2 Cholesteryl Ester Transfer Protein and Lipid Metabolism and Cardiovascular Diseases

Helena C. F. Oliveira and Helena F. Raposo

Abstract

In this chapter, we present the major advances in CETP research since the detection, isolation, and characterization of its activity in the plasma of humans and several species. Since CETP is a major modulator of HDL plasma levels, the clinical importance of CETP activity was recognized very early. We describe the participation of CETP in reverse cholesterol transport, conflicting results in animal and human genetic studies, possible new functions of CETP, and the results of the main clinical trials on CETP inhibition. Despite major setbacks in clinical trials, the hypothesis that CETP inhibitors are anti-atherogenic in humans is still being tested.

Keywords

Cholesteryl ester transfer protein (CETP) · Lipoprotein metabolism · Lipid metabolism · CETP inhibitors · Cardiovascular diseases

2.1 Introduction

Cholesteryl ester transfer protein (CETP) was first isolated from plasma and characterized in 1978 [1, 2]. Its activity consists of promoting a net transport of cholesteryl ester (CE) from HDL to VLDL and LDL in exchange for triglycerides (TG). This plasma activity was found in many species, including primates, rabbits, hamsters, reptiles, and fishes, but it is not present in mice, rats, and dogs [3, 4]. The protein purification [5] and cDNA cloning in 1987 [6] and human gene cloning in 1990 [7] fomented investigations on CETP. Since then, a growing interest in CETP research has been observed (Fig. 2.1).

Chemical purification showed that CETP is a plasma hydrophobic glycoprotein consisting of 476 amino acids with an apparent molecular weight of 66–74 kDa [5]. Protein purification and gene cloning allowed for the production of recombinant protein, antibodies, and transgenic mouse models that were very important for further studies on the structure, function, gene expression regulation, human polymorphism screening, and relationship with cardiovascular diseases [8]. However, it took approximately 30 years after the isolation of CETP plasma activity to resolve its molecular structure by Qiu and collaborators in 2007 [9]. This milestone finding allowed for the elucidation of the CETP mechanism of action and a more refined drug design targeting CETP inhibition. In fact, the current view supports the formation of a ternary complex of CETP with donor and acceptor lipoproteins, allowing the transfer of neutral lipids through a hydrophobic tunnel inside its structure [10].

H. C. F. Oliveira (✉) · H. F. Raposo
Department of Structural and Functional Biology, Biology Institute, State University of Campinas, Campinas, SP, Brazil
e-mail: ho98@unicamp.br

X.-C. Jiang (ed.), *Lipid Transfer in Lipoprotein Metabolism and Cardiovascular Disease*, Advances in Experimental Medicine and Biology 1276, https://doi.org/10.1007/978-981-15-6082-8_2

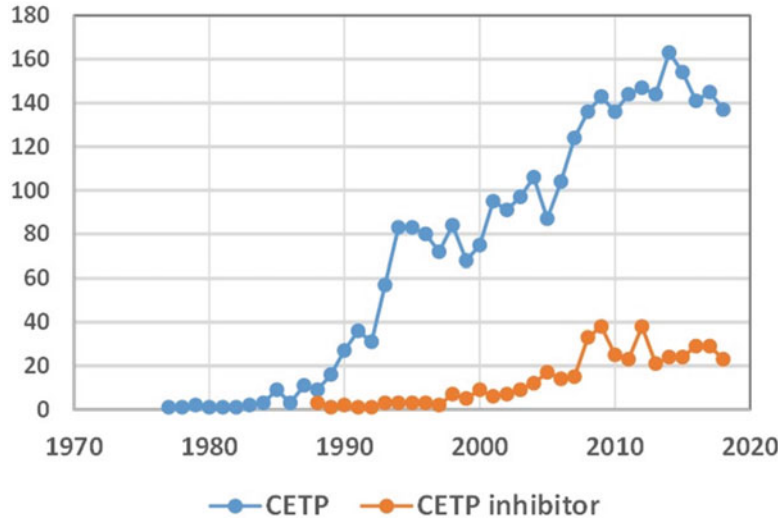

Fig. 2.1 Number of scientific publications through the years with the keywords cholesteryl-ester-transfer-protein or CETP (blue line) and cholesteryl-ester-transfer-protein inhibitor or CETP inhibitor (orange line). PubMed searches were performed on January 13, 2020, limiting the results by the presence of the keywords in the field: Title and Abstract

The human CETP gene is located on chromosome 16 (16q13), spans 25 kb, and contains 17 exons. It is conserved in chimpanzees, rhesus monkeys, chickens, zebrafish, and frogs. Orthologs of the human CETP gene have been described in 217 organisms (Gene ID: 1071, https://www.ncbi.nlm.nih.gov/gene/1071). There is a major CETP gene alternative splicing product that may occur at high frequency [11]. The alternatively spliced product, missing exon 9, is in-frame, and the loss of 60 amino acids in the central region of the protein leads to its inactivation [12]. Cotransfection of wild-type (full length) and exon 9-deleted constructs significantly reduces wild-type secretion and activity [12, 13].

