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Perspective

Oral Cholesteryl Ester Transfer Protein (CETP) Inhibitors: A Potential New Approach for Treating Coronary Artery Disease

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Introduction

Cholesteryl ester transfer protein (CETP), a plasma glycoprotein, mediates the transfer of neutral lipids in the blood among various lipoproteins.[1,2] Because of their poor water solubility, neutral lipids such as triglycerides (TG), free cholesterol (FC), and cholesteryl esters (CE) generally are not freely circulating in plasma but instead are packaged together and transported through the body in larger lipoprotein particles that have amphipathic lipids and proteins as surface components. Low-density lipoprotein (LDL) and high-density lipoproteins (HDL) particles help move cholesterol to and from the periphery, respectively. The entire transport process is highly regulated in terms of lipoprotein production and the transfer of lipids to and from these particles.

CETP facilitates the transfer of CE from HDL containing apolipoprotein-A (apo-A) to apo-B-containing lipoproteins such as very-low-density lipoprotein (VLDL) and LDL with a balanced exchange of TG. For every CE acquired from HDL and transferred to LDL or VLDL, CETP takes up one TG molecule from LDL or VLDL and transfers it to HDL. Thus, CETP plays a critical role in both CE and TG transfer among lipoproteins. A CETP inhibitor would thus be expected to raise plasma HDL cholesterol (HDLc) levels, lower LDL cholesterol (LDLc), and provide a potential therapeutic benefit for patients with coronary artery disease (CAD).

As discussed in more detail below, whether CETP inhibition might induce an antiatherogenic response by raising HDLc or have proatherogenic effects by interfering with reverse cholesterol transport (RCT) has been the subject of considerable debate and controversy.[2−5] Important insights from studies over the past several years have added to our understanding of the multiple mechanisms by which HDL achieves its atheroprotective effects[6] and the complex physiological and pathophysiological roles CETP plays in lipoprotein metabolism. However, the field still remains controversial. Recently, a number of potent, selective, and orally active CETP inhibitors have also been identified that raise HDLc in animals and more clearly define the role of CETP in preclinical models of atherosclerosis.[2] Several of these new CETP inhibitors have been brought forward and evaluated in clinical trials, and two have now independently demonstrated phase II proof of concept by dramatically increasing HDLc levels by 50% or more in healthy volunteers and patients with low HDLc.[7,8] Thus, for the first time, pharmacological agents are available that have the potential to elevate HDLc in patients with low HDLc to about the same extent that the current standard of care drugs reduce LDLc in hyperlipidemic patients. These exciting clinical results have generated renewed interest in CETP inhibition as a potential new therapeutic strategy for treating CAD, and more than 750 related patents or publications have appeared in Chemical Abstracts since 2000. It remains to be seen whether prolonged treatment with these new agents leads to a profound reduction in secondary coronary events across a broader patient population without disrupting normal lipoprotein balance and function. This Perspective summarizes the current status of orally active CETP inhibitors as potential new treatments for CAD.

Low HDLc as an Independent Risk Factor for CAD

Atherosclerosis describes the underlying progression in arterial dysfunction and remodeling that restricts blood flow to vessels in the peripheral vasculature and is ultimately manifested as CAD. This remodeling is accompanied by the buildup of

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lipid-laden, cholesterol-rich fatty deposits within the vessel wall that arise from the retention of modified LDL particles by monocytes, macrophages, and T cells in the intimal space below the endothelial lining. The resulting atherosclerotic plaques are vulnerable to rupture and thrombus formation. The loss of oxygen supply to the heart produces cellular and tissue injury culminating in chest pain (angina), heart attack, and/or myocardial infarct (MI).[9]

While smoking, hypertension, age, obesity, and family history all contribute to CAD, dyslipidemia is one of the most prominent risk factors for this disease. Epidemiological studies have established correlations between various forms of dyslipidemia and the accompanying risk of developing CAD. Hyperlipidemia, usually attributed to high levels of LDLc, is associated with a significant risk for CAD. Nearly 60% of the circulating cholesterol in human plasma is present as LDLc, and most of the higher overall total plasma cholesterol levels found in CAD patients is associated with LDLc. Historically, in healthy human subjects total plasma cholesterol levels are believed to be in the normal range when they occur below 200 mg/dL with accompanying LDLc levels below 130 mg/dL and HDLc above 40 mg/dL. In contrast, CAD patients have total plasma cholesterol concentrations of 250−300 mg/dL or more with a corresponding increased plasma LDLc.

Compounds that target either cholesterol biosynthesis such as the HMG-CoA reductase inhibitors[10] (statins) or agents that limit cholesterol absorption, e.g., ezetimibe,[11] significantly reduced LDLc and lowered the incidence of coronary events in hyperlipidemic patients. In several prospective statin trials, lowering LDLc by 28−35% led to a corresponding 24−34% reduction in primary or secondary CAD mortality and morbidity in this patient population.[12] For every 1% lowering of LDLc, an approximate 1% reduction in CAD risk was observed.[10,12] As a result, statins have become first line therapy for lowering total plasma cholesterol levels. However, typically more than 60% of the statin-treated patients in these controlled trials continued to develop cardiovascular disease and failed to experience a therapeutic benefit.[13] Most of these nonresponders also had low HDLc levels.[14] Thus, there appears to be a large unmet medical need for newer therapies that would provide additional benefit to this sizable patient population that is nonresponsive to statins.

In contrast, several epidemiological studies have demonstrated an inverse relationship between serum HDLc levels and the incidence of ischemic heart disease in both middle-aged men and the elderly.[15] In the Framingham cohort,[15a] a higher prevalence (19%) of heart disease occurred in subjects in this group solely because of low (<35 mg/dL) HDLc compared to the prevalence of disease (12%) in those that only had elevated (>130 mg/dL) LDLc. Within this CAD patient population, 60% had low HDLc (<35 mg/dL) and elevated LDLc (>130 mg/dL), 25% had low HDLc (<35 mg/dL) with acceptable LDLc (<130 mg/dL) and TG (<200 mg/dL), while 15% had elevated LDLc (>130 mg/dL) with acceptable HDLc (35−59 mg/dL) and TG levels (<200 mg/dL). Thus, low levels of HDLc represent a significant independent risk factor in CAD irrespective of whether patients have elevated LDLc, and this has been confirmed in a recent National Cholesterol Education Program (NCEP) report.[16] A risk prediction model developed from the Framingham cohort[17] showed that a nearly 50% higher cardiovascular risk was associated with male patients having HDLc levels below 35 mg/dL compared to those in the normal range (35−59 mg/dL). An even higher (2-fold) associated risk was observed in women with HDLc below 35 mg/dL. In contrast, a significantly reduced risk (0.61 vs 1.00) was present in both men and women having HDLc levels above 60 mg/dL. Thus, HDLc levels above 60 mg/dL are considered to be cardioprotective. HDLc is now recognized as one of the best predictors of cardiovascular risk in women of all ages and in men after middle-age.[20]

