhibition generally results in the accumulation of relatively large HDL particles, which may be less effective in accepting cholesterol from peripheral tissues. In this regard, it may be noted that such large HDL are associated with increased atherosclerosis in SR-BI knock out mice.

In this issue of *Atherosclerosis, Thrombosis, and Vascular Biology*, Kee et al have addressed the question whether CETP inhibition will compromise the overall removal of HDL-CE from plasma. Hereto, rabbits were fed a chow diet supplemented with an oral CETP inhibitor that resembles torcetrapib with respect to enhancing the association of CETP with its substrates, thereby creating a nonproductive CETP-lipoprotein complex. The CETP inhibitor (5 mg/kg) resulted in a strong decrease in CETP activity (≈90%) and an increase in HDL cholesterol (≈100%) and apoAI (≈60%). By compartmental modeling of the decay curves resulting from intravenous injection of [*H]*CE-labeled native HDL (nHDL) or reconstituted spherical HDL (rHDL), the authors determined the rate of HDL-CE removal from plasma via the direct pathway and indirect pathway. In untreated rabbits, the indirect pathway contributed 25% and 47% of the total HDL-CE removal, as judged from the fitted decay curves of nHDL and rHDL, respectively. As expected, CETP inhibition resulted in a virtually complete inhibition of the transfer of [*H]*CE from HDL to apoB-containing lipoproteins. Concomitantly, the direct clearance pathway was increased by 18% (nHDL) or 65% (rHDL). These combined effects led to a total HDL-CE removal rate that was not significantly lower as observed in the untreated rabbits. The effects of CETP inhibition on HDL-CE fluxes are summarized in the accompanying figure.

Although Kee et al convincingly show that the direct pathway of HDL-CE removal is increased on CETP inhibition, the implication of this effect for RCT and atherosclerosis development should be interpreted with care for several reasons. First, although CETP inhibition does not affect the total HDL-CE removal rate significantly, a consistent trend toward reduction (13%) is observed using either nHDL or rHDL, which may reach statistical significance by using larger groups of rabbits. It should especially be noted that in the study using labeled autologous HDL (nHDL), which accurately reflects the behavior of the endogenous HDL-CE pool, the total HDL-CE removal rate under CETP-inhibited

A. basal

B. CETP inhibition

Schematic diagram of reverse cholesterol transport. Kee et al have studied the effect of CETP inhibition on HDL-CE clearance pathways (see boxed squares). By kinetic analysis, they observed that HDL-CE is cleared from the circulation both directly (75%; presumably by selective delivery to the liver) and indirectly (25%; after transfer of HDL-CE to apoB-containing lipoproteins) (panel A). Inhibition of CETP, which almost completely blocks the indirect uptake route, increased the rate of CE removal via the direct pathway (from 1.37 to 1.61 μmol/L per min), resulting in an unaltered overall serum clearance of HDL-CE (panel B). See text for further details.
conditions (1.61 μmol/L per min) is just in between the values of the direct removal (1.37 μmol/L per min) and total removal (1.84 μmol/L per min) as observed in untreated rabbits. Second, by investigation of the impact of CETP inhibition on the serum clearance of HDL, the authors focus on only one of the final steps of RCT. It remains to be investigated whether CETP inhibition affects the uptake of cholesterol from extra-hepatic cells, and in particular from macrophages in the arterial wall. It is also not clear from this study whether the actual hepatic uptake of CE and subsequent fecal excretion of the cholesterol will be affected by CETP inhibition. A recent study in humans showed that CETP inhibition alone, by using torcetrapib, did not affect fecal excretion.38 However, in combination with atorvastatin, CETP inhibition resulted in a significantly reduced fecal output of bile acids (−16%) and neutral sterols (−8%).38 This is consistent with the recent observation that, in humans, apoB-containing lipoproteins are mainly responsible for the CE output via lipoproteins to the liver,32 and would imply that pharmacological CETP inhibition could indeed affect the normal uptake of CE via the LDL receptor pathway, which should be addressed by future studies.

In conclusion, Kee et al37 observed that CETP inhibition in rabbits does not necessarily decrease the overall removal rate of HDL-CE from plasma. However, the impact of this observation on RCT, including the critical efflux of cholesterol from the arterial wall, is still obscure. The effects of CETP inhibition on the integrative RCT from macrophages to the liver and feces in vivo, and on other functional parameters of HDL, including its antioxidant, antithrombotic, and anti-inflammatory properties,39 should thus be subject of future investigation. In addition, given the recent evidence that the cholesterol output from plasma may differ between rabbits and humans,34 additional research will be required to elucidate whether these findings can be extrapolated to the clinical setting. The effects of CETP inhibition on (surrogate) cardiovascular end points in humans are eagerly awaited, and those data will ultimately indicate whether CETP inhibitors can become new powerful therapeutic tools for both raising HDL cholesterol and decreasing atherosclerosis, most likely in combination with drugs that lower apoB-containing lipoproteins, such as statins or fibrates.

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