The role of plasma lipid transfer proteins in lipoprotein metabolism and atherogenesis

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Abstract The plasma lipid transfer proteins promote the exchange of neutral lipids and phospholipids between the plasma lipoproteins. Cholesteryl ester transfer protein (CETP) facilitates the removal of cholesteryl esters from HDL and thus reduces HDL levels, while phospholipid transfer protein (PLTP) promotes the transfer of phospholipids from triglyceride-rich lipoproteins into HDL and increases HDL levels. Studies in transgenic mouse models and in humans with rare genetic deficiencies (CETP) or common genetic variants (CETP and PLTP) highlight the central role of these molecules in regulating HDL levels. Human CETP deficiency is associated with dramatic elevations of HDL cholesterol and apolipoprotein A-I levels, while PLTP variants with increased expression are associated with higher HDL levels. A recent meta-analysis suggests that common CETP alleles causing reduced CETP and increased HDL levels are associated with reduced coronary heart disease. The failure of a clinical trial with the CETP inhibitor torcetrapib may have been related in part to off-target toxicity. Ongoing phase 3 clinical trials with other CETP inhibitors may help to clarify if this strategy can ultimately be successful in the treatment of atherosclerosis.—Masson, D., X-C. Jiang, L. Lagrost, and A. R. Tall. **The role of plasma lipid transfer proteins in lipoprotein metabolism and atherogenesis.** *J. Lipid Res.* 2009. **50:** S201–S206.

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CHOLESTERYL ESTER TRANSFER PROTEIN

Discovery and characterization of CETP

More than 40 years ago in this journal, Nichols and Smith (1) described a factor in human plasma that was able to stimulate the reciprocal exchange of triglycerides and cholesteryl esters between lipoprotein subclasses. The first biochemical characterization of a plasma lipid transfer protein came 13 years later (2). Complete purification of cholesteryl ester transfer protein (CETP) (3) and the subsequent cloning of the CETP cDNA (4) were achieved in 1987. These studies indicated that mature CETP is a 476 amino acid protein (74 kDa) with a highly hydrophobic amino acid content and four N-linked glycosylation sites. Neutralizing CETP monoclonal antibodies showed that this molecule was responsible for all cholesteryl ester (CE) and triglyceride (TG) transfer activity in human plasma and that inhibition of activity in rabbits resulted in increased HDL levels and a reduced content of CE in VLDL (5). Shortly thereafter, the elucidation of human genetic deficiency of CETP established the key role of this molecule in human lipoprotein metabolism (6). Recently, the crystal structure of CETP revealed an elongated boomerang shaped molecule with the curvature of the concave surface likely fitting to the convex curvature of the lipoprotein surface (7) (**Fig. 1**). A unique 60 Å long hydrophobic tunnel with two distinct openings traverses the core of the protein. This tunnel can be filled with two CE molecules and plugged by two phospholipid molecules at each end. It has been proposed that upon CETP binding at the lipoprotein surface, phospholipids bound at the mouth of the tunnel merge into the phospholipid monolayer and allow neutral lipids to enter and exit the tunnel. A C-terminal amphipathic helix (Fig. 1, black arrow) recognized by the neutralizing CETP monoclonal antibody (5) is situated at the mouth of the N-terminal entrance to the lipid binding tunnel and may play a role in facilitating entry of neutral lipid into CETP. The CETP structure is consistent with the earlier findings that CETP binds neutral lipids and shuttles them between plasma lipoproteins in a carrier-mediated mechanism (8).

Abbreviations: apo, apolipoprotein; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; LPS, lipopolysaccharide; PLTP, phospholipid transfer protein; TG, triglyceride.

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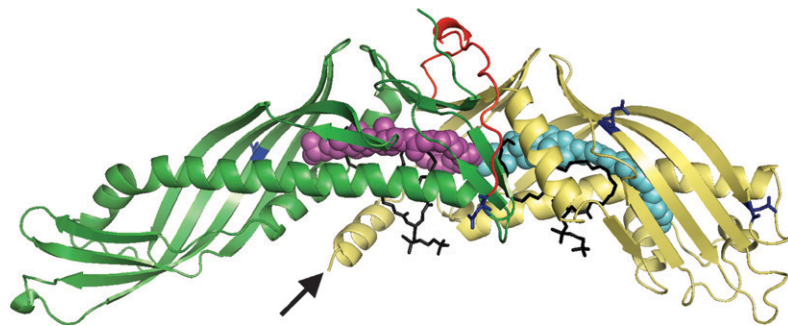e-mail: dm2561@columbia.edu or david.masson@chu-dijon.fr