Several clinical studies have demonstrated that a reduction in CAD events is positively correlated with treatments that raised HDLc. The controlled Helsinki heart trial monitored coronary event rates in over 4000 asymptomatic middle-aged men with dyslipidemia for 5 years and showed that correcting low levels of HDLc with gemfibrozil led to a 34% decrease in coronary events.[18] For each 1 mg/dL rise in HDLc among the study subjects, the average response was a 3% decrease in the risk of new CAD. Similarly, in the VA-HIT trial, 2531 men with CAD having HDLc ≤ 40 mg/dL, LDLc ≤ 140 mg/dL, and TG ≤ 300 mg/dL were treated for an average of 5 years with gemfibrozil.[19] In this study, LDLc was not changed significantly, TG was lowered 31%, HDLc was raised 6%, and the combined number of CAD events was lowered 24% (a 2−3% decrease in CAD for every 1% rise in HDLc). In comparison, prospective statin trials showed about a 1% reduction in CAD risk for every 1% decrease in LDLc.[10,12] While statin therapy effectively lowered LDLc by up to 45%, these agents generally yield a very modest increase in HDLc levels of usually less than 10%.[10,20] Unfortunately, attributing the event reductions observed in the VA-HIT trial exclusively to HDL elevations is confounded by the concomitant reductions in triglycerides. In contrast, no significant reductions in coronary events were observed in patients treated with bezafibrate for more than 6 years in the bezafibrate infarction program (BIP) trial, even though bezafibrate treatment increased average HDLc by 18% and lowered TG by 21%.[21]

Niacin (nicotinic acid, pyridine-3-carboxylic acid) has also been used to elevate HDLc by as much as 30%, but side effects such as flushing were common and limited patient compliance. Similar increases in total HDLc of 26%−28% have been achieved with fewer side effects following long-term treatment with NIASPAN, an extended release form of nicotinic acid, either alone[22] or with simvastatin.[23] Prolonged treatment with immediate-release niacin alone had some clinical benefit in reducing total mortality, but side effects often limit treatment.[24] To date, there have been no large population secondary prevention trials correlating HDLc increases higher than 30% through direct pharmacological intervention with an improved cardiovascular benefit.

Atheroprotective Effects of HDL

HDL exerts its cardiovascular benefit through several atheroprotective mechanisms that are the subject of ongoing in vitro and in vivo studies.[6] Most importantly, HDL mediates the RCT pathway, the one mechanism by which excess cholesterol is removed from peripheral tissues and delivered to the liver for elimination via the bile into the gut (Figure 1).[25] Since most tissues are unable to break down free cholesterol, this RCT process thus represents an important mechanism for regulating cholesterol pools and maintaining cholesterol homeostasis. Both the macrophages and underlying smooth muscle cells involved in atherosclerotic lesion formation acquire cholesterol by internalization of FC from modified LDL. The key role that HDL plays in mediating the RCT process from these cholesterol-rich cells in the atherosclerotic lesion is regarded as the primary mechanism by which HDL reduces or prevents progression of atherosclerosis.[6,20,25] As shown in Figure 1, by transferring

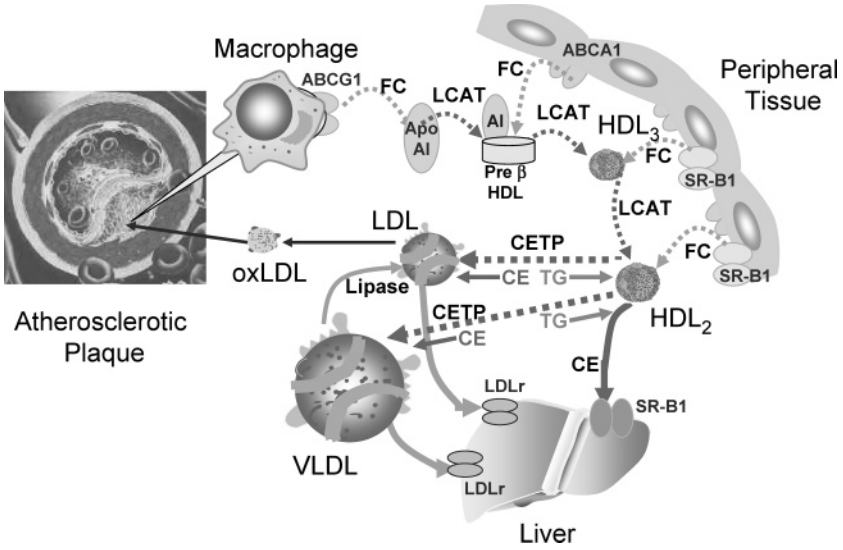


Figure 1. Model for CETP in reverse cholesterol transport. Free cholesterol (FC) in peripheral tissues or atherosclerotic plaque is removed via ABC transporters through their interaction with apo-AI. The esterification of cholesterol with fatty acids by the LCAT reaction drives the growth of HDL particles first via discoidal preβ-HDL and then to mature spherical HDL particles via smaller HDL_3 and larger HDL_2. Spherical HDL_2 and HDL_3 particles and mature HDL do not interact well with ABCA1 in peripheral tissue but can access additional FC through apo-AI-mediated interaction with related specific macrophage ABCG1 transporters or the SR-B1 scavenger receptors found in peripheral cells. CEs can be delivered directly to the liver by the interaction of HDL particles with the hepatic SR-B1 receptor or indirectly following the CETP process that transfers CE from HDL to LDL and VLDL particles in exchange for TG. The CE in VLDL and LDL particles can then be taken up in the liver by the hepatic LDL receptor (LDLr).[136]

CE from HDL to VLDL and LDL for elimination in the liver, CETP mediates an important step in the RCT pathway and facilitates removal of excess cholesterol from the periphery to the liver.

A better understanding of HDL assembly and the individual components of RCT has evolved over the past few years as several individual steps have been elucidated. Notably, HDL consists of a heterogeneous population of lipoprotein particles that vary by their density, shape, size, lipoprotein composition, and charge.[6] Several subclasses of HDL particles have been observed that can be separated by gel filtration or size exclusion chromatography, including a discoidal pre-β-HDL and two spherical subclasses HDL_2 and HDL_3. HDL_3 particles have higher densities and smaller diameters than HDL_2 particles. Pre-β-HDL particles contain mostly apo-A (>90%) and have only a small relative percentage of CE and FC.[6e]

HDL particle assembly is a complex process involving several intermediates that occurs throughout the circulation. Mature HDL assembly begins when lipid-free apo-AI accepts FC at sites in the liver, intestine, endothelial cells, or macrophages by a receptor-mediated process involving ATP-binding cassette (ABC) transporters, primarily ABCA1 (Figure 1).[6f] The resulting FC associated with apo-AI is esterified to CE by the action of lecithyl cholesterol acyl transferase (LCAT), which utilizes apo-AI as a cofactor. This LCAT-assisted fatty acid esterification process drives the formation of the discoidal pre-β-HDL particles. These pre-β-HDL particles also accept more FC from ABCA1 and develop further into spherical HDL_2 and HDL_3 particles, following the action of LCAT. Spherical HDL_2 and HDL_3 particles and mature HDL do not interact well with ABCA1 but can access additional FC through apo-AI-mediated interaction with related specific macrophage ABCG1 transporters or the SR-B1 scavenger receptors found in peripheral cells. Intact spherical HDL_2 and HDL_3 particles and mature HDL can deliver FC and CE to the liver by a similar apo-AI-mediated interaction with the hepatic SR-B1 receptor. Alternatively, CE is transferred from HDL (primarily via HDL_2) to either VLDL or LDL by a process mediated by CETP, and subsequently the CE in these apo-B-containing particles is delivered to the liver via the LDL receptor (LDLr). The resulting triglyceride-enriched HDL particles are subject to catabolism in the liver by various lipases, which regenerates apo-AI to restart the cycle. While ABCA1 and ABCG1 promote cholesterol efflux from cells to the various forms of HDL, the SR-B1 receptors mediate bidirectional transport of cholesterol both in the liver and in the periphery.