Structurally, CETP belongs to the BPI-like protein family, which includes bactericidal/permeability-increasing protein (BPI), LPS (lipopolysaccharide)-binding protein (LBP), phospholipid transfer protein (PLTP), and CETP. While BPI and LBP are known to be involved in innate immunity against bacteria through their ability to sense lipopolysaccharides, PLTP and CETP are characterized by lipid exchange among plasma lipoproteins [14]. These proteins are also classified within a large family of extracellular and intracellular membrane contact site proteins that contain the tubular lipid binding protein (TULIP) domain [15].

2.2 Reverse Cholesterol Transport

Much of the scientific interest in CETP arises from the fact that it causes a reduction in HDL cholesterol plasma levels. A well-documented negative correlation between plasma concentrations of HDL and cardiovascular risk has been established since the late 1970s [16, 17]. Although the protective mechanisms of HDL were not known at that time, the concept that it was the main player in a process called reverse cholesterol transport (RCT) was emerging. In this process, first proposed by Glomset [18], HDL facilitates the uptake of cholesterol from peripheral tissues and its transport to the liver for catabolism and excretion, thus avoiding accumulation of cholesterol in the plasma compartment and hence in the arteries (Fig. 2.2). In theory, CETP may play a dual role in RCT. It may add an alternative route for delivering cholesterol to the liver via LDL receptor and LDL-receptor related protein (LRP) pathways (often called indirect RCT). However, if these pathways are malfunctioning, CETP activity results in increased LDL cholesterol levels and a risk of atherosclerosis development (Fig. 2.2).

The engineering of CETP transgenic mice in the early 1990s [19–21] was very useful to study its functions, gene expression regulation, and

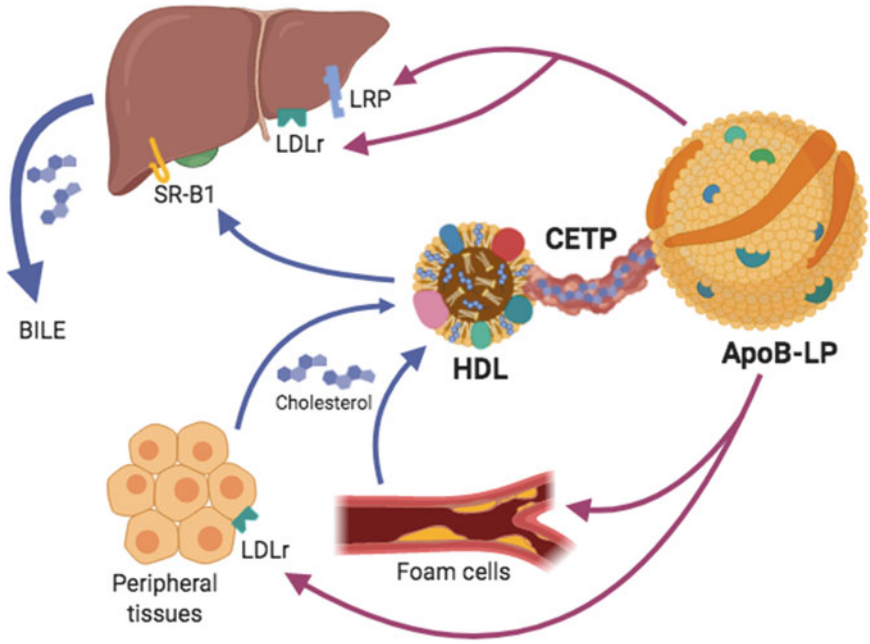

Fig. 2.2 Simplified overview of reverse cholesterol transport (RCT). The first step of RCT is the removal of cholesterol from cell membranes through the interaction of HDL subspecies with ABCA1/G1 membrane transporters. Then, the enzyme lecithin cholesterol acyl transferase (LCAT) esterifies cholesterol on the surface of HDL, which then enters into the hydrophobic core of the HDL particle. HDL-cholesteryl ester has two fates: it is either directly and selectively delivered to steroidogenic tissues (liver, adrenal, and gonadal) via scavenger receptor class B type I (SRBI) or it is transferred to VLDL and LDL (apoB-LP) in exchange for triglycerides (TG) by the action of CETP. The VLDL + LDL enriched in CE are then taken up mainly by the liver through LDL receptors (LDLr) and LDL receptor-related proteins (LRP). The pathway that includes CETP is termed indirect RCT. Once in the liver, cholesterol and cholesterol-derived bile acids are secreted into the bile and excreted from the body via the feces

susceptibility to diseases. In humans, CETP mRNA is mostly expressed in the liver, spleen, and adipose tissue [6]. These data are confirmed in the Human Protein Atlas that analyzed 27 tissue samples from 95 human individuals [22] and included lymph nodes and placenta as high CETP-expressing tissues. Hepatic synthesis is the major source of CETP found in the plasma of primates [23], with Kupffer cells being the predominant source in humans [24]. Human CETP transgenic mice expressing a natural promoter-driven CETP minigene reproduced a similar human pattern of tissue expression [20]. Thus, it was possible to study gene regulation in this model. For instance, CETP gene expression was shown to be upregulated by dietary cholesterol [20, 25, 26], thyroid hormones [27], and fish oil and fibrate treatment [28], while it is downregulated by corticosteroids [29] and hyperinsulinemia [30]. When CETP is expressed in hyperlipidemic atherosclerosis-prone models, such as the LDL receptor knockout mouse or apo E knockout mouse, an acceleration of diet-induced atherosclerosis is observed [31–33]. On the other hand, other experimental mouse model data support the concept that CETP may have a protective role against atherosclerosis in conditions where LDL receptor function is preserved. For instance, CETP expression decreased atherosclerotic lesions in hypertriglyceridemic mice [34], when cholesterol esterification rates were high due to LCAT overexpression [35, 36], in castrated mice [37, 38], in diabetic mice [39, 40], and in SR-BI knockout mice [41, 42].

2.3 Human Genetic Studies

The importance of plasma CETP in lipoprotein metabolism was long ago demonstrated by the discovery of CETP-deficient subjects with marked hyperalphalipoproteinemia in the Japanese population [43]. Among several mutations of the CETP gene, two are common mutations in that population: an intron 14 splicing defect (Int14 + 1 G --> A) and an exon 15 missense mutation (D442G). Although elevated levels of HDL cholesterol are a marker for protection against atherosclerosis, subjects with CETP deficiency show a variety of abnormalities in the composition and function of HDL that impair RCT [44]. Epidemiological studies in Japanese Americans living in Hawaii and Japanese in the Omagari area of Japan, where the intron 14 splicing defect is markedly frequent, have shown a relatively increased incidence of coronary atherosclerosis in CETP deficiency [45, 46]. On the other hand, the TaqIB polymorphism-B2 allele, with low CETP mass and moderate increases in HDL cholesterol, has been associated with a decreased risk for coronary heart disease in many studies, including the Framingham Offspring Study [46, 47]. However, controversy remains, since subsequent reports of the Framingham Heart study and Honolulu Heart Program presented discrepant results [48]. A more recent meta-analysis confirmed an association between the TaqIB polymorphism and the risk of myocardial infarct. This study suggests that the B2B2 genotype of the CETP TaqIB polymorphism is a protective factor against the development of myocardium infarct [49]. However, not all CETP-reducing polymorphisms are protective. A large meta-analysis of selected studies with three common and three uncommon CETP polymorphisms evaluated the associations of CETP genotypes, phenotypes, lipid levels, and coronary risk. The authors concluded that three CETP genotypes that are associated with moderate inhibition of CETP activity (and, therefore, modestly higher HDL-C levels) show weak (but significant) inverse associations with coronary risk, compatible with the expected reductions in risk for equivalent increases in HDL-cholesterol concentration [50]. Another meta-analysis suggests that two (out of seven) CETP polymorphisms (rs708272 [C>T] and rs1800775 [C>A]) may contribute to myocardium infarct susceptibility, especially among Caucasians [51]. Thus, it seems that the associations between CETP levels (due to gene polymorphisms) and cardiovascular diseases may be population-specific, highly dependent on the phenotype (level of CETP activity, LDL and HDL concentration/composition), and highly influenced by environmental factors.

2.4 Possible New Function of CETP: Anti-inflammatory

Similar to its family members, BPI and LBP, CETP may have an anti-inflammatory function that may be relevant not only for atherogenesis but also for immune responses [8]. Experimentally, inhibition of CETP with torcetrapib did not reduce atherosclerosis beyond the statin effect but induced more proinflammatory lesions in hypertriglyceridemic apoE3Leiden/CETP-expressing mice [52]. Normolipidemic human CETP-expressing mice were protected from mortality induced by bacterial lipopolysaccharide acute infusion [53] or polymicrobial sepsis induced by cecal ligation and puncture [54]. Regarding humans, the first large-scale trial with the CETP inhibitor torcetrapib was interrupted because of increased mortality due to several reasons, including infections (22% of noncardiovascular deaths) [55]. In addition, the mortality rate of patients with sepsis correlated with a reduction in plasma CETP concentrations [56]. However, this issue is still enigmatic. A recent report described that a gain-of-function variant (s1800777-A) of CETP promotes a profound reduction in HDL levels and reduced survival in patients with sepsis when compared with noncarriers [57]. Therefore, CETP anti-inflammatory effects may be a direct nonlipid transporting function or indirectly mediated by modulation of HDL size, composition, and concentration [58].

2.5 Does CETP Have an Intracellular Function?

As a lipid-binding and transport protein, hypothetically, CETP may play an intracellular physiological role.

Zhang and collaborators showed that transient transfection of CETP cDNA into COS-7 cells induced higher cholesterol efflux compared with mock-transfected cells, while lipid uptake was not affected. Conversely, the efflux of free cholesterol from macrophages obtained from CETP-deficient subjects was significantly decreased compared with that from normal subjects [59]. These data suggest that local intracellular CETP expression in macrophages plays an anti-atherogenic function, facilitating the removal of cholesterol from the cells.

Because the protein product of the major alternative splice variant of CETP (exon 9 deleted) is retained within the endoplasmic reticulum (ER) [12], Lira and collaborators studied whether the expression of CETP variants could induce ER stress [13]. Transient CETP transfections were performed in a human liposarcoma cell line (SW872) and a human embryonic kidney cell line (HEK293S). Surprisingly, not only the alternative spliced variants of CETP but also the full-length protein expression caused an induction of genes linked to the ER stress response [13]. Thus, although CETP is a secreted protein, intracellular CETP plays a complex role in modulating ER stress or the unfolded protein response.

Morton and collaborators have shown that CETP expression modulates cholesterol and triglyceride homeostasis in the SW872 human liposarcoma cell line. They showed that short-term partial inhibition of CETP in these cells using antisense oligonucleotides induces a phenotype characterized by inefficient mobilization of CE stores leading to CE accumulation [60]. In subsequent studies, CETP was chronically repressed with stably transfected oligonucleotides. CETP-deficient cells had decreased CE and TG biosynthesis and inefficient CETP-mediated translocation of CE and TG from the ER to their site of storage [61, 62]. On the other hand, when they stably overexpressed full-length CETP, SW872 cells accumulated 50% less TG due to a decrease in TG synthesis and a higher TG turnover rate, resulting in the formation of smaller and more metabolically active lipid droplets [63]. In agreement, Zhou and collaborators previously showed that transgenic mice expressing CETP under the control of an adipocyte-specific promoter (aP2) exhibited decreased adipocyte lipid content and size [64]. More recently, Izem et al. showed that exon 9-deleted CETP (the nonsecreted isoform) inhibits full-length CETP synthesis and promotes cellular triglyceride storage [65].

2.6 CETP Inhibition as a Target to Decrease Cardiovascular Diseases

From most human and experimental studies, it is clear that CETP is important clinically and has been a target for drug development. Dozens of review articles on CETP inhibition have been published in the last 5 years, most of them debating the viability of this strategy because of minor or no benefits of the tested molecules in reducing cardiovascular events so far. Despite the setbacks, the hypothesis that CETP inhibitors are anti-atherogenic in humans is still being tested. A summary of the results of the main trials with CETP inhibitors is presented in Box 2.1 and will be discussed below.

The concept that in vivo CETP inhibition could be anti-atherogenic was experimentally demonstrated in rabbits. CETP was inhibited with antisense oligodeoxynucleotides against CETP, reducing CETP mass and increasing HDL cholesterol (HDL-C), resulting in a reduced aortic lesion area [66]. In addition, anti-CETP immunotherapy in rabbits was able to reduce CETP activity, increase HDL, decrease LDL, and diminish atherosclerotic lesions [67].

Box 2.1 Main CETP Inhibitor Trials

Torcetrapib (Pfizer)
Atorvastatin + torcetrapib (vs. atorvastatin only)
Trial **ILLUSTRATE**
24-month follow-up
HDL-C increased by 61%
LDL-C decreased by 20%
Increase in systolic blood pressure of 4.6 mm Hg
From small to no favorable effect on atheroma volume (Nissen et al. 2007) [68]
Trial **ILLUMINATE**
12-month follow-up
HDL-C increased by 72.1%
LDL-C decreased by 24.9%
Increase in systolic blood pressure (5.4 mm Hg)
Increase in apo-CIII and aldosterone levels
Increase in mortality by CVD, cancer, and infections/sepsis (Barter et al. 2007) [55]
Dalcetrapib (Hoffmann-La Roche)
Trial: **dal-OUTCOMES**
Dalcetrapib (vs. placebo)
31-month follow-up
Increased HDL (25%), blood pressure (0.6 mm Hg), and C-reactive protein (hsPCR)
No changes in LDL and aldosterone
No benefits on CVD events/mortality.
Possible benefits in genetically defined population (ADCY9 gene, adenylate cyclase, AA allele) (Schwartz et al. 2012) [69]
Evacetrapib (Eli Lilly)
Trial: **ACCELERATE**
Evacetrapib (vs. placebo)
28-month follow-up
HDL-C increased by 132%
LDL-C decreased by 37%
Increased cholesterol efflux
Increased blood pressure (1.2 mm Hg) and hsPCR
No CVD benefits in 28 months (Lincoff et al. 2017) [70]
Anacetrapib (Merck)
Trial: **REVEAL**
Anacetrapib (vs. placebo) in patients receiving intensive atorvastatin therapy
4.1-year follow-up
HDL-C increased by 104% (midpoint)
LDL-C decreased by 41% (midpoint)
Slightly higher blood pressure (0.7 mm Hg)
No significant differences in the risk of death, cancer, or other serious adverse events
Associated with a lower incidence of new-onset diabetes (11%)
Lower incidence of major coronary events (10.8 vs. 11.8% CVD events) (HPS3/TIMI55–REVEAL Collaborative Group, Bowman L, et al. 2017) [71]
TA-8995 (obicetrapib) – funded by Dezima and undertaken by Xention
Trial: **TULIP**
Nine treatments with TA-8995 alone and combined with statins
Analyses at 12 weeks of treatment
LDL-C reduced by 45.3% (apoB reduced by 33.7%)
HDL-C increased by 179% (apoA-1 increased by 63.4%)
Combined with statins: decrease in LDL-C from 39.8% to 50.2% (Hovingh et al. 2015)
Increased preβ-HDL and cell cholesterol efflux (van Capelleveen et al. 2016) [79]

2.6.1 Torcetrapib

The first pharmacological molecule designed to inhibit CETP was torcetrapib. In two trials, ILLUSTRATE and ILLUMINATE, patients received atorvastatin + torcetrapib or atorvastatin alone and were followed for 12 or 24 months [55, 68]. Compared to atorvastatin monotherapy, torcetrapib + atorvastatin was able to increase HDL-C (61–72%) and decrease LDL-C (20–25%). However, an important side effect

was the elevation of systolic blood pressure by approximately 5 mm Hg. There was a small or no favorable effect on atheroma volume [68]. Because torcetrapib-treated patients showed an increased number of cardiovascular events and death from both cardiovascular and noncardiovascular causes [55], the clinical trial was terminated prematurely. The increase in aldosterone levels and blood pressure was considered off-target toxicity rather than a CETP inhibition effect per se, increasing expectations for further generation of CETP inhibitors.

2.6.2 Dalcetrapib

The second molecule to go on to clinical trial was dalcetrapib (dal-OUTCOMES). Although the increase in systolic blood pressure was modest, dalcetrapib was less efficient at increasing HDL-C and reducing LDL-C, so the risk of major cardiovascular outcomes was not significantly altered. Dalcetrapib is considered a relatively weak inhibitor of CETP, meaning that a more potent CETP inhibitor could still be effective regarding clinical benefits in cardiovascular diseases (CVD) [69].

2.6.3 Evacetrapib

A potent CETP inhibitor, evacetrapib, was then evaluated in the ACELLERATE trial [70]. Indeed, evacetrapib caused marked favorable changes in the lipoprotein profile, increasing HDL-C by approximately 130% and reducing LDL-C by 37% compared to placebo. However, there were no significant benefits for CVD risks and events, and the trial was stopped due to futility at 28 months of treatment. Some could raise the possibility that longer treatment could show CVD improvements.

2.6.4 Anacetrapib

The most successful CETP inhibitor to date is anacetrapib. It in fact reduced the incidence of major coronary events [71]. Anacetrapib was added to intensive statin treatment in the REVEAL trial, a much larger and longer trial than the previous ones. Patients were followed up for 4 years, and at midpoint, HDL-C was increased by 104% in the anacetrapib group. The incidence of the primary outcome was reduced in the anacetrapib group (10.8% vs. 11.8% in the placebo group). Although there was no significant difference between groups during the first year of follow-up, the incidence of major coronary events after 1 year was significantly lower in the anacetrapib group (rate ratio, 0.88; 95% CI, 0.81 to 0.95; $P = 0.001$). Anacetrapib is a highly lipophilic drug that accumulates in adipose tissue, explaining its prolonged elimination profile [72]. No serious adverse events were identified, and there was only a slightly higher blood pressure (0.7 mm Hg) in the group of patients receiving anacetrapib. Another unexpected good finding of anacetrapib was its association with a lower incidence of new-onset diabetes cases. A recent meta-analysis of CETP inhibitor trials showed that CETP inhibitor therapy was associated with a significant 12% reduction in the incidence of diabetes and concluded that the improvement in glucose metabolism is at least in part related to the increase in HDL-C concentration [73].

Although CETP inhibitors are expected to increase HDL-C levels, their impact on reducing LDL-C has gained special attention. In the anacetrapib trial, LDL-C levels were reduced by 40%, as indicated by the "direct method" or by 17% when measured by beta-quantification. This discrepancy discloses the importance of understanding what different methods for LDL cholesterol quantification truly quantify. It is important to discover differential inhibitor effects across the whole spectrum of atherogenic apoB-containing lipoproteins [74]. CETP inhibition may also reduce the concentrations of triglyceride-rich remnant lipoproteins rather than affect size-specific LDL particles [75]. Regarding the mechanism of action, anacetrapib reduces LDL-C levels by increasing its catabolism, while the LDL-apoB-100 production rate is unchanged [76].

2.6.5 TA-8995 (Obicetrapib)

Another promising CETP inhibitor compound is TA-8995 (obicetrapib), which is well tolerated and shows beneficial effects on lipid and apolipoprotein profiles in healthy subjects and patients with mild dyslipidemia [77, 78]. TA-8995 was tested in a randomized, double blind, placebo-controlled phase 2 trial (TULIP) [78]. The safety and efficacy of TA-8995 were tested as a monotherapy and combined with statins. This CETP inhibitor reduces LDL cholesterol and apoB levels by 45% and 34%, respectively, conferring an additional decrease in LDL-C in combination with statins. HDL-C and apoA-1 increased up to 179% and 63%, respectively. TA-8995 also reduces lipoprotein(a) ranging from 27% to 37% [78]. TA-8995 is now registered in a new clinical trial (*ClinicalTrials.gov identifier (NCT number): NCT02241772*) to evaluate its effects on subjects with elevated Lp (a). A subsequent study advanced the possible mechanisms of TA-8995. Plasma samples from TULIP trial patients treated with TA-8995 were shown to dose-dependently increase total and ABCA1-specific cholesterol efflux capacity from the J774 macrophage cell line. These findings suggest that TA-8995 not only increases HDL-C and pre-Beta1-HDL particle levels but also promotes the functional properties of these particles. Whether these changes in HDL particle composition and functionality have a beneficial effect on cardiovascular outcome requires formal testing [79]. Thus, a cardiovascular disease outcome trial with TA-8995 is still needed to translate these effects into a reduction in cardiovascular disease events.

2.7 Concluding Remarks

Major advances in understanding CETP biology were obtained after protein purification, gene cloning, and molecular structure resolution. Studies in animal models have elucidated CETP functions, gene expression regulation, and relationships with diseases. Apart from its HDL-reducing systemic action, intriguing reports raise the possibility that CETP may have relevant local and novel nonlipid transfer functions. Animal and human studies suggest that CETP is important clinically and worthy as a target for drug development. The pros and cons of inhibiting CETP were discussed. Despite major setbacks in clinical trials, the hypothesis that CETP inhibitors are anti-atherogenic in humans is still being tested.

Acknowledgments We thank our co-authors on the original studies reviewed here, as well as other groups for their original contributions to the field. HCFO and HFR research was supported by Fundação de Amparo a Pesquisa do Estado de São Paulo (Fapesp grants #2017/17728-8, # 2015/17555-0 and # 2013/07607-8) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq grants #300937/2018-0)

References

1. Chajek T, Fielding CJ (1978) Isolation and characterization of a human serum cholesteryl ester transfer protein. Proc Natl Acad Sci U S A 75:3445–3449
2. Pattnaik NM, Montes A, Hughes LB, Zilversmit DB (1978) Cholesteryl ester exchange protein in human plasma isolation and characterization. Biochim Biophys Acta 530:428–438
3. Ha YC, Barter PJ (1982) Differences in plasma cholesteryl ester transfer activity in sixteen vertebrate species. Comp Biochem Physiol B 71:265–269
4. Tsutsumi K, Hagi A, Inoue Y (2001) The relationship between plasma high density lipoprotein cholesterol levels and cholesteryl ester transfer protein activity in six species of healthy experimental animals. Biol Pharm Bull 24:579–581
5. Hesler CB, Swenson TL, Tall AR (1987) Purification and characterization of a human plasma cholesteryl ester transfer protein. J Biol Chem 262:2275–2282
6. Drayna D et al (1987) Cloning and sequencing of human cholesteryl ester transfer protein cDNA. Nature 327:632–634
7. Agellon LB et al (1990) Organization of the human cholesteryl ester transfer protein gene. Biochemistry 29:1372–1376
8. Oliveira HCF, de Faria EC (2011) Cholesteryl ester transfer protein: the controversial relation to atherosclerosis and emerging new biological roles. IUBMB Life 63:248–257
9. Qiu X et al (2007) Crystal structure of cholesteryl ester transfer protein reveals a long tunnel and four bound lipid molecules. Nat Struct Mol Biol 14:106–113

10. Charles MA, Kane JP (2012) New molecular insights into CETP structure and function: a review. J Lipid Res 53:1451–1458
11. Inazu A et al (1992) Alternative splicing of the mRNA encoding the human cholesteryl ester transfer protein. Biochemistry 31:2352–2358
12. Quinet E, Yang TP, Marinos C, Tall A (1993) Inhibition of the cellular secretion of cholesteryl ester transfer protein by a variant protein formed by alternative splicing of mRNA. J Biol Chem 268:16891–16894
13. Lira ME, Loomis AK, Paciga SA, Lloyd DB, Thompson JF (2008) Expression of CETP and of splice variants induces the same level of ER stress despite secretion efficiency differences. J Lipid Res 49:1955–1962
14. Alva V, Lupas AN (2016) The TULIP superfamily of eukaryotic lipid-binding proteins as a mediator of lipid sensing and transport. Biochim Biophys Acta 1861:913–923
15. Wong LH, Levine TP (2017) Tubular lipid binding proteins (TULIPs) growing everywhere. Biochim Biophys Acta Mol Cell Res 1864:1439–1449
16. Miller GJ, Miller NE (1975) Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. Lancet Lond Engl 1:16–19
17. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR (1977) High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am J Med 62:707–714
18. Glomset JA (1968) The plasma lecithins: cholesterol acyltransferase reaction. J Lipid Res 9:155–167
19. Agellon LB et al (1991) Reduced high density lipoprotein cholesterol in human cholesteryl ester transfer protein transgenic mice. J Biol Chem 266:10796–10801
20. Jiang XC, Agellon LB, Walsh A, Breslow JL, Tall A (1992) Dietary cholesterol increases transcription of the human cholesteryl ester transfer protein gene in transgenic mice. Dependence on natural flanking sequences. J Clin Invest 90:1290–1295
21. Marotti KR et al (1993) Severe atherosclerosis in transgenic mice expressing simian cholesteryl ester transfer protein. Nature 364:73–75
22. Fagerberg L et al (2014) Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol Cell Proteomics MCP 13:397–406
23. Quinet E, Tall A, Ramakrishnan R, Rudel L (1991) Plasma lipid transfer protein as a determinant of the atherogenicity of monkey plasma lipoproteins. J Clin Invest 87:1559–1566
24. Wang Y et al (2015) Plasma cholesteryl ester transfer protein is predominantly derived from Kupffer cells. Hepatol Baltim Md 62:1710–1722
25. Oliveira HC et al (1996) Human cholesteryl ester transfer protein gene proximal promoter contains dietary cholesterol positive responsive elements and mediates expression in small intestine and periphery while predominant liver and spleen expression is controlled by 5′-distal sequences. Cis-acting sequences mapped in transgenic mice. J Biol Chem 271:31831–31838
26. Luo Y, Tall AR (2000) Sterol upregulation of human CETP expression in vitro and in transgenic mice by an LXR element. J Clin Invest 105:513–520
27. Berti JA et al (2001) Thyroid hormone increases plasma cholesteryl ester transfer protein activity and plasma high-density lipoprotein removal rate in transgenic mice. Metabolism 50:530–536
28. Raposo HF, Patrício PR, Simões MC, Oliveira HCF (2014) Fibrates and fish oil, but not corn oil, up-regulate the expression of the cholesteryl ester transfer protein (CETP) gene. J Nutr Biochem 25:669–674
29. Masucci-Magoulas L et al (1995) Decreased cholesteryl ester transfer protein (CETP) mRNA and protein and increased high density lipoprotein following lipopolysaccharide administration in human CETP transgenic mice. J Clin Invest 95:1587–1594
30. Berti JA et al (2003) Cholesteryl ester transfer protein expression is down-regulated in hyperinsulinemic transgenic mice. J Lipid Res 44:1870–1876
31. Plump AS et al (1999) Increased atherosclerosis in ApoE and LDL receptor gene knock-out mice as a result of human cholesteryl ester transfer protein transgene expression. Arterioscler Thromb Vasc Biol 19:1105–1110
32. Westerterp M et al (2006) Cholesteryl ester transfer protein decreases high-density lipoprotein and severely aggravates atherosclerosis in APOE*3-Leiden mice. Arterioscler Thromb Vasc Biol 26:2552–2559
33. Van Eck M et al (2007) Important role for bone marrow-derived cholesteryl ester transfer protein in lipoprotein cholesterol redistribution and atherosclerotic lesion development in LDL receptor knockout mice. Circ Res 100:678–685
34. Hayek T et al (1995) Decreased early atherosclerotic lesions in hypertriglyceridemic mice expressing cholesteryl ester transfer protein transgene. J Clin Invest 96:2071–2074
35. Föger B et al (1999) Cholesteryl ester transfer protein corrects dysfunctional high density lipoproteins and reduces aortic atherosclerosis in lecithin cholesterol acyltransferase transgenic mice. J Biol Chem 274:36912–36920
36. Berti JA, de Faria EC, Oliveira HCF (2005) Atherosclerosis in aged mice over-expressing the reverse cholesterol transport genes. Braz J Med Biol Res Rev Bras Pesqui Medicas E Biol 38:391–398
37. Cazita PM et al (2003) Cholesteryl ester transfer protein expression attenuates atherosclerosis in ovariectomized mice. J Lipid Res 44:33–40
38. Casquero AC et al (2006) Atherosclerosis is enhanced by testosterone deficiency and attenuated by CETP expression in transgenic mice. J Lipid Res 47:1526–1534

39. Kako Y, Massé M, Huang L-S, Tall AR, Goldberg IJ (2002) Lipoprotein lipase deficiency and CETP in streptozotocin-treated apoB-expressing mice. J Lipid Res 43:872–877
40. MacLean PS et al (2003) Cholesteryl ester transfer protein expression prevents diet-induced atherosclerotic lesions in male db/db mice. Arterioscler Thromb Vasc Biol 23:1412–1415
41. Harder C, Lau P, Meng A, Whitman SC, McPherson R (2007) Cholesteryl ester transfer protein (CETP) expression protects against diet induced atherosclerosis in SR-BI deficient mice. Arterioscler Thromb Vasc Biol 27:858–864
42. El Bouhassani M et al (2011) Cholesteryl ester transfer protein expression partially attenuates the adverse effects of SR-BI receptor deficiency on cholesterol metabolism and atherosclerosis. J Biol Chem 286:17227–17238
43. Koizumi J et al (1985) Deficiency of serum cholesteryl-ester transfer activity in patients with familial hyperalphalipoproteinaemia. Atherosclerosis 58:175–186
44. Yamashita S et al (2000) Molecular mechanisms, lipoprotein abnormalities and atherogenicity of hyperalphalipoproteinemia. Atherosclerosis 152:271–285
45. Zhong S et al (1996) Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. J Clin Invest 97:2917–2923
46. Nagano M et al (2004) Molecular mechanisms of cholesteryl ester transfer protein deficiency in Japanese. J Atheroscler Thromb 11:110–121
47. Ordovas JM et al (2000) Association of cholesteryl ester transfer protein-TaqIB polymorphism with variations in lipoprotein subclasses and coronary heart disease risk: the Framingham study. Arterioscler Thromb Vasc Biol 20:1323–1329
48. Quintão ECR (2010) Letter by Quintao regarding article, 'Association of circulating cholesteryl ester transfer protein activity with incidence of cardiovascular disease in the community'. Circulation 122:e420; author reply e421
49. Cao M, Zhou Z-W, Fang B-J, Zhao C-G, Zhou D (2014) Meta-analysis of cholesteryl ester transfer protein TaqIB polymorphism and risk of myocardial infarction. Medicine (Baltimore) 93:e160
50. Thompson A et al (2008) Association of cholesteryl ester transfer protein genotypes with CETP mass and activity, lipid levels, and coronary risk. JAMA 299:2777–2788
51. Wang Q et al (2014) Seven functional polymorphisms in the CETP gene and myocardial infarction risk: a meta-analysis and meta-regression. PloS One 9: e88118
52. de Haan W et al (2008) Torcetrapib does not reduce atherosclerosis beyond atorvastatin and induces more proinflammatory lesions than atorvastatin. Circulation 117:2515–2522
53. Cazita PM, Barbeiro DF, Moretti AIS, Quintão ECR, Soriano FG (2008) Human cholesteryl ester transfer protein expression enhances the mouse survival rate in an experimental systemic inflammation model: a novel role for CETP. Shock Augusta Ga 30:590–595
54. Venancio TM et al (2016) CETP lowers TLR4 expression which attenuates the inflammatory response induced by LPS and polymicrobial sepsis. Mediators Inflamm 2016:1784014
55. Barter PJ et al (2007) Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med 357:2109–2122
56. Grion CMC et al (2010) Lipoproteins and CETP levels as risk factors for severe sepsis in hospitalized patients. Eur J Clin Invest 40:330–338
57. Trinder M et al (2019) Cholesteryl ester transfer protein influences high-density lipoprotein levels and survival in sepsis. Am J Respir Crit Care Med 199:854–862
58. Blauw LL, Wang Y, Willems van Dijk K, Rensen PCN (2020) A novel role for CETP as immunological gatekeeper: raising HDL to cure sepsis? Trends Endocrinol Metab. https://doi.org/10.1016/j.tem.2020.01.003
59. Zhang Z et al (2001) Expression of cholesteryl ester transfer protein in human atherosclerotic lesions and its implication in reverse cholesterol transport. Atherosclerosis 159:67–75
60. Izem L, Morton RE (2001) Cholesteryl ester transfer protein biosynthesis and cellular cholesterol homeostasis are tightly interconnected. J Biol Chem 276:26534–26541
61. Izem L, Morton RE (2007) Possible role for intracellular cholesteryl ester transfer protein in adipocyte lipid metabolism and storage. J Biol Chem 282:21856–21865
62. Greene DJ, Izem L, Morton RE (2015) Defective triglyceride biosynthesis in CETP-deficient SW872 cells. J Lipid Res 56:1669–1678
63. Izem L, Greene DJ, Bialkowska K, Morton RE (2015) Overexpression of full-length cholesteryl ester transfer protein in SW872 cells reduces lipid accumulation. J Lipid Res 56:515–525
64. Zhou H et al (2006) Adipose tissue-specific CETP expression in mice: impact on plasma lipoprotein metabolism. J Lipid Res 47:2011–2019
65. Izem L, Liu Y, Morton RE (2020) Exon 9-deleted CETP inhibits full length-CETP synthesis and promotes cellular triglyceride storage. J Lipid Res. https://doi.org/10.1194/jlr.RA120000583
66. Sugano M et al (1998) Effect of antisense oligonucleotides against cholesteryl ester transfer protein on the development of atherosclerosis in cholesterol-fed rabbits. J Biol Chem 273:5033–5036
67. Rittershaus CW et al (2000) Vaccine-induced antibodies inhibit CETP activity in vivo and reduce aortic lesions in a rabbit model of atherosclerosis. Arterioscler Thromb Vasc Biol 20:2106–2112

68. Nissen SE et al (2007) Effect of torcetrapib on the progression of coronary atherosclerosis. N Engl J Med 356:1304–1316
69. Schwartz GG et al (2012) Effects of dalcetrapib in patients with a recent acute coronary syndrome. N Engl J Med 367:2089–2099
70. Lincoff AM et al (2017) Evacetrapib and cardiovascular outcomes in high-risk vascular disease. N Engl J Med 376:1933–1942
71. HPS3/TIMI55–REVEAL Collaborative Group et al (2017) Effects of anacetrapib in patients with atherosclerotic vascular disease. N Engl J Med 377:1217–1227
72. Krishna R et al (2017) Chronic administration of anacetrapib is associated with accumulation in adipose and slow elimination. Clin Pharmacol Ther 102:832–840
73. Masson W et al (2018) Therapy with cholesteryl ester transfer protein (CETP) inhibitors and diabetes risk. Diabetes Metab 44:508–513
74. Holmes MV, Ala-Korpela M (2019) What is 'LDL cholesterol'? Nat Rev Cardiol 16:197–198
75. Kettunen J et al (2019) Lipoprotein signatures of cholesteryl ester transfer protein and HMG-CoA reductase inhibition. PLoS Biol 17:e3000572
76. Millar JS et al (2016) Anacetrapib lowers LDL by increasing ApoB clearance in mildly hypercholesterolemic subjects. J Clin Invest 126:1603–1604
77. Ford J et al (2014) Tolerability, pharmacokinetics and pharmacodynamics of TA-8995, a selective cholesteryl ester transfer protein (CETP) inhibitor, in healthy subjects. Br J Clin Pharmacol 78:498–508
78. Hovingh GK et al (2015) Cholesterol ester transfer protein inhibition by TA-8995 in patients with mild dyslipidaemia (TULIP): a randomised, double-blind, placebo-controlled phase 2 trial. Lancet Lond Engl 386:452–460
79. van Capelleveen JC et al (2016) Effects of the cholesteryl ester transfer protein inhibitor, TA-8995, on cholesterol efflux capacity and high-density lipoprotein particle subclasses. J Clin Lipidol 10:1137. e3–1144.e3