Fig. 1. Structural model of human CETP (courtesy of Dr. Xiayang Qiu, Pfizer). N-terminal domains are in green, C-terminal domains are in yellow, and the linker is in red. The two CE molecules are in magenta and cyan, and phospholipids are represented as black bonds. CETP displays an elongated boomerang shape with the curvature of the concave surface fitting to the convex curvature of the lipoprotein surface. A unique hydrophobic tunnel filled with two CE molecules traverses the core of the protein. This tunnel is plugged by two phospholipid molecules at each end. A C-terminal helix (black arrow) is situated at the mouth of the N-terminal entrance to the hydrophobic tunnel and was previously identified as the target of the CETP neutralizing antibody TP-2.

Role of CETP in lipoprotein metabolism

In humans and monkeys, CETP is expressed in liver (parenchymal and nonparenchymal cells), small intestine (enterocytes), adipose tissue, and spleen (4). There is also significant expression of CETP in macrophages, and bone marrow transplantation experiments using as donors mice expressing the human CETP transgene under the control of its natural promoter suggest that macrophages make a significant contribution to plasma CETP levels (9). CETP gene expression is stimulated by dietary cholesterol and endogenous hypercholesterolemia as a result of activation of liver X receptor/retinoid X receptor transcription factors bound to the proximal promoter of the human CETP gene (10). In human plasma, CETP is present at concentrations of ∼2 μg/mL, with moderately higher levels in dyslipidemic subjects, and is mainly associated with high density lipoproteins (5). Plasma CE transfer activity is dependent on both CETP concentration and the ability of CETP to interact with lipoproteins. This interaction can be stimulated by free fatty acids generated during hydrolysis of dietary TGs (5) or inhibited by specific apolipoproteins such as apoC-I or apoF (11, 12). Due to the mechanism of lipid transfer, CETP can only promote the net mass transfer of lipids between lipoprotein subclasses that have different CE/TG ratios. Therefore, CETP facilitates the transfer of CEs from CE-rich LDL and HDL toward VLDL. CETP promotes the reciprocal enrichment of LDL and HDL with TGs derived from VLDL (5). Unlike CEs, TGs can be hydrolyzed in the plasma compartment through the action of lipases. Subsequent hydrolysis of TG in LDL promotes their remodeling with the generation of small dense LDL with a smaller neutral lipid core. A similar mechanism also occurs in HDL, and hydrolysis of large-triglyceride-enriched HDL generates smaller HDL3 together with the release of lipid poor apolipoprotein A-I (5).

ROLE OF CETP IN ATHEROSCLEROSIS

Predictions from lipoprotein physiology

By transferring CEs from HDL toward apoB-containing lipoproteins, CETP decreases the concentration of HDL cholesterol and apoA-I and increases the concentration of CE in VLDL and remnants. In addition, CETP activity raises levels of LDL cholesterol and apo B, most likely due to downregulation of hepatic LDL receptors (5). Small, dense LDLs generated by CETP and lipases may be particularly atherogenic because of increased affinity for artery wall proteoglycans and increased susceptibility to oxidation. In contrast with these pro-atherogenic effects, the remodeling of HDL particles by CETP is accompanied by the release of lipid-poor apoA-I, which is the preferential acceptor for ABCA1-mediated cholesterol efflux from macrophage foam cells. However, this effect may be offset by a decrease in large HDL particles that promote cholesterol efflux in part via the ABCG1 pathway (13). In the steady state, CETP activity appears not to change the overall efficiency of reverse cholesterol transport, though the lipoproteins mediating reverse cholesterol transport are likely different (i.e., primarily remnants and LDL in the presence of CETP activity and HDL in CETP deficiency). Any beneficial effect of CETP inhibition likely accrues from decreased cholesterol uptake and increased cholesterol efflux in macrophage foam cells and in vascular cells of atherosclerotic plaques (14).

Animal studies

Interestingly, CETP is not present in all animal species. Introduction of the human CETP gene in transgenic mouse models has led to varied effects on atherosclerosis. CETP expression increased atherosclerosis in hypercholesterolemic mouse models, such as apoE and LDL-receptor-deficient mice (15, 16), and in a mouse model of mixed

hyperlipidemia expressing apoE(Leiden) (17). By contrast, in hypertriglyceridemic apoCIII Tg mice, expression of CETP can be either non- or anti-atherogenic (18). In mice, CETP activity appears to be pro-atherogenic when it causes both reduced HDL levels and increased levels of CE in remnants and LDL, while it is nonatherogenic in a setting of hypertriglyceridemia and prominent accumulation of small lipid-poor, apoA-I-rich HDL particles.

HUMAN STUDIES

Genetic CETP deficiency

Genetic CETP deficiency was discovered in Japanese families with increased HDL levels (6, 19). Homozygous deficient subjects display dramatic increases in HDL-C (approximately + 100–200%) as well as decreases in LDL-C and apoB levels (approximately −40%). HDL from homozygous CETP-deficient subjects are very large and enriched in apoE and show an enhanced ability to promote cholesterol efflux from macrophages in part through the ABCG1 pathway (13). LDL particles from CETP-deficient subjects are heterogeneous in size and display a reduced affinity for the LDL receptor. In a cross-sectional survey of Japanese/American men, heterozygotes for CETP gene defects had an increased risk for coronary heart disease (20). However, this finding was not confirmed in a subsequent prospective study in the same population, in which a trend to a lower incidence of stroke and coronary heart disease (CHD) was apparent in men with heterozygous CETP deficiency (21).

CETP polymorphisms

Although many studies have demonstrated associations between CETP single-nucleotide polymorphisms in Caucasian populations and small changes in plasma CETP and HDL concentration, the relationship between these polymorphisms and susceptibility to atherosclerotic cardiovascular disease has been inconsistent. A recent meta-analysis of three different single nucleotide polymorphisms (SNPs) in the CETP gene (two of them in linkage disequilbrium) in 113,833 subjects, including 27,196 cases of CHD, showed a significant or borderline significant reduction in CHD for the CETP alleles associated with lower CETP and higher HDL levels (22) (**Fig. 2A**). The protective effect of HDL elevation associated with lower CETP levels was similar
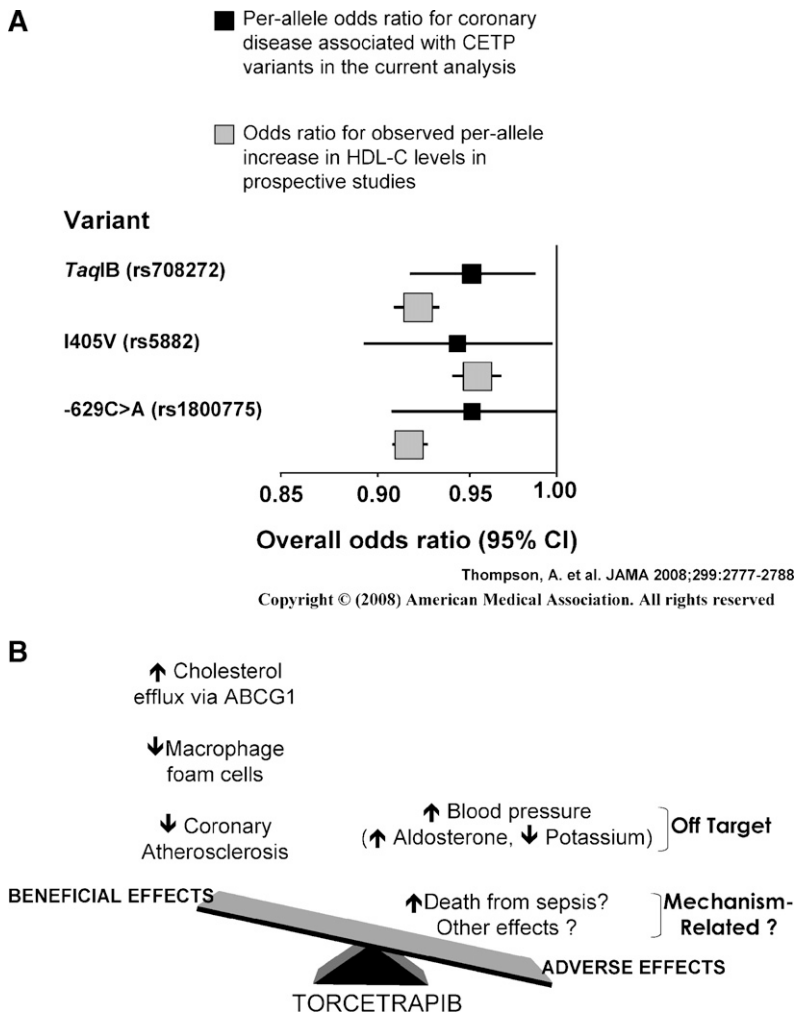
Fig. 2. Relationship of CETP with atherosclerosis. A: Observed per-allele odds ratios for coronary disease with CETP variants versus odds ratios derived from prospective studies of HDL-C levels. A significant reduction in CHD is observed for the CETP alleles associated with lower CETP and higher HDL levels. Interestingly, the protective effect of HDL elevation associated with lower CETP levels is similar to that afforded by HDL elevation in prospective epidemiological studies. B: The ILLUMINATE study was stopped prematurely as a result of an excess of deaths and cardiovascular disease in the group receiving torcetrapib. Potential adverse effects of torcetrapib include off-target effects, such as increases in blood pressure, sodium, bicarbonate, and aldosterone levels, as well as a decrease in potassium levels. However mechanism-related adverse effects cannot be ruled out. Beneficial effects would include increase in cholesterol efflux via ABCG1 resulting in decrease foam cell formation and a decrease coronary atherosclerosis. In the ILLUSTRATE study, there was an inverse relationship between change in HDL and change in percentage of atheroma volume in the group receiving torcetrapib.

to that afforded by HDL elevation in prospective epidemiological studies. One caveat to the conclusions of this meta-analysis is that findings may have been influenced by publication bias. Recently, a genotype score of nine validated SNPs that are associated with modulation in levels of LDL or HDL cholesterol, including CETP TaqIB, was found to be an independent risk factor for incident cardiovascular disease, and in this analysis, HDL- and LDL-associated SNPs acted independently (23). Overall, the evidence linking HDL-associated SNPs with CHD is weaker than that for LDL, consistent with the idea that LDL is the cause of atherosclerosis, while HDL is a modifying factor. Notably, these studies show either no effect of CETP genetic variants on CHD or a protective effect, but there is no consistent relationship linking reduced CETP levels to increased CHD.

CETP inhibitors

All CETP inhibition strategies have been effective at increasing HDL levels and decreasing atherosclerosis in rabbits (24). Clinical trials in humans using CETP inhibitors, such as torcetrapib, have shown marked increases in HDL and moderate reductions in LDL (25). Unfortunately, a large phase 3 clinical trial called ILLUMINATE involving torcetrapib was stopped prematurely as a result of an excess of deaths and cardiovascular disease in the group receiving torcetrapib and atorvastatin compared with atorvastatin alone (26). The reasons for the adverse outcome are uncertain. Torcetrapib administration was associated with a number of undesirable off-target effects that could have contributed to increase mortality/morbidity (Fig. 2B). They included increases in blood pressure, sodium, bicarbonate, and aldosterone levels as well as a decrease in potassium levels (26). It seems that these adverse effects were molecule specific and not related to CETP inhibition (27). The mechanism of hypertension appears to result in part from an increased production of adrenal steroids, including aldosterone and cortisol (27). Post hoc analysis of the ILLUSTRATE study showed that while the majority of torcetrapib-treated patients demonstrated no regression of coronary atherosclerosis, a significant regression of coronary atherosclerosis was observed in patients in the highest HDL-C quartile. In this post hoc analysis, there was a continuous inverse relationship between HDL levels and the percentage of atheroma volume (28). Moreover, this relationship was only clearly seen in the group receiving the CETP inhibitor, strongly suggesting that in patients achieving high HDL levels, HDL particles were functional in promoting regression of atherosclerosis.

Two other CETP inhibitors, anacetrapib (Merck) and dalcetrapib (Roche), are in advanced clinical studies (29, 30). Unlike torcetrapib, these agents do not appear to cause hypertension. Dalcetrapib has a distinct mechanism of action compared with anacetrapib and torcetrapib and likely covalently modifies a Cys-H group in the N-terminal part of the lipid binding tunnel of CETP. Anacetrapib is a more potent CETP inhibitor than dalcetrapib and produces larger effects on HDL and LDL levels. Ongoing phase 3 clinical studies with various CETP inhibitors may help to determine if the addition of CETP inhibitors to statins can lead to a reduction in atherosclerotic cardiovascular disease.

PHOSPHOLIPID TRANSFER PROTEIN

Discovery and characterization

Earlier biochemical studies showed that a second lipid transfer protein distinct from CETP was present in human plasma (31). This protein was unable to transfer neutral lipids between LDL and HDL, but unlike CETP promoted net mass transfer of phospholipids from phospholipid vesicles or lipolyzed VLDL particles into HDL (32). Cloning of the cDNA revealed that phospholipids transfer protein (PLTP) is a 476 amino acid protein (Mr 81 kDa) with six N-linked glycosylation sites (33). PLTP displays an ~25% amino acid identity with CETP and with two other proteins, the lipopolysaccharide (LPS) binding protein and the bactericidal permeability increasing protein, involved in the defense of the organism against LPS from gram negative bacteria. The four proteins comprise the lipid transfer/LPS binding family. They also share significant homology with the PLUNC proteins that could be involved in the local innate immune response against bacteria in oral, nasal, and respiratory epithelia (34). Similar to CETP, PLTP probably acts as a carrier that shuttles phospholipids between lipoprotein particles. In addition to phospholipids, PLTP is able to transfer other amphipatic compounds, such as free cholesterol, LPS, and vitamin E (35, 36).

Role of PLTP in lipoprotein metabolism

In contrast with CETP, PLTP is widely expressed in organs and cells (33). High levels of PLTP mRNA are especially seen in the brain, the lung, and the gonads, suggesting specific functions of PLTP in these organs. PLTP gene expression is controlled by nuclear receptors such as farnesoid X receptor and liver X receptor (37). In addition to promoting transfer of phospholipids from VLDL and chylomicrons into HDL (38), PLTP may contribute to the remodeling of HDL particles. In vitro studies suggest that when the lipid composition of a particle is altered by phospholipid transfer, its apolipoproteins are destabilized. This induces the fusion of two remnant particles and the loss of an apoAI molecule (39). Thus, PLTP activity may contribute to generation of large α-HDL as well as pre-β-HDL particles. Vitamin E transfer mediated by PTLP is also important in lipoprotein metabolism because PLTP contributes to a decrease the vitamin E content of circulating lipoproteins and to an increase their oxidability (36). Alteration of vitamin E content of liver may also contribute regulating apoB lipoprotein production, perhaps by influencing reactive oxygen species generation and efficiency of insulin signaling in hepatocytes (40). In addition, PLTP may also work within cells to add lipids to nascent apoB, thus limiting apoB degradation and increasing VLDL production (41).

Animal models

In contrast with CETP, PLTP is present in all animal species and is expressed at high levels in mouse. In human apoAI transgenic mice, PLTP overexpression resulted in a moderate increase in HDL cholesterol and apoA-I as well as a more pronounced increase in pre β-HDL (42). Gene knockout of PLTP resulted in ∼50% reductions of apoA-I and HDL cholesterol and phospholipids levels (38). Surprisingly, in apoB-transgenic and apoE-deficient backgrounds, PLTP deficiency resulted in markedly decreased atherosclerosis. This was explained by a decrease in the production and levels of apoB-containing lipoprotein, an increase in their vitamin E content, and a decrease in their susceptibility to oxidation (36, 41). Reduction of cholesterol absorption may have also contributed to the protective effect of PLTP deficiency (43). In PLTP transgenic mouse models, PLTP overexpression increased atherosclerosis susceptibility (44). In fact, the effects of PLTP on atherosclerosis probably result from a balance between systemic deleterious effects and local protective effects. As underscored by recent bone marrow transplantation studies, local PLTP expression in macrophages could be protective as long as systemic PLTP levels are not markedly elevated (45, 46).

Human studies

To date, no genetic deficiency has been reported for PLTP. Importantly, two recent studies have identified SNPs near the PLTP gene that are associated with HDL (47) and with TG levels for one variant (48). Interestingly, the variant associated with higher HDL and lower TGs also correlated with higher PLTP transcript levels in the liver (48). This is the first evidence for a direct link between PLTP and lipoprotein levels in humans. The effects on HDL levels are consistent with the transgenic mouse studies (38, 42), but the apparent lowering of TG levels in association with increased PLTP expression was not predicted.

FUTURE DIRECTIONS

Clinical trials involving CETP inhibitors with minimized toxic off-target side effects will likely show whether this strategy can be successfully used in the treatment of human atherosclerosis. However, major challenges remain concerning optimal dosage, selection of patient groups who may benefit from this therapy, and the difficulty of showing incremental effects of adding a new therapy to potent statins. While the recent genome wide association studies have indicated a role of PLTP in human lipoprotein metabolism, the elucidation of loss-of-function mutations would be a major step toward the elucidation of the role of this protein in human lipoprotein metabolism and atherosclerosis.

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