High-density lipoprotein mass, cholesteryl ester transport protein, and macrophage reverse cholesterol transport: from the bedside back to the bench

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In December 2006, the ILLUMINATE study was unexpectedly terminated because of excess mortality in the treatment group. In this study, which enrolled more than 15 000 patients, the leading cholesteryl ester transport protein (CETP) inhibitor Torcetrapib, plus Atorvastatin, was compared with Atorvastatin plus placebo in a high cardiovascular risk population. An impressive response with a 72.1% increase in high-density lipoprotein (HDL)–cholesterol accompanied by a 24.9% decrease in low-density lipoprotein (LDL)–cholesterol was demonstrated. However, a 25% increased rate of cardiovascular events and a 40% increase in cardiovascular death occurred, and mortality due to non-cardiovascular events was doubled. Other trials failed to show any benefit of Torcetrapib in the postponement of atherosclerosis in the common carotid and coronary arteries. Off-target effects of CETP inhibition were demonstrated and thorough discussions of cholesterol metabolism followed. In effect, the ILLUMINATE study brought a dramatic end to three decades of enthusiasm about the ‘Gordian knot’ between the unlimited protection of high HDL mass, measured by its cholesterol content, against cardiovascular events. Also, the traditional assumption that HDL elevation, such as in the case of CETP inhibition, will lead to the same protective effect was seriously challenged.

Reverse cholesteryl ester transport (RCT) refers to the process that returns cholesterol from extra-hepatic peripheral tissues to the liver for excretion. The anti-atherogenic capacity of HDL relies on its ability to support cholesterol efflux from cholesterol-loaded arterial macrophages, which play a central role in the development of atherosclerosis. HDL can act via a direct pathway that delivers cholesteryl ester (CE) to the liver by scavenger receptor class B type-1 (SR-B1) or by an indirect pathway that transfers CE to apo-B-containing lipoproteins (VLDL, LDL) in exchange for triglycerides. This pathway leads to decreased levels of HDL and smaller HDL particles. (Noteworthy is the opposite composition of CE-rich, large HDL particles which may compromise their function.)

Cholesteryl ester transport protein participates in both pathways and ultimately is responsible for the recycling and generation of lipid-poor apo A1 cholesterol-acceptor molecules, which are lipidated by the orchestrated interaction with both ATP-binding cassette transporter A1 (ABCA1) and the enzyme lethicin cholesterol acyltransferase. CETP involvement in atherogenesis is complex. Whether it is pro- or anti-atherogenic depends on species characteristics and the metabolic environment. Mice lack CETP, and in CETP transgenic models there have been mixed results. Combined with an atherogenic background, introduction of CETP to knock-out (KO) models leads to moderate to severe atherosclerosis. The opposite occurs in the case of a hypertriglyceridemic background. In rabbit, the CETP inhibition leads to reduced atherosclerosis. Unlike humans, rabbits express CETP but are deficient in hepatic lipase, which is needed for the delipidation of the particle (in addition to endothelial lipase). This may limit the regeneration of delipidated Apo A-1 for interaction with ABCA1. In humans, genetic deficiency in CETP increases HDL and the risk of coronary artery disease. In cases of extremely high HDL (hyperalphalipoproteinemia), the lipoproteins conferred pro-atherogenic features that were corrected by the addition of pure CETP to the plasma. Recently, HDL from CETP-deficient subjects was shown to markedly increase cholesterol efflux from macrophages. This proved to be an ABCG1-dependent pathway (an oxysterol-induced macrophage RCT system activated by the liver-X-receptors).

The article by Tchoua et al. in this issue enters the stage at a time when more information on the role of CETP in RCT and atherosclerosis is critical. The authors approach two important issues in HDL–CETP action and inhibition: the first relates to the impact on intracelulular lipid metabolism and the second relates to HDL functionality as a cholesterol transport: from the bedside back to the bench.
acceptor. Intracellular cholesterol metabolism in macrophages and hepatocytes was not affected by CETP inhibition. Furthermore, cholesterol efflux was fully explained by variations in HDL-C levels, arguing against impairment of HDL functionality by CETP inhibition. In respect to in vivo cholesterol efflux, the authors compared two animal species. One is a mouse model in which only the direct pathway of cholesterol delivery to the liver via SR-B1 exists (as a result of lack of CETP). The other is a newly introduced hamster model in which both the direct and indirect pathways operate, similar to the case in humans. For cholesterol efflux analysis, a validated model by Rader et al. was used to assess cholesterol and bile acid excretion in faeces. The CETP activity and inhibition were investigated in the mouse model without increasing HDL. The insertion of human CETP resulted in elevated cholesterol levels in plasma and liver, and augmented faecal bile acids. Concomitantly, the HDL to non-HDL ratio was decreased. Thus, the operation of the alternative pathway via apo-B-containing lipoproteins and LDL receptors led to enhanced RCT in this species. Torcetrapib reduced the cholesterol excretion in faeces as well as the ratio of HDL to non-HDL cholesterol in plasma, suggesting that Torcetrapib was pro-atherogenic in this setting. In the hamster model, in contrast to mice, CETP inhibition resulted in enhanced cholesterol excretion with a significant increase in the HDL to non-HDL ratio. Thus, in this species, RCT was promoted by Torcetrapib.

On the basis of these types of experiments, CETP metabolism in humans may be better illuminated. The concept that CETP belongs to a system that facilitates RCT is strengthened by mice expressing CETP combined with LDL and SR-B1 KO. With this background, CETP promoted macrophage RCT despite a reduction in HDL. Active LDL receptors were required for this effect. In humans, similar to the hamster model, CETP inhibition enhances the cholesterol flow to the liver by increasing HDL cholesterol availability. This mechanism may be counterbalanced by shutting off the transfer of cholesterol to apo-B lipoproteins, limiting RCT by LDL receptors. In the case of statins, which are unique in their efficient LDL reducing capacity by up-regulation of LDL receptors, a modest increase in HDL-C is evident. This HDL elevation follows a decrease in CETP mass and activity. In support of this concept, Atorvastatin treatment of mice expressing human CETP resulted in increased HDL-C by reducing CETP and CE transfer from HDL to LDL.

In summary, the contribution of HDL to different cholesterol pathways in humans is open for further research and debate. Obtaining additional data on diverse metabolic backgrounds with dysfunctional HDL/high triglycerides/small dense LDL, which are prevalent in obesity, metabolic syndrome, and diabetes, will be necessary. Also, the effects of raising HDL on its anti-oxidative and anti-inflammatory properties are not well defined. Meanwhile, the unpredicted behaviour of CETP in relation to RCT and atherosclerosis teaches us a lesson. It is an excellent time to go back and forth between the bedside and the bench.

References