HDL also has beneficial antioxidant and anti-inflammatory properties and has been demonstrated to improve endothelial dysfunction. Oxidized LDL (oxLDL) contributes to the emerging atherosclerotic plaque by increasing the lipid content of macrophages leading to foam cell development. HDL contains two antioxidant enzymes, paraoxonase and platelet-activating factor acetylhydrolase, that not only inhibit the oxidation of LDL but also help degrade oxidized phospholipids within oxLDL.[6,26] Moreover, HDL and its associated apo-A protect endothelial cells from damage induced by oxLDL.[26] HDL also displays anti-inflammatory properties, since pretreatment of human endothelial cells with HDL inhibits the cytokine-induced expression of cell adhesion molecules.[6]

Alternatively, overall HDL functionality may be more important than absolute HDLc levels, since recent data indicate that HDL has both proinflammatory and anti-inflammatory properties. HDL isolated and characterized from the plasma of a small group of CAD patients exhibited a more proinflammatory profile in vitro on the basis of its lipid peroxide content and ability to alter LDL-induced monocyte chemotaxis than HDL obtained from age and sex-matched controls.[27] These preliminary results suggest that the proinflammatory characteristics of HDL from CAD patients may be a better predictor of overall risk than absolute HDLc levels. However, no large trials have yet been done to confirm this hypothesis.[27] A number of other studies have indicated that either infusion or overexpression of apo-A in animals produced profound reductions in atherosclerotoic lesions.[6] In contrast, humans having genetic deficiencies in apo-AI have very low levels of plasma HDL and exhibit premature CAD.[28]

The National Cholesterol Education Panel recently raised the threshold for treatment intervention in those CAD patients at risk because of low HDLc levels to include subjects having HDLc below 40 mg/dL.[16] In these patients, it is still uncertain to what extent HDLc must be raised to produce a cardiovascular benefit, but elevations of 50% or more may be required.[20] Current therapies offer only modest improvements and are often limited by side effects.[20] A clear need exists to identify safer and more effective HDLc-raising drugs for use as monotherapy or in conjunction with other lipid-lowering agents. Several new strategies are under investigation that might lead to more pronounced HDLc elevations.[6,20] These include targeting enzymes associated with lipoprotein metabolism (e.g., hepatic triglyceride lipase, lipoprotein lipase, LCAT, or phospholipid transfer protein (PLTP)), modulating SR-B1, administering apo-AI or other HDL apolipoproteins such as apo-AI_{Milano}, activating agonists of nuclear receptors, or inhibiting CETP. This Perspective focuses on orally active CETP inhibitors as a potential new approach for elevating HDLc.

Physiological Role of CETP in Atherosclerosis

Whether CETP contributes to atherosclerosis or plays an antiatherogenic role has been the subject of an ongoing scientific debate.[2−5] In human plasma, CETP plays a potentially proatherogenic role by moving CE from HDL into VLDL and LDL particles, thereby lowering atheroprotective HDLc and raising proatherogenic VLDLc and LDLc. This equilibrium should be driven by the overall plasma concentration of CETP, and higher plasma CETP protein levels should therefore induce greater amounts of proatherogenic non-HDLc. On the other hand, CETP also plays a potentially key antiatherogenic role in the RCT process (Figure 1). By transferring CE from HDL to LDL and VLDL, CETP helps to remove excess cholesterol from peripheral tissues (including atherosclerotic plaque) and increases the amount of cholesterol in these apo-B lipoproteins that are taken up by the liver through the hepatic LDLr. Higher CETP plasma levels should therefore facilitate cholesterol removal by the RCT pathway. As discussed in more detail below, CETP also plays a key role in HDL particle remodeling.

CETP Plasma Levels and CAD Risk. In the EPIC-Norfolk cohort study of apparently healthy European men and women who developed CAD, CETP plasma levels were positively correlated with LDLc and negatively correlated with HDLc. In this study, the risk of CAD increased as the plasma levels of CETP increased, and the associated higher cardiovascular risk was most pronounced in subjects with elevated triglycerides.[29] Similarly, in a 2-year study of nearly 300 Caucasian subjects with familial hypercholesterolemia and elevated LDLc, plasma CETP levels were positively correlated with a more atherogenic lipid profile and increased progression of atherosclerosis. Subjects in this study having higher CETP plasma levels displayed decreased HDLc, increased LDLc and TG levels, a reduced HDL particle size, and smaller, more dense proatherogenic LDL particles.[30] Also in this study, treatment with statins improved the lipoprotein profile, but their effect was lessened in subjects with high plasma CETP protein levels.

Human CETP Deficiency. Originally, interest in CETP as a potential therapeutic target for CAD began with reports describing a small Japanese male population genetically deficient in CETP having hyperalphalipoproteinemia, i.e., markedly elevated HDLc, as much as 3- to 6-fold higher than the normal range.[31] A modest reduction in LDLc and changes in lipoprotein particle size (larger HDL and smaller, more heterogeneous LDL particles) and composition were also observed in these subjects. CETP deficiency arising from two common specific mutations in the CETP gene occurs frequently in the Japanese population, particularly in subjects with elevated HDLc, and accounts for nearly 10% of the HDLc variability in this population.[32]

Several polymorphisms in the CETP gene leading to reduced CETP plasma levels have also been identified, although relating them to CAD has led to variable results. The Honolulu heart study followed more than 3000 elderly Japanese-American men with CETP deficiency arising from two different CETP gene mutations and found that the relationship between CETP deficiency and CAD risk was complex and possibly dependent upon the accompanying HDLc and TG levels. The prevalence of disease was found to be higher in men with CETP gene mutations (21%) than in subjects without mutations (16%) even though subjects with mutations had reduced CETP levels and an overall higher average of HDLc. Those subjects with HDLc levels greater than 60 mg/dL had a significantly reduced cardiovascular risk, approaching that typically seen in normolipidemic subjects.[33] Nevertheless, a small subset of subjects having heterozygous CETP genetic defects with about half the normal plasma concentration of CETP and with HDLc levels between 41 and 60 mg/dL appeared to have increased cardiovascular risk in this study, although the number of events was small.[34] However, the findings for this heterozygous population were not confirmed in a recent 7-year follow-up study.[35] No statistically significant relationship between heterozygous mutations in the CETP gene and CAD was observed in the follow-up study, although a prospective analysis indicated a nonsignificant trend toward a lower CAD incidence in subjects with CETP mutations. Examples of CETP deficiency have now been identified in other Asian and Caucasian populations, and at least 10 mutations in the CETP gene have been associated with hyperalphalipoproteinemia. Indeed, it has been estimated that more than 30% of Japanese hyperalphalipoproteinemia cases can be attributed to CETP deficiency.[36]

One of the most widely studied CETP polymorphisms is Taq1B within intron 1. Analysis for CETP polymorphism in the Framingham offspring study identified subjects with the B1B1 allele who had higher CETP levels and lower HDLc levels than either B1B2 or B2B2 subjects. In men, the B2B2 allele was associated with significantly reduced CETP activity and elevated HDLc and thus resembled a mild form of the CETP-deficient phenotype. The presence of the B2B2 allele in men was also correlated with a markedly reduced risk for CAD,[37] but the cardiovascular benefits of the B2B2 allele were not observed in women. A similar correlation between the B2B2 allele with reduced CETP activity, elevated HDLc, and a reduced risk for CAD has recently been observed within the VA-HIT cohort.[38] Conversely, in a recent prospective clinical study of healthy, middle-aged U.S. men, carriers of the B2 allele of the Taq1B polymorphism in the CETP gene had higher HDLc but did not have a lower risk of MI.[39] Likewise, those populations exhibiting hyperalphalipoproteinemia were only protected against atherosclerosis below a certain HDLc level.[40] Other genetic polymorphisms in the CETP gene have been identified, but their relationship to CAD risk is inconsistent. For example, an analysis of a Danish population group associated different CETP mutations with decreased HDLc in both men and women and revealed a possibly paradoxical decreased risk of ischemic heart disease in women.[41]

There are no reports in the literature that CETP deficiency, lower CETP plasma protein levels, or reduced CE transfer activity leads to any overt mechanism-based toxicity. To the contrary, several studies in both Caucasian[42] and Asian[35]

populations indicated that subjects with exceptional longevity have elevated HDLc and lower LDLc, with larger particle sizes occurring in both HDL and LDL fractions. The isoleucine to valine variant at position 405 in the CETP gene is one of the candidate polymorphisms contributing to this special phenotype that is characterized by reduced CETP serum concentrations and a lower prevalence of disease, including CAD, in centenarians.[42] However, another study of more than 250 Japanese centenarians showed that the B2B2 allele of the CETP Taq1B polymorphism was consistently associated with higher HDLc levels, both in centenarians and in younger controls, but the allelic frequency did not differ between the two groups.[43] A separate study followed subjects from the Omagari district in northern Japan that have both CETP gene mutations and hyperalphalipoproteinemia.[36] In this study, those subjects having especially high HDLc (>70 mg/dL) had greater ischemic changes in their electrocardiograms than those having HDLc below 70 mg/dL, thus indicating that CETP deficiency may be proatherogenic in some subjects.

CETP in RCT. CETP also plays a potentially key antiatherogenic role in RCT.[25,44] By regulating HDLc plasma levels and the remodeling of HDL particles, CETP is an important component of this RCT pathway. By exchange of CE for TG in HDL, the resulting TG-enriched HDL particles are more susceptible to hydrolysis by hepatic lipases, which generates smaller HDL particles. CETP helps to reduce the overall HDL particle size, and smaller HDL particles are more efficient at promoting cholesterol eflux from macrophages, the initial step in the RCT process.[45] The enlarged, less dense HDL particles obtained from CETP-deficient subjects are enriched in cholesterol, apo-A, and apo-E, and these particles are less subject to lipase-mediated catabolism. The larger apo-E-enriched HDL particles from homozygous CETP-deficient subjects have an impaired ability to remove free cholesterol from macrophages.[46] CETP is also expressed in macrophages within atherosclerotic lesions and may initiate the early RCT process.[47] In addition, the remodeling of HDL by CETP and hepatic lipase leads to an enhanced uptake of CE from these lipoprotein particles by cells expressing the SR-B1 receptor.[48] Studies of cholesterol flux and the RCT pathway in either humans or animals are difficult,[6d] so there are few reports correlating CETP with cholesterol flux via the RCT process. One recent study has shown, however, that the RCT process was unaffected in transgenic mice expressing high levels of plasma simian CETP activity.[49]

CETP Expression in Animals. The induction of diet-induced atherosclerosis generally is difficult in rodent species that have low LDL and VLDL plasma concentrations. Mice and rats carry most of their cholesterol in atheroprotective HDL particles and are naturally deficient in CETP.[50] In contrast, animals such as rabbits naturally express high CETP activity and are particularly susceptible to diet-induced atherosclerosis. However, transgenic mice have been created expressing human or simian CETP. These CETP-transgenic mice produced profound reductions in HDLc levels[51] and a correspondingly enhanced ability to develop atherosclerotic lesions either alone[52] or when back-crossed into knock-out strains for the murine LDL receptor or apo-E.[53] In contrast, a possible antiatherogenic role for CETP was supported by studies in ovariectomized mice that have more severe atherosclerosis because of suppressed estrogen levels. CETP expression in ovariectomized transgenic mice lowered both plasma LDL and HDL lipoprotein levels and produced a concomitant 50% reduction in aortic atherosclerotic lesion area.[54]

Numerous animal studies also support the hypothesis that inhibition of CETP activity has a beneficial effect on HDLc levels and attenuates atherosclerosis. For example, iv administration of TP-2, a CETP-neutralizing monoclonal antibody, to hamsters produced a dramatic 60% reduction in CETP activity with a concomitant 30−40% elevation of HDLc in both normal and hypercholesterolemic animals.[55] Administration of TP-2 to hamsters also induced a shift to larger HDL particles and smaller LDL particles, producing a lipoprotein profile similar to that observed in CETP-deficient Japanese subjects. In another study, cholesterol-fed rabbits treated with antisense oligodeoxynucleotides designed against CETP had elevated HDLc and displayed significantly reduced aortic lesion areas.[56] Rabbits immunized with a CETP peptide-based vaccine had reduced CETP plasma activity and altered lipoprotein profiles where HDLc was raised by 42% and LDL was lowered by 24% versus controls, and the surface area of aortic atherosclerotic lesions was reduced by nearly 40%.[57]

On the basis of the current understanding of the combined data, it is evident that the role CETP plays in atherosclerosis is highly complex and may depend on several factors including (a) the plasma expression level of CETP and any genetic mutations that are present, (b) the concurrent lipidemic profile of VLDLc, LDLc, and HDLc, (c) plasma TG levels, and (d) the overall metabolic state. Pharmacological intervention in patients may therefore also prove to be complicated and dependent on individual characteristics. Despite these concerns, there is intense interest in identifying potent and selective CETP inhibitors to determine whether they could represent a new therapeutic approach for improving the HDLc/LDLc ratio and provide an important therapeutic benefit to CAD patients with low HDLc levels.[2−6] It is quite common within the CAD population for subjects to have elevated CETP plasma protein levels that are 2- to 3-fold higher than the concentrations typically found in the plasma of normal subjects (1−3 μg/mL).[58] Consequently, CETP inhibitors may be particularly beneficial for CAD patients whose plasma CETP protein levels exceed levels that are required to maintain the normal RCT process.

CETP as a Target for Drug Discovery

The available kinetic data[59] suggest that the CETP-facilitated lipid transfer process occurs via a "ping-pong mechanism". That is, CETP interacts individually with either HDL or LDL, and there is no evidence that a HDL−CETP−LDL ternary complex forms in plasma. Thus, CETP binds and dissociates from the lipoprotein particles and exchanges neutral lipids from within the lipoprotein core to the hydrophobic binding sites contained within the interior of CETP. CETP can be found associated with a variety of lipoproteins in plasma depending on the sex and hyperlipidemic state of the source. While CETP can interact with VLDL, LDL, and other lipoproteins in plasma, the majority of CETP is usually found associated with HDL particles containing apo-AI alone, and only 1% of the CETP in plasma is found in the free, unassociated form.[60] No cofactors are utilized in the transfer process, and no energy is evolved as part of neutral lipid transfer. Thus, this overall dynamic transfer process is mediated by the equilibrium defined by the CETP protein concentration, as well as the relative concentrations of lipoproteins and their associated neutral lipids present at any given time in plasma.

As a transfer protein, CETP presents several challenges for drug design. Since CETP is not an enzyme, there is no chemistry of catalysis, and as a result, no transition state or tightly bound intermediate is stabilized by the protein and available to use

for inhibitor design. Nearly 50% of the 476 amino acids in the mature CETP protein are hydrophobic residues. However, since CETP is readily soluble in water, many if not most of these hydrophobic amino acids must be organized away from the water-accessible surface and directed toward the interior of the protein, presumably to form the putative CE and TG binding sites.[60] CETP is homologous to three other related lipid-binding proteins: PLTP, lipopolysaccharide binding protein, and bactericidal permeability increasing protein (BPI). While detailed three-dimensional structural information on CETP is lacking, a working model for the protein has been proposed on the basis of its homology to BPI.[62] This model predicts a flattened "boomerang" shape for CETP where the longest dimension is about 4 times longer than the other two dimensions. This shape is likely optimal to facilitate the interaction of CETP with the spherical lipoprotein particles such as LDL and HDL. This model also predicted separate and distinct binding sites for the CE and TG ligands, but the precise three-dimensional size and shapes of these sites are uncertain. The CETP carboxy terminus has been implicated in the lipid transfer process because the TP-2 antibody directed at the C-terminal helix prevented lipid transfer. The structural model suggested that this amphipathic helix is positioned to block access to the N-terminal lipid-binding pocket, effectively limiting solvent exposure to this hydrophobic surface when CETP is in solution and not bound to lipoprotein. However, when CETP binds to lipoprotein, this helix may interact with the phospholipid surface and reposition itself to make the hydrophobic interior containing the CE and TG binding sites accessible and facilitate neutral lipid transfer.

While there are likely separate binding sites for both CE and TG in CETP, the precise stoichiometries and absolute affinities for each of these ligands are unknown, although CETP appears to have a higher relative intrinsic affinity for CE over TG.[62] These ligands cannot be bound too tightly; otherwise, the facilitated transfer would be difficult. Thus, the ligand−protein interactions in each of these sites must be limited. The three-dimensional structure of cholesteryl myristate, a typical CE found abundantly in HDL, presents a very hydrophobic and an extended flat surface with only two heteroatoms available for interactions with the CETP protein.[63] In support of this observation, substrate-based inhibitors synthesized from a cholesterol template displayed very weak inhibition.[2] The conformational flexibility of the TG likely presents more three-dimensionality as well as several more heteroatom interactions at this site, but no TG analogues have been evaluated as CETP inhibitors for comparison. In any case, one might expect that hydrogen-bond donor groups and polar or charged functionalities may not be compatible with the interior hydrophobic ligand binding sites in CETP. The challenge then is to identify potential CETP inhibitors that selectively and potently act on CETP without interfering with PLTP transfer or disrupting lipoprotein integrity. Moreover, compounds that can bind to this highly hydrophobic surface will likely exhibit higher than desired clogP properties that may introduce other issues related to overall drug development such as difficult formulations and limited or inconsistent oral bioavailability.

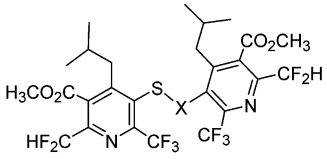
Overexpressed recombinant human CETP is readily available, and several assays have been developed to monitor CETP activity for high-throughput screening systems. A common in vitro assay relies on a differential precipitation assay to measure the rate and amount transferred from preformed and [^{3}H]CE-enriched HDL to unlabeled LDL particles.[64] Following incubation with buffered CETP and inhibitors in aqueous DMSO solution, LDL is differentially precipitated with dextran sulfate and magnesium chloride. After transfer to a filter plate and following filtration, the amount of radiolabel contained in LDL is measured using liquid scintillation counting. Alternatively, various fluorescence-based assays using fluorescent-labeled CE analogues have also been used.[64] Fluorescent CE analogues are self-quenched in a donor phosphatidylcholine emulsion. Following incubation in plasma and CETP-mediated transfer to VLDL, the fluorescence can be measured in VLDL. Sufficient concentrations of CETP are also present in human serum so that transfer activity can be monitored under more physiologically relevant conditions.[65] In many inhibitor classes, it is common to observe a significant shift to weaker potency when the activity is measured in the presence of human plasma or serum, presumably due to protein binding. For clarity in the discussion below, the assay used to determine any in vitro IC_{50} data will always be accompanied by a notation of either "buffer" or "human plasma" to designate the assay employed to measure the activity. Historically, identifying CETP inhibitors that exhibit sufficiently potent activity in the presence of human serum to justify more advanced pharmacological evaluation in animals has been especially challenging.[2] Despite these difficulties, in the past 5 years, remarkable progress has been made in identifying several potent inhibitor classes that retain their activity in the presence of human serum and have been optimized to demonstrate oral pharmacological proof of concept in animal models. While nearly every major pharmaceutical company has investigated CETP at some point in time as a potential drug target, the scientific effort in the field is currently led by a few key players, including Pfizer, Japan Tobacco, Bayer, and the former Searle/Pharmacia group (now Pfizer).

Discovery of Potent CETP Inhibitors

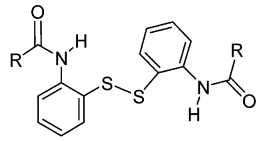
Since the initial reports connecting CETP deficiency to HDL elevation first appeared in the late 1980s, both industrial and academic researchers have been interested in identifying potent, reversible classes of CETP inhibitors. For nearly a decade this proved to be a particularly frustrating and futile exercise. While multiple classes of both natural product and synthetic inhibitors were identified, most exhibited only modest IC_{50} values in the 10−50 μM range under buffered conditions in vitro.[2] In all cases, further optimization of this activity could not be achieved, and analogues with submicromolar potency proved to be elusive. More importantly, no compounds were identified at that time that could modulate CETP activity in the presence of human serum at sufficiently low micromolar concentrations to warrant pharmacological testing in animal models. Remarkable progress has occurred particularly over the past 5 years such that multiple classes of inhibitors have now been identified that not only inhibit CETP-mediated transfer with at least nanomolar IC_{50} values under buffered conditions but also display low micromolar to low nanomolar activity in the presence of human plasma.

Thiol-Based Inhibitors. Human CETP is monomeric and contains seven cysteine (Cys) residues, suggesting that at least one of these thiol-containing amino acids is unpaired and available for modification.[1,62] Several thiol-modifying inhibitors of CETP have been identified, and their sites of action have been mapped to specific CETP Cys residues.[2,66] Both Cys^{13} (near the ligand binding site) and Cys^{333} have been implicated as the unpaired cysteine residues, but the data to date are dependent on the reagents used for modification.[66,67] Several hydrophobic disulfides, including the dithiobis[nicotinic acid ester][67] derivative **1a** (SC-71952, Monsanto/Searle) and the bis[*o*-amidophenyl]disulfides[68] **2a**,**b** (Japan Tobacco), have been identified as low-micromolar CETP inhibitors in vitro.

Detailed biochemical studies of the time-dependent interaction of **1a** with CETP have been reported. The potency of **1a** increased dramatically following preincubation with CETP. In the absence of preincubation, **1a** had an IC_{50} of 1−2 μM under buffered conditions, but after 24 h of preincubation with protein, the potency increased nearly 200-fold to give an IC_{50} of 0.010 μM. Thus, **1a** was shown to inhibit CETP by a two-step process involving first fast, reversible binding followed by a time-dependent and irreversible inactivation.[2b,67] Interestingly, the time-dependent inactivation by **1a** was completely lost when tested against the Cys13Ala mutant protein, implicating Cys^{13} as the site of action for this class and positioning this inhibitor near the putative CETP ligand binding sites.[62] The critical importance of the disulfide moiety to the overall activity of **1a** was shown by comparison with the corresponding thioether analogue **1b**, which was significantly less active (IC_{50} = 19 μM, buffer) in the absence of preincubation and did not exhibit time-dependent inhibition activity.

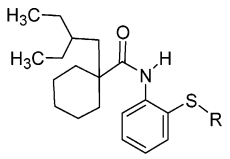


1a: X= S: SC-71952
IC_{50} = 1-2 μM, buffer
1b: X= CH_2: IC_{50} = 19 μM, buffer



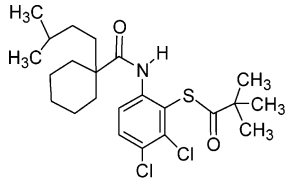
2a: R = CH_3: IC_{50} = 500 μM, human plasma
2b: R = α-CH_3-cyclohexyl: IC_{50} = 8 μM, human plasma

The most extensively studied and advanced of these sulfur-based inhibitors is represented by the thiol ester **3a** (JTT-705, Japan Tobacco).[68] The bulky, highly substituted amido moiety in **3a** was optimized starting from **2a**, where the α-methylcyclohexyl moiety in **2b** (IC_{50} = 8 μM) was nearly 60-fold more potent in human plasma assays than the original simple acetamide lead **2a** (IC_{50} = 500 μM). Both the ortho orientation of the phenyl ring substituents and the need for the secondary amide in analogues of **2a** were shown to be required for activity. Unfortunately, these disulfides were not orally bioavailable, and the corresponding free thiol **3b**, though equally active, proved to be unstable. Simple ester derivatives of **3b** were orally absorbed, exhibited comparable activity as the free thiol in human plasma, and were shown to hydrolyze easily back to **3b**. The isobutyryl ester analogue **3a** (IC_{50} = 6 μM, human plasma) provided the optimal combination of chemical stability and oral absorption, thus acting as a potential prodrug for **3b**. Administering rabbits with a single oral dose of **3a** at 30 mg/kg inhibited 95% of the CETP in the isolated rabbit plasma versus the vehicle alone, and this inhibition persisted for up to 9 h. Compound **3a** was also shown to have no significant interaction with or inhibitory effects on other lipid transfer proteins (PLTP) or enzymes involved with lipid metabolism such as HMG-CoA reductase or acyl-CoA/cholesterol acyltransferase (ACAT).[69] As a result, **3a** has been extensively evaluated in more advanced pharmacological models as discussed below.[70] U.S. patents have recently been issued claiming both the generic class and specific compound **3a**.[71]



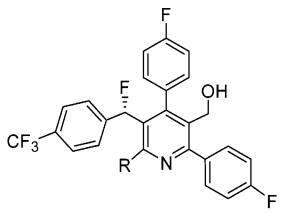
3a: R = $(CH_3)_2CHC(O)$: JTT-705
IC_{50} = 6 μM, human plasma
3b: R = H: IC_{50} = 3 μM, human plasma

Further biochemical characterization of the CETP inhibition exhibited by **3a** and related analogues also showed that their inhibitory activity, like that of **1a**, was lost when evaluated against the Cys13Ala mutant protein.[69] Thus, both classes of hydrophobic disulfide inhibitors appear to target the same Cys residue in the CETP protein for activity. Recently, additional SAR studies of **3a** have been reported that explored modifications to the central benzene ring and identified the related 4,5-dichloropivaloyl thioester analogue **4** (IC_{50} = 2 μM, human plasma) having the extended α-(3-methylbutyl)cyclohexyl side chain as a slightly more potent inhibitor than **3a**, although only in vitro data were disclosed.[72]



4: IC_{50} = 2 μM, human plasma

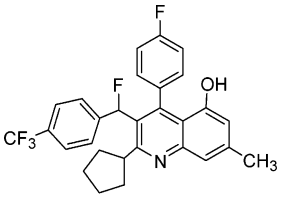
5,6,7,8-Tetrahydroquinolines and Related Inhibitors. The first work in this area was reported by investigators from Bayer, who have recently described the discovery, key SAR findings, and lead optimization efforts for both their first- and second-generation CETP inhibitors.[73] Starting from an initial series of highly functionalized 5-hydroxymethylpyridine based[74] and benzyl alcohol based[75] leads, the pentasubstitued pyridine **5a** was identified as a lead candidate with nanomolar potency (IC_{50} = 0.013 μM, buffer). In this series, the apical 4-fluorophenyl group at position 4 and the fluorinated 4-trifluoromethylbenzyl moiety at position 3 were retained from earlier series and shown to be important for overall activity. However, the aliphatic substituent at position 2 was sensitive to change in that the corresponding isopropyl derivative **5b** was about 40-fold less potent (IC_{50} = 0.500 μM) in its racemic benzylic fluoride form. Many of these early pyridine leads from Bayer appear to be derived from synthetic intermediates of cerivastatin.



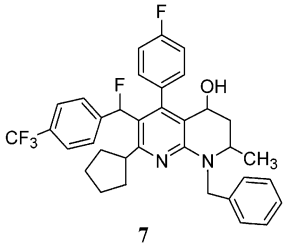
5a: R = *cyclo*-C_5H_9: IC_{50} = 0.013 μM, buffer
5b: R = *i*-Pr (rac): IC_{50} = 0.500 μM, buffer

Unfortunately, **5a** was metabolically unstable, and their efforts turned to potentially more stable derivatives that rigidified the primary alcohol by incorporating various bicyclic structures into the pyridine core. Multiple series of related fused bicyclic heterocycles were investigated,[2] and early patented examples include both the 5-hydroxyquinoline **6** (IC_{50} = 0.18 μM)[76] and

the naphthyridine derivative **7**,[77] having submicromolar activity against CETP under buffered conditions and oral activity in animals. In each of these series, the apical 4-fluorophenyl group at position 4 and the 4-trifluoromethylbenzylic fluoride residue at position 3 were again retained from earlier series and were required to maintain the overall activity of this series. These have been further optimized to provide unresolved racemic examples of the related highly substituted secondary and tertiary tetrahydroquinolinols **8a**,**b** (IC_{50} = 0.006 μM) with cyclic aliphatic groups at the 2-position and containing geminal small aliphatic groups at the 7-position of the saturated ring that exhibit significantly greater potency than either **6** or **7**.[78] In several of these bicyclic series, small branched aliphatic groups, e.g., isopropyl, can now replace the cyclic aliphatic substituent at the pyridine 2-position and still retain high potency, although detailed SAR studies and the accompanying biological data were largely confined to the limited information presented in the context of their patent applications.



6: IC_{50} = 0.18 μM, buffer



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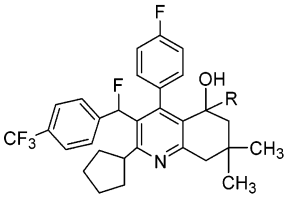
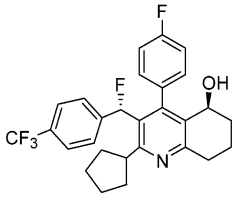
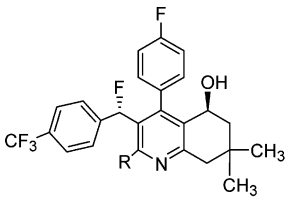


8a: R = H: IC_{50} = 0.006 μM, buffer
8b: R = CH_3: IC_{50} = 0.006 μM, buffer

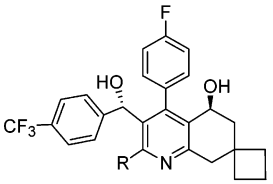


9: IC_{50} = 0.100 μM, buffer

The added potency of this tetrahydroquinolinol series came at the price of the additional chiral center. More insights into the key chiral relationship between the two asymmetric centers that contributes to the activity of this tetrahydroquinolinol series have recently been reported.[73] In particular, the racemic anti-tetrahydroquinolol isomer **9** (IC_{50} = 0.100 μM, buffer) was identified as an initial lead for optimization. Replacing the benzylic fluoride moiety in **9** with alcohol, carbonyl, or amino groups significantly lowered potency, and even the corresponding desfluoro derivative was less active (IC_{50} = 0.600 μM, buffer). However, when individual methyl groups were introduced at each of the remaining positions on the saturated ring, incorporation of a single methyl group at the 7-position was found to be particularly beneficial (IC_{50} = 0.020 μM, buffer). When two methyl substituents were added at the 7-position to reduce the number of chiral centers, the geminally substituted analogue **10a** was identified with very high potency (IC_{50} = 0.009 μM, buffer).[73] Comparable in vitro potency data have been reported for **10a** both as a racemic diastereomer and in its enantiomerically pure form, thus indicating the assay limitations at this potency. In this series, the related 2-isopropyl analogue **10b** was about 2-fold less active (IC_{50} = 0.018 μM, buffer). After further evaluation, **10a** was chosen as Bayer's first clinical candidate.[73,78] U.S. patents have been issued for **10a** describing a chiral synthesis for its preparation as well as compound per se and pharmaceutical composition claims.[73,79] Interestingly, the chiral benzylic fluoride is introduced in the last step from the corresponding chiral alcohol by reaction with DAST that proceeds unexpectedly with retention of configuration.[73]



10a: R = *cyclo*-C_5H_9:IC_{50} = 0.009 μM, buffer
10b: R = *i*-Pr: IC_{50} = 0.018 μM, buffer

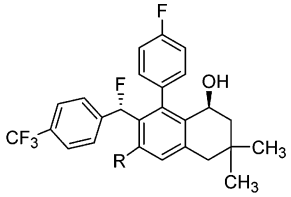


11a: R = *i*-Pr: IC_{50} = 0.012 μM, buffer
11b: R = *cyclo*-C_5H_9: IC_{50} = 0.009 μM, buffer

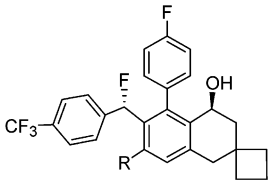
In contrast to the original SAR discussed above for the geminal 7,7-dimethyl series **10a**,**b**, a recent Bayer patent application described the related spirocyclic secondary tetrahydroquinolindiols **11a**,**b** as extremely potent CETP inhibitors (IC_{50} = 0.009−0.012 μM) with interesting pharmacological properties, where the key enantiomeric benzylic fluoride moiety has now been replaced with an alcohol group. Interestingly in this spirocyclic series, the presence of either a cyclic or small branched acyclic substituent at the 2-position was equally well tolerated for potency.[80]

The corresponding chiral geminal 7,7-dialkyl **12a**,**b** and spirocyclic **13a**,**b** tetrahydronaphthalene derivatives have also been described with similar low nanomolar in vitro activity.[73,81] These partially saturated naphthalene systems were prepared by an elegant domino aldol reaction. The synthesis of **13a** has been reportedly carried out on a kilogram scale without chromatographic purification, and the product was isolated during scale-up as a pure enantiomer in greater than 99% ee after crystallization.[73] Following further biological evaluation, **13a** was selected as Bayer's second-generation clinical candidate.[73]

1,2,3,4-Tetrahydroquinolines and Related Inhibitors. Researchers at Pfizer have identified a completely distinct but related series of 4-amino-substituted 1,2,3,4-tetrahydroquinoline carbamates where the partial saturation occurs in the heterocyclic

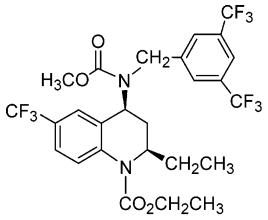


12a: R = *i*-Pr: IC_{50} = 0.003 μM, buffer
12b: R = *cyclo*-C_5H_9: IC_{50} = 0.003 μM, buffer

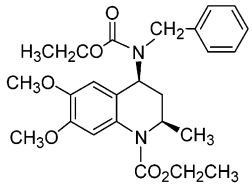


13a: R = *i*-Pr: IC_{50} = 0.003 μM, buffer
13b: R = *cyclo*-C_5H_9: IC_{50} = 0.003 μM, buffer

ring rather than the carbocyclic ring found in the Bayer leads. Optimization of this series led to an exciting new potent (IC_{50} = 0.05 μM, human plasma) chiral CETP inhibitor, **14** (torcetrapib, CP-529,414), for clinical development. The discovery and early optimization of torcetrapib have been reported[82] starting from the initial 6,7-dimethoxy-4-aminotetrahydroquinoline[83] lead **15** that had micromolar potency (IC_{50} = 10 μM) under buffered conditions and retained its activity in the presence of human plasma (IC_{50} = 25 μM).[82]

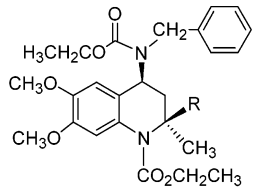


14: torcetrapib
IC_{50} = 0.050 μM, human plasma

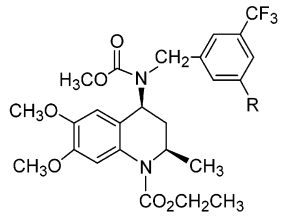


15: IC_{50} = 10 μM, buffer

The positioning of the 2*R*-methyl substituent in **15** was preferred for activity over the corresponding 2*S*-methyl enantiomer **16a** (IC_{50} = 50 μM, buffer). In contrast to the geminally disubstituted Bayer analogues **8**−**10** that retained their potency, addition of a geminal 2,2-dimethyl group as in **16b** significantly reduced activity (IC_{50} > 100 μM) in the Pfizer series. Overall activity was relatively insensitive to changes in either of the carbamate ester groups. However, the exocyclic 4-aminomethyl carbamate was about 2-fold more potent than the corresponding ethyl carbamate. In contrast, the introduction of one or more trifluoromethyl substituents into the benzylic ring of **15** significantly increased potency. The monotrifluoromethyl analogue **17a** (IC_{50} = 0.5 μM buffer; IC_{50} = 1.6 μM, human plasma) was approximately 20-fold more potent than **15**, and the 3,5-bistrifluoromethyl derivative **17b** was about 100-fold more potent (IC_{50} = 0.005 μM buffer; IC_{50} = 0.100 μM, human plasma) than **15**.[82]



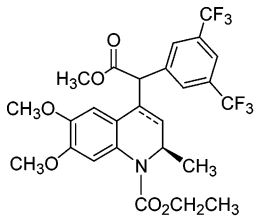
16a: R = H: IC_{50} = 50 μM, buffer
16b: R = CH_3: IC_{50} > 100 μM, buffer



17a: R = H: IC_{50} = 0.50 μM, buffer
17b: R = CF_3: IC_{50} = 0.005 μM, buffer

Various electron-donating and electron-withdrawing substituents were also explored at positions 6 and 7 of the unsaturated ring, and again, a single 6-trifluoromethyl group increased potency relative to the 6,7-dimethoxy analogue. Further optimization of the 2-methyl substituent showed that the cis orientation between the 2*R*-alkyl and 4*S*-amino substituents was optimal and identified the chiral 2*R*-ethyl derivative **14** as the compound having the best pharmacokinetic properties, compared to either the chiral 2*R*-methyl or 2*R*-isopropyl analogues. Additional biochemical studies indicate that torcetrapib is a potent, reversible inhibitor of CETP and that the binding of **14** to CETP enhances its interaction with HDL.[82]

Patent applications describing these series began appearing in 2000 and continue to be issued.[84] While several of these applications contain numerous chemical examples, there is little accompanying biological data to infer additional SAR information. The first evaluation of the sensitivity of naturally occurring CETP variants to inhibition by a CETP inhibitor was recently reported using torcetrapib as the prototypical inhibitor, and all of the nine missense protein variants tested showed essentially the same level of inhibition by torcetrapib.[85] Examples of the related deazatetrahydro- and deazadihydroquinolines **18** and various tetrahydroquinoxaline derivatives **19a**,**b** have also been described, although no biological data for these compounds have been reported.[86]



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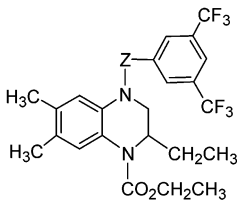
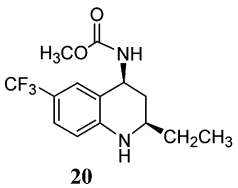


19a: Z = CH_2
19b: Z = C(O)



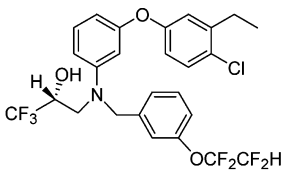
20

Nevertheless, the announced phase III clinical development program for torcetrapib will be the most extensive in Pfizer's

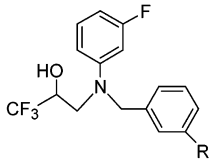
history.[87] A recent publication[88f] and several U.S. patents described the process to make torcetrapib via the key chiral intermediate **20**,[88] and a competing application describing the preparation of several key intermediates by a Japanese company, Kaneka, has also appeared.[89] The synthesis of isotopically labeled **14** has also been described to support various drug metabolism and distribution studies.[90] Patent applications describing processes to isolate anhydrous torcetrapib have also appeared, including the conditions required to isolate single crystalline, polymorphic forms.[91] The high hydrophobicity (log P > 4.5) and negligible water solubility reported[82] for torcetrapib may also necessitate the use of atypical special formulation technologies to achieve consistent oral exposure and clinical efficacy, and more than a dozen patent applications describing novel formulation strategies for this compound have recently appeared, including several solid dispersion methods as well as a controlled release dosage form.[92]

N,N-Disubstituted Trifluoro-3-amino-2-propanol Inhibitors. In contrast to the fused bicyclic templates utilized at Bayer and Pfizer, investigators at Searle/Pharmacia have reported the discovery of an extremely potent acyclic inhibitor class, the trifluoro-3-(tertiary amino)-2-propanols.[93] The most potent example reported for this series is the chiral *R*-enantiomer **21** (SC-591), which contains an unusual 3-(1,1,2,2-tetrafluoroethoxy)benzylamine substituent. The discovery and detailed SAR studies for this unique series starting from the initial weakly active (IC_{50} = 40 μM, buffer; IC_{50} > 200 μM, human plasma) achiral screening lead **22a** have been described.[94] Modification of the 3-trifluoromethyl group in **22a** demonstrated that the activity of **22a** could be improved by introducing either a 3-trifluoromethoxy **22b** (IC_{50} = 12 μM, buffer) or a 3-tetrafluoroethoxy **22c** (IC_{50} = 4.5 μM, buffer) group, whereas the simple 3-methyl derivative **22d** was considerably less active (IC_{50} > 100 μM, buffer). Similarly, replacement of the 3-fluoroaniline with a 3-phenoxyaniline group increased the overall potency by nearly 10-fold in the 3-trifluoromethoxybenzylamine analogue **23a** (IC_{50} = 1.5 μM, buffer). An even more dramatic 30-fold increase in potency was observed in the 3-tetrafluoroethoxybenzylamine derivative **23b** (IC_{50} = 0.14 μM, buffer; IC_{50} = 5.6 μM, human plasma, SC-75744), the first submicromolar inhibitor in this series that displayed low micromolar activity in the presence of human plasma. Thus, as the potency of the series increased, the importance of the extended 3-tetrafluoroethoxy moiety to the activity became more apparent. Both haloalkoxy and halogenated thioethers were preferred for potency in the benzyl ring over simple alkoxy substituents such as the 3-methoxy analogue **23c** (IC_{50} = 15 μM, buffer). Various heterocyclic mimics of this 3-tetrafluoroethoxy moiety were also explored, and a 2-furyl group (IC_{50} = 0.48 μM, buffer) was identified as a possible replacement, although it was about 4-fold less active than **23b**, and all other related heterocyclic groups were less potent.[95]

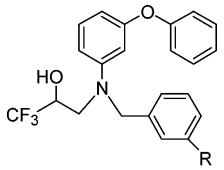
Interestingly, repositioning the 3-phenoxyaniline in **23a** to the corresponding 4-position significantly reduced activity in the 4-substituted regioisomer (IC_{50} = 25 μM, buffer). On the other side of the molecule, analogues of **23a** that replaced the trifluoromethyl moiety attached to the secondary carbinol group with simple vinylic, benzylic, or phenyl groups were significantly less potent (IC_{50} > 100 μM, buffer), while the corresponding *n*-propyl derivative was slightly less active (IC_{50} = 3.6 μM, buffer) than **23a**. Thus, both the trifluoromethyl group from the propanol and the 1,1,2,2-tetrafluoroethoxybenzylic amine moiety jointly contributed to the overall potency.



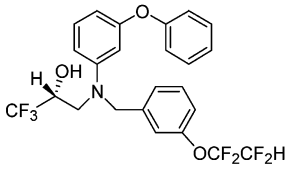
21: IC_{50} = 0.003 μM, buffer



22a: R = CF_3: IC_{50} = 40 μM, buffer
22b: R = OCF_3: IC_{50} = 12 μM, buffer
22c: R = OCF_2CF_2H: IC_{50} = 4.5 μM, buffer
22d: R = CH_3: IC_{50} > 100 μM, buffer



23a: R = OCF_3: IC_{50} = 1.5 μM, buffer
23b: R = OCF_2CF_2H: IC_{50} = 0.14 μM, buffer
23c: R = OCH_3: IC_{50} = 15 μM, buffer



24: IC_{50} = 0.02 μM, buffer

When the chiral analysis of **23b** was performed, it was unexpectedly found to contain a 7:1 mixture of enantiomers rather than the expected 1:1 mixture. All of the activity for CETP inhibition resided in the minor *R*-enantiomer **24** isolated from this mixture, and **24** exhibited very good submicromolar potency in both the buffer- and plasma-based assays (IC_{50} = 0.02 μM, buffer; IC_{50} = 0.6 μM, human plasma). Interestingly, