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Cholesteryl ester transfer protein (CETP) inhibition beyond raising HDL cholesterol levels

Pathways by which modulation of CETP activity might alter atherogenesis

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ABSTRACT

Raising high-density lipoprotein (HDL) cholesterol is a promising strategy in the struggle to prevent cardiovascular disease, and cholesteryl ester transfer protein (CETP) inhibitors have been developed to accomplish this. The first results are encouraging, and in rabbits, inhibition of CETP reduces atherosclerosis. Because human data regarding the reduction of atheroma burden require more time, the biochemical mechanisms underlying the putative atheroprotection of CETP inhibitors are currently dissected, and several pathways have emerged. First, CETP inhibition increases HDL cholesterol and reduces low-density lipoprotein (LDL) cholesterol levels consistent with CETP lipid transfer activity and its role in reverse cholesterol transport (RCT). This coincides with putative beneficial increases in both HDL and LDL size. However, many aspects regarding the impact of CETP inhibition on the RCT pathway remain elusive, in particular whether the first step concerning cholesterol efflux from peripheral tissues to HDL is influenced. Moreover, the relevance of scavenger receptor B-I and consequently the central role of HDL in human RCT is still unclear. Second, CETP inhibition was shown recently to increase antioxidant enzymes associated with HDL, in turn associated with decreased oxidation of LDL. Atheroprotection in man is currently anticipated based on the improvement of these biochemical parameters known to influence atherosclerosis, but final confirmation regarding the impact of CETP inhibition on cardiovascular outcome will have to come from trials evaluating clinical end points.

Abbreviations – ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; ABCG4, ATP-binding cassette transporter G4; CAD, coronary artery disease; CETP, cholesteryl ester transfer protein; HDL, high-density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LDL, low-density lipoprotein; RCT, reverse cholesterol transport
CETP beyond raising HDL cholesterol

**REVIEW**

*Introduction*

Inhibition of cholesteryl ester transfer protein (CETP) holds promise as a novel approach to prevent coronary artery disease (CAD) because of its profound effect on high-density lipoprotein (HDL) cholesterol levels. The scope of this review is to discuss the impact of CETP inhibition beyond this primary effect. The physiological function of CETP in the circulation, that is transfer of neutral lipids (cholesteryl esters and triglycerides) between lipoproteins, and the causes and effects of natural variation in CETP activity, has been the subject of many reviews on CETP and CETP inhibition (1-6). Therefore, we have chosen to summarize the current understanding of lipoprotein remodelling by CETP (schematically presented in the figure). Importantly, in dyslipidemias characterized by increased concentrations of triglyceride-rich particles (mainly very low-density lipoprotein (VLDL) and VLDL remnants), the cholesteryl ester transfer from HDL shifts from LDL toward VLDL, particularly large VLDL1 (7, 8) (figure, panel 2). This is considered to be pro-atherogenic because cholesteryl ester-enriched VLDLs become substrates for hepatic lipase, resulting in the generation of deleterious small dense LDL (9). Recently, increased CETP activity was indeed associated with increased risk for CAD in subjects with elevated triglyceride levels (10). Taking this together, it could be hypothesized that in hypertriglyceridemic individuals, CETP inhibition may be anti-atherogenic by reducing small dense LDL formation. Recent data on CETP inhibition in rabbits and humans show that pharmacological inhibition of CETP has similar effects on the lipid profile as naturally occurring CETP deficiency (11). Notably, next to the marked increases in HDL cholesterol and HDL size, the CETP inhibitor torcetrapib induced a significant increase in LDL size, caused by both an increase in large LDL particles and a decrease in small LDL particles, suggesting a less atherogenic lipid profile (12). Recently, similar effects were reported for JTT-705 (13). In this review, we focus on how changes in lipoprotein concentrations and lipoprotein neutral lipid content after CETP inhibition may influence cholesterol exchange processes between the periphery, the circulation, and the liver, thereby covering the reverse cholesterol transport (RCT) pathway. We furthermore discuss the effects of CETP inhibition on fecal sterol excretion. Finally, we briefly touch on the effects of CETP inhibition on the anti-inflammatory and anti-oxidative properties of HDL.

*CETP inhibition and RCT*

RCT is generally invoked to provide the rationale for the anti-atherogenic properties of HDL. It concerns the removal of excess cholesterol from peripheral tissues for elimination by the liver and secretion into bile (figure) (14). Many of its players have been identified, and their particular functions are confirmed by in vitro and animal studies (15). Panel 1 of the figure illustrates the most direct route for removal of cholesterol in humans: the ATP-binding cassette transporter A1 (ABCA1) shuttles cholesterol from peripheral cells to lipid-poor apolipoprotein A-I. Next, lecithin:cholesterol acyltransferase (LCAT) esterifies free cholesterol, and the resulting cholesteryl esters induce maturation of HDL particles. These HDLs can deliver cholesterol to the liver via the
scavenger receptor B-I as shown in mice (16). In humans and rabbits, the presence of CETP in the circulation creates a major diversion from this route. In exchange for triglycerides, CETP facilitates transfer of cholesteryl esters from HDL to apolipoprotein B-containing particles, which can also be taken up via the LDL receptor. Ultimately, excess liver cholesterol can be excreted as neutral sterols and bile acids into bile for removal via the feces. Although this model is widely accepted, it is of note that there exists very little experimental evidence that HDL is indeed central to this dynamic process in humans. Nevertheless, RCT provides an excellent tool to discuss the effects of CETP inhibition on HDL metabolism, and we review the role of CETP in each step of this pathway.

**Cholesterol efflux from peripheral tissues to HDL**

One of the atheroprotective properties of HDL is its ability to act as an acceptor of cholesterol from peripheral cells, or specifically macrophage foam cells in the arterial wall. This initial step in RCT depends mainly on the presence of cholesterol transporters in the cell membrane and the presence and avidity of extracellular acceptor particles, mostly thought to be HDL (17). CETP has been suggested to affect this process by changing the cholesterol efflux capacity of donor cells and by modulating the uptake capacity as well as availability of initial acceptor particles (figure, panel 3).

**Cholesterol donor capacity of cells**

A role for CETP in the cellular cholesterol donor capacity was proposed after it was shown that CETP is expressed by monocyte-derived macrophages present in fatty streaks and atherosclerotic plaques of human origin (18, 19). Zhang et al also reported that blood-borne macrophages of a CETP-deficient subject were less efficient in cholesterol efflux compared with a control subject, and that COS-7 cells transiently overexpressing CETP showed an increased efflux capacity to medium, whereas influx capacity was unaltered (19). In contrast to this potentially anti-atherogenic role for CETP in macrophages, suppression of CETP synthesis in a liver cell line (HepG2) enhanced cholesterol efflux to HDL, whereas this was unaltered in an adipose tissue cell line (SW872) (20, 21). Considering the crucial role of macrophage foam cells in atherogenesis, further research into the exact function of CETP produced by these cells is warranted.

**Cholesterol acceptor capacity of HDL particles**

By changing the neutral lipid composition and size of HDL, CETP may affect the avidity of these particles for cholesterol. In CETP-deficient individuals (2, 22, 23) and after CETP inhibition (12, 24-27), average HDL size increases, and the cholesterol content of mainly large HDL2 particles is increased (12, 28). Indeed, it was reported that plasma from homozygotes for CETP null defects had a reduced acceptor capacity for cholesterol from lipid-laden macrophages (28, 29). These investigators concluded that the large cholesteryl ester-rich HDL particles in CETP deficiency are defective as cholesterol acceptors. These effects can be understood today when considering that ABCA1 only mediates cholesterol and phospholipid efflux to lipid-free or lipid-poor apolipoprotein
A-I (30, 31). In CETP deficiency, apolipoprotein A-I synthesis is furthermore unchanged (32), and recycling of HDL is thought to be compromised (33). Thus, acceptor capacity in the form of lipid-poor apolipoprotein A-I may be attenuated. However, other molecules that transport lipids across the cell membrane of macrophages, such as scavenger receptor B-I, ABCG1 and ABCG4, were recently shown to also stimulate cholesterol efflux, but instead to larger HDL particles (HDL2) (31, 34-37). This suggests that these transporters may accommodate cellular cholesterol efflux to the larger HDL particles as observed in CETP deficiency and after CETP inhibition. Indeed, with the present knowledge of ABCA1 and scavenger receptor B-I transporters, Miwa et al recently readdressed cholesterol efflux in CETP deficiency using both serum and HDL from 3 homozygotes and 3 heterozygotes for a CETP null mutation (the CETP Int14 mutation) (38). Their results suggest that the ABCA1 pathway functions normally and is preferred under reduced CETP activity as seen in heterozygotes, whereas the scavenger receptor B-I pathway is activated in complete absence of CETP. These data furthermore suggest that cholesteryl ester-rich HDL particles are still efficient cholesterol acceptors. In vitro studies on cholesterol efflux have shown that the most important property of acceptor particles is the phospholipid content (39). Also in plasma of hypertriglyceridemic subjects, it was observed that HDL-phospholipid and not low HDL cholesterol levels determined efflux from Fu5AH cells (40). In this respect, it is interesting that increases in total HDL-phospholipid content have been observed after CETP inhibition in both humans and rabbits, but how this relates to efflux acceptor capacity has not been addressed to date (25, 26, 41, 42).

Availability of cholesterol acceptor particles

It was postulated that the driving force of cellular cholesterol efflux is not the concentration of the initial acceptors (lipid-poor apolipoprotein A-I or HDL) but the presence of secondary acceptors (LDL and VLDL) and proteins and enzymes, such as LCAT, CETP, and lipases (phospholipid transfer protein, hepatic lipase, lipoprotein lipase), which redistribute cholesterol to secondary acceptors and recycle initial acceptors (39). This suggests that CETP enhances cellular cholesterol efflux rates, but evidence that relates CETP activity to efflux rates is spurious. In one in vitro study, it was indeed demonstrated that cholesterol efflux from red blood cells was highly correlated with LCAT and CETP activities in postprandial plasma as well as the concentration of secondary acceptors, such as chylomicrons, VLDL, and LDL, but not with the initial acceptor HDL (43). However, in other in vitro studies, no association between CETP activity in plasma and cholesterol efflux from Fu5AH cells was observed (44, 45). A cholesterol efflux enhancing role for CETP was also observed in human CETP transgenic mice but not in rabbits. Serum of human transgenic apolipoprotein A-I/CETP mice showed an increased relative efflux efficiency per HDL particle compared with transgenic apolipoprotein A-I mice, as assayed by measuring efflux from both cholesterol-loaded Fu5AH cells and fibroblasts (46, 47). After CETP inhibition, HDL from JTT-705–treated rabbits was equally efficient in accepting cholesterol from acetylated LDL-loaded J774 macrophages compared with HDL from control rabbits (41). This is most likely related to the observed increase in
apolipoprotein A-I synthesis in rabbits after CETP inhibition (42). Interestingly, it has been shown that in patients with low HDL cholesterol, torcetrapib decreased the fractional catabolic rate of apolipoprotein A-I, whereas the synthetic rate was unaltered (26). This illustrates that the extrapolation of data from animals to man, even if these animals possess endogenous CETP, is challenging. Together, it is difficult to establish the role of CETP in the important initial step in the RCT pathway, which in vivo occurs within the confinement of the arterial wall. Here, we depend on artificial systems in which only 1 factor that contributes to cholesterol efflux can be assessed at once. For example, scavenger receptor B-I mediated efflux is usually addressed in the rat hepatoma cell line Fu5AH, whereas for ABCA1 expression, fibroblasts or cAMP-stimulated macrophages are generally used (15). There is accumulating evidence that both cholesterol efflux pathways work complementarily, and respond diversely to, for example, the phospholipid content of acceptor particles or metabolic changes (36, 48, 49). Moreover, because the relative contributions of ABCA1, scavenger receptor B-I (and human homologue CLA-1) (50, 51), ABCG1, and ABCG4 in macrophage efflux are not firmly established in human physiology (17), we wish to refrain from predicting to what extent CETP inhibition will affect the initial step in the RCT pathway.

Uptake and storage of cholesterol in adipose tissue
Adipose tissue is an important storage and sensing organ for cholesterol homeostasis, and the metabolic consequences of adiposity play a role in the progression of atherosclerosis (52, 53). Moreover, both adipocytes and adipose tissue-derived macrophages secrete inflammatory proteins that may contribute to the development of a systemic inflammatory state (54). Therefore, it is of interest to underline that in addition to the liver, adipose tissue is the second major site of CETP production (55). Maybe, the adipocyte may quickly regulate plasma CETP levels in response to physiological changes (56). Indeed, CETP gene expression in adipose tissue is significantly upregulated by dietary cholesterol as observed in hamster and human adipose tissue (21, 55). There are indications that CETP plays a role in the selective uptake of HDL cholesteryl esters in adipose tissue, which could accommodate removal of cholesterol from the circulation (57, 58). Vassiliou and McPherson showed in vitro that extracellular CETP (mimicking CETP in the circulation) accounts for 14% of cholesteryl ester-selective uptake in adipose tissue independent of the presence of apolipoprotein B receptors and scavenger receptor B-I. These authors propose that this so-called lateral cholesterol transport and storage may be anti-atherogenic, and that CETP inhibitors may influence this process. This was addressed recently in liver cells, as is discussed below (59). CETP may also play a role in intracellular cholesterol homeostasis; Izem and Morton showed that suppression of CETP synthesis in the adipose tissue cell line SW872 causes cholesteryl ester accumulation (21). CETP activity is moreover positively correlated with body mass index and waist-to-hip ratio, but it is unclear whether this is cause or consequence (60, 61). Interestingly, a significant weight gain was observed in rabbits receiving JTT-705 in combination with a severe hypercholesterolemic diet for 3 months compared with controls on the same diet (62). However, this was not observed in a previous study on a milder hypercholesterolemic diet (63). Given this
information, it could be inferred that inhibition of CETP gives rise to a reduction in lipid uptake in adipose tissue, but whether this will have an impact on cholesterol homeostasis, systemic inflammatory status, and atherosclerosis is unclear. Therefore, it would be of interest to study the effect of CETP inhibition on adipose tissue, inflammatory markers, and body weight changes.

**Uptake of cholesterol by the liver**

In mice, which lack CETP, ±70% of circulating cholesteryl esters is taken up directly from HDL by the liver (64), which is mediated by scavenger receptor B-I (65). Similar data have been obtained in the hamster, a low-CETP animal (66). As soon as higher CETP levels come into play, the delivery route of cholesteryl esters to the liver changes dramatically. In rabbits, 70% of cholesteryl esters are transferred from HDL to apolipoprotein B-containing particles and subsequently taken up in the liver by the LDL receptor and related receptors and only 30% via apolipoprotein A-I–containing particles (67). In humans, a recent kinetic analysis by Schwartz et al indicates that irreversible cholesteryl ester output from lipoproteins to the liver comes from apolipoprotein B-containing particles and not from HDL (68). This would suggest that HDL-mediated cholesterol uptake in the liver does not play an important role in humans. This could mean that CETP inhibition will affect the normal uptake of cholesteryl esters in the human liver via the LDL pathway. Therefore, we discuss the effects of CETP on both HDL and LDL receptor–mediated pathways as illustrated in the figure, panel 4.

**Scavenger receptor B-I/CLA-1**

CLA-1, the human homologue of scavenger receptor B-I, is expressed mainly in adrenal tissues, liver, and reproductive organs and was shown to have receptor affinity for all lipoproteins, including native HDL, LDL, and VLDL, but also modified oxidized LDL (ox-LDL) and acetylated LDL (69, 70). However, data on the relationship between HDL remodelling by CETP and cholesterol uptake by scavenger receptor B-I are contradicting. Kinoshita et al observed that in scavenger receptor B-I overexpressing Chinese hamster ovary cells, the uptake of cholesteryl esters per HDL particle is increased from cholesteryl ester-rich HDL of CETP-deficient individuals compared with normal HDL (71). This suggests that scavenger receptor B-I may efficiently take up cholesterol from cholesteryl ester-enriched HDL after loss of CETP function. In contrast, CETP enhances cholesteryl ester uptake in the liver of mice overexpressing human LCAT or apolipoprotein A-I (72, 73), leading to less atherosclerosis in human transgenic LCAT/CETP mice (72). Moreover, the enhanced liver uptake was directly from HDL and not attributable to transfer to apolipoprotein B-containing particles (73). Because the role of CLA-1 in humans is not established, the implications of CETP inhibition for this cholesterol uptake route in the liver cannot be properly discussed.

**LDL receptor**

As already indicated, the main route for delivery of cholesteryl esters to the liver in humans is thought to be mediated by apolipoprotein B-containing lipoproteins. Remarkably, increased
apolipoprotein B catabolism has been observed in homozygous CETP deficient subjects compared with unaffected controls (74). This is consistent with upregulation of the LDL receptor in rabbits injected with antisense CETP oligonucleotides (75) and downregulation of the LDL receptor in mice that overexpress human CETP (76). It has been suggested that upregulation of the LDL receptor in homozygous CETP deficiency is a compensatory mechanism to counteract reduced affinity for the LDL receptor of the observed polydisperse apolipoprotein B-containing particles (77). Depending on the CETP inhibitor, marked (12, 25) or modest (24, 27) reductions in LDL cholesterol have been shown in humans. A similar effect was also observed when CETP inhibitors were used in combination with statins (12, 26, 27). Whether this is also attributable to upregulation of the LDL receptor remains to be determined. Alternatively, reduced LDL cholesterol levels after CETP inhibition can also be explained by decreased CETP-mediated transfer of cholesteryl esters from HDL to LDL. In this case, cholesteryl esters are not removed from the circulation via LDL, and the influx into the liver may in fact be reduced. In this respect, it is interesting to note that after CETP inhibitor treatment, LDL size increases, particularly by a decrease in small LDL, which may suggest that the remaining LDL particles are less atherogenic (12, 13). As an alternative pathway, the uptake of cholesteryl esters by the liver could also be mediated via apolipoprotein E-containing HDL, because CETP inhibition is associated with increased apolipoprotein E levels (25, 27, 78). It has been shown that apolipoprotein E-containing HDL particles can be taken up by members of the LDL receptor family (79-81), but also by scavenger receptor B-I (82, 83). Furthermore, Yamashita et al showed that apolipoprotein E-rich HDL from CETP-deficient individuals had a higher affinity for LDL receptors on fibroblasts than LDL itself (84). Although Clark et al did not confirm that the increase of apolipoprotein E levels can be accounted for by an apolipoprotein E-enrichment of HDL, it can be hypothesized that CETP inhibitors may lead to enhanced removal of cholesteryl esters via apolipoprotein E-containing HDL particles (25, 78).

Receptor-independent selective uptake of HDL cholesterol
In 1987, Granot et al reported that addition of CETP to liver cells (HepG2) in vitro enhanced selective uptake of HDL cholesterol (85). Later, this was attributed to the indirect effect of CETP-mediated transfer of HDL cholesterol to other lipoproteins before uptake because this presumed that selective uptake of HDL cholesterol could be blocked by antibodies against the LDL receptor and other apolipoprotein B and E receptors (86). In contrast, Gauthier et al recently observed that exogenous CETP enhanced the uptake of HDL cholesterol in mouse hepatocytes of both LDL receptor-null mice and scavenger receptor B-I _/_, mice in vitro (59). Torcetrapib partially inhibited this selective uptake mediated by endogenous CETP in these experiments. In vivo, in adenoviral CETP-expressing mice, high doses of intravenously injected torcetrapib attenuated CETP-mediated decrease in total cholesterol only partially. The authors suggest that the remaining decrease in total cholesterol was attributable to hepatic cholesterol clearance by cell-associated CETP, and that this anti-atherogenic function of CETP may not be inhibited by torcetrapib. Nevertheless, the overall importance in humans needs to be established because kinetic analysis
CETP beyond raising HDL cholesterol

by Schwartz suggests that selective uptake of HDL cholesterol in man plays a minor role in total hepatic uptake of cholesteryl esters (68). In summary, CETP inhibition is likely to influence the hepatic uptake of cholesteryl esters from the circulation via apolipoprotein E-rich HDL, maybe in combination with upregulation of the LDL receptor.

Secretion of cholesterol by the liver into the intestine

The ultimate step in the removal of cholesterol from the body is the excretion of neutral sterols and bile acids in the feces, which can be upregulated in humans by the infusion of apolipoprotein A-I or reconstituted HDL (87, 88). These studies support the idea that HDL is central to the removal of (peripheral) cholesterol in humans, and it was hypothesized that increasing HDL cholesterol and apolipoprotein A-I levels by CETP inhibition may also increase fecal cholesterol excretion. However, Brousseau et al did not observe a difference in fecal sterol excretion, even after torcetrapib induced a 100-125% increase in HDL cholesterol in patients with low HDL cholesterol at baseline (26). Similarly, in rabbits, attenuation of CETP activity by red pepper was shown to slightly increase triglyceride excretion, but did not affect cholesterol excretion (89). In human transgenic CETP mice, it has also been observed that liver cholesterol levels and cholesterol synthesis are strongly affected by infusion of reconstituted HDL, but this had no effect on fecal excretion (90). A lack of change in fecal cholesterol secretion was also observed in ABCA1 knockout mice that have almost no HDL cholesterol in the circulation (91). Together, these data suggest that the earlier steps in the RCT pathway (ie, the enrichment of HDL with cholesteryl esters), and, consequently, the increase of HDL cholesterol, have little to do with the removal of cholesterol from the human body as a final step in RCT. On the other hand, it could be that fecal sterol excretion does not accurately reflect changes in hepatic cholesterol influx after CETP inhibition. This hypothesis is supported by the observation that rats metabolize cholesteryl esters taken up via HDL more efficiently into bile acids than cholesteryl esters from LDL (92, 93). Finally, it may be that torcetrapib did not induce fecal sterol excretion to the same extent as a large intravenous dose of apolipoprotein A-I, but there may have been a reduction in fecal sterol excretion, which was too small to detect. Although there is no evidence that CETP inhibition stimulates fecal cholesterol excretion, overexpression of human CETP in mice has been shown to enhance fecal cholesterol excretion (94). This effect could be related to the increased cholesteryl esters content of the liver of these mice, but these investigators also observed a small increase in the expression of ABCG5. Because this transporter is known to affect hepatic sterol excretion, this suggests that CETP can affect cholesterol output in this model, albeit not along the classical route of RCT (94). In summary, these CETP inhibition data support the view that HDL may not represent the major vehicle to deliver cholesteryl esters to the liver for subsequent elimination in bile in humans. There is no consensus regarding the significance of this final excretion step with respect to maintaining whole body flux in RCT, neither whether this final step in RCT is actually connected to and reflects what is assumed the most important step for the atherosclerosis process (ie, cholesterol efflux from macrophages) (95, 96).
This leaves the question whether CETP inhibition may affect atherogenesis by altering RCT as yet unanswered.

**CETP inhibition and anti-inflammatory and anti-oxidative properties of HDL**

It has been long recognized that apart from its role in RCT, HDL possesses properties that may reduce atherosclerosis by attenuating inflammation of the vascular wall and by preventing oxidation of LDL (97). For details, we refer to a recent review by Barter (98). It was recently shown that reconstituted HDL can effectively block neutrophil infiltration in the carotid artery of the rabbit (99), an early event of inflammation of the vessel wall (100). Other investigators reported apolipoprotein A-I to inhibit T-cell activation of macrophages, which, in turn, will reduce the production of inflammatory cytokines and chemokines (101). In addition, through prevention of LDL oxidation, a key event in atherogenesis, HDL has also been shown to inhibit the formation of foam cells (102, 103). Most of this effect is currently attributed to enzymes that are associated with HDL (figure, panel 5), but also, physicochemical properties of HDL subfractions have been implicated (104-106). First, apolipoprotein A-I was shown to prevent LDL oxidation (102, 103). Second, paraoxonase-1, exclusively present on HDL, protects LDL from oxidation but also enhances cholesterol efflux from macrophages and, as such, may play a dual role in the protection against atherosclerosis (107-109). Other HDL-associated enzymes that may reduce propagation of oxidation of LDL are platelet-activating factor acetylhydrolase (PAF-AH or lPPLA2) (110) and LCAT (106, 111). Because of the strong impact of CETP inhibition on HDL, it is plausible that CETP inhibition modifies the anti-inflammatory and anti-oxidative properties of HDL. Currently, there are no data on the effects of CETP inhibitors on changes in the anti-inflammatory properties of HDL. Anti-oxidative properties can be indirectly addressed by measuring resistance of LDL to oxidation or antibody levels against ox-LDL, which, however, does not give information on the underlying mechanism. In 78 postmenopausal women, CETP activity and oxidation of LDL were weakly correlated (61). In vitro, LDL incubated in plasma containing a CETP inhibiting antibody was more resistant to oxidation, indicating that CETP inhibition might reduce oxidative modification of lipoproteins (112). In contrast, it was also shown that CETP may be anti-atherogenic because it prevented cholesterol loading of ox-LDL (113). Studies in mice have also provided equivocal data. Introduction of the human CETP gene in mice did not affect ox-LDL antibodies in the circulation (114). However, in ovariectomized mice, a lack of CETP resulted in higher ox-LDL antibody levels, suggesting that CETP is protective under these physiological conditions. Data on anti-oxidative enzymes in CETP deficiency or after CETP inhibition are scarce. Elevation of paraoxonase-1 activity was observed in 1, but not in a second homozygous CETP-deficient individual (115). Moreover, when adjusted for HDL cholesterol or apolipoprotein A-I levels, lower specific paraoxonase-1 activity was observed in CETP-deficient individuals compared with controls. This could be connected to the observation that human apolipoprotein A-II can displace paraoxonase-1 from HDL particles taken the increased LpAI:AII fraction in CETP deficiency (28, 116, 117). Zhang et al recently showed that paraoxonase-1 and PAF-AH increased significantly
after treating rabbits with JTT-705 (78), but it should be noted that this was not observed by Huang et al in a previous report (62). Recently, Bisoendial et al have assessed paraoxonase-1 activity and auto-antibodies against ox-LDL after CETP inhibition with JTT-705 in patients with very low HDL cholesterol (13). The data indicate that CETP inhibition improves the anti-oxidative status of these individuals, but the sample size is small, and further studies are warranted.

Impact of CETP inhibition on atherogenesis and concluding remarks

The purpose of CETP inhibition therapy is to protect against CAD by reducing atherosclerosis or stabilizing vulnerable plaques. Awaiting the first human data, studies with rabbits have provided a basis for optimism. Already in 1998, Sugano et al observed markedly reduced atherosclerosis in Japanese white rabbits on a high-cholesterol diet (0.3%) using an antisense strategy that inhibits CETP expression (75). Furthermore, 3 different vaccines known to induce antibodies against CETP have been shown to reduce atherosclerosis in New Zealand white rabbits on a cholesterol-rich diet (118-120). Pharmacological inhibition with JTT-705 prevented atherosclerosis progression in Japanese white rabbits on a 0.2% cholesterol diet (63). However, no significant effects were observed in a different study in such rabbits that received a similar dose of JTT-705, but were on a 0.25% cholesterol diet (62). This may be explained by more severe diet-induced hypercholesterolemia in the latter rabbits, but the treatment period was also shorter. More recently, pharmacological inhibition of CETP with torcetrapib also prevented atherosclerosis in New Zealand white rabbits on a 0.2% cholesterol/10% coconut oil diet (121). It is a major challenge to determine what mechanisms are primarily responsible for the atheroprotective effect of CETP inhibition. Of course the marked increase in HDL cholesterol levels is put forward; but which functions of HDL are responsible for atheroprotection? Moreover, as illustrated in this review, CETP does not only affect HDL metabolism but also LDL metabolism. To protect the arterial wall against atherosclerosis, the following processes are thought important: (1) preventing inflammation, and internalization/modification of LDL to attenuate foam cell formation; (2) stimulating cholesterol efflux from lipid-laden macrophages; and (3) enhancing LDL uptake by the liver, thereby limiting LDL modification and plasma residence time of this atherogenic lipoprotein. The current literature provides evidence that CETP inhibition may affect each of these processes. At the same time, this review shows that many aspects regarding the functions of CETP in human metabolism and the importance of human RCT are still elusive. The first issue that remains to be resolved is the role of CETP in adipose tissue. Second, with respect to the classical concept of RCT, there is concern that CETP inhibition reduces the flux of cholesterol through RCT both by decreased recycling of HDL acceptor particles and by diminished hepatic cholesterol uptake via the LDL receptor. The unchanged fecal cholesterol excretion after CETP inhibition may be considered an indication of this. However, it can also indicate that HDL is not central to liver cholesterol uptake in man. Importantly, this does not rule out that HDL serves as an initial acceptor of cholesterol from lipid-laden macrophages in the intima. There remains a strong need to determine what parameter can give us in vivo information on this crucial first step of RCT, rather than waiting for changes in
arterial wall morphology or atheroma burden. Furthermore, it is not clear what the relative
correlation of RCT to human atheroprotection is, especially when compared with the anti-
flammatory and anti-oxidation properties of HDL. Finally, this review has addressed the complex
biology of CETP in RCT, but it remains to be seen whether this is relevant when it comes to
atheroprotection. Despite all uncertainties, the impact of CETP inhibition on raising HDL
cholesterol and decreasing both LDL cholesterol and small dense LDL illustrates its potential to
reduce atherosclerosis in man. Moreover, CETP inhibition may protect against atherosclerosis by
improving the anti-inflammatory and antioxidant actions of HDL, as demonstrated previously in
rabbits and now for the first time in man. These data suggest that CETP inhibition goes beyond
raising HDL cholesterol levels alone.
**Figure.** Simplified schematic representation of the effects of CETP inhibition on the RCT pathway and protection against inflammation and oxidation. The numbered flashes indicate impact areas of CETP inhibition, which are further detailed in the respective panels. Flashes with upward or downward arrows indicate up- and down-regulation, respectively. Black arrows indicate transfer of free cholesterol (FC), cholesteryl esters (CE) or triglycerides (TG), open arrows indicate lipoprotein particle conversion. 1. RCT. ABCA1 shuttles FC and phospholipids (PL) across the cell membrane to lipid poor apolipoprotein A-I. LCAT esterifies FC into CE, which are internalized into the core of maturing HDL. CETP transfers CE from HDL to apolipoprotein B lipoproteins (VLDL) in exchange for TG. CETP inhibition results in accumulation of CE-rich HDL and decreases LDL-CE. Normally, CE-rich LDL is taken up by the liver via the LDL-receptor, and TG in TG-rich HDL is hydrolyzed by hepatic lipase (HL), resulting in regeneration of lipid poor apolipoprotein A-I. 2. Under hypertriglyceridemic conditions, CETP preferentially transfers CE from HDL to TG-rich particles (TRL). This results in CE-rich VLDL, which are a substrate for HL resulting in the formation of small dense LDL (sdLDL) 3. Efflux of cholesterol from macrophages. ABCA1 shuttles FC and PL to lipid-poor apolipoprotein A-I particles. Scavenger receptor B1 (SR-BI) (or human homologue CLA-1) can transfer FC to various sized HDL particles, ABCG1 and ABCG4 may specifically transfer cholesterol to larger HDL particles (HDL2). CETP inhibition increases the HDL2 fraction, and may also increase lipid poor apolipoprotein A-I. The total impact of CETP inhibition on cholesterol efflux is unclear. 4. Uptake of HDL-CE and LDL by the liver. LDL is taken up by the liver via the LDL-receptor (LDL-R). CETP inhibition decreases LDL cholesterol, but expression of the LDL-R may be upregulated by CETP inhibition. Specific uptake of CE from HDL by the liver is accommodated by SR-BI in mice, and perhaps by CLA-1 in humans. Apolipoprotein E containing HDL is increased after CETP inhibition. This may be taken up by the liver by CLA-1, the LDL-R or other receptors. 5. Anti-oxidative action of HDL. HDL protects LDL from oxidation into oxLDL. Several enzymes present on HDL have been shown to account for this such as apolipoprotein A-I, paraoxonase, PAF-AH, LCAT. CETP inhibition increases these proteins, except for the latter. Apolipoprotein A-II, which is also increased by CETP inhibition has been shown to displace paraoxonase from HDL. The ultimate effect of CETP inhibition on oxidation of LDL is not known.
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Chapter 10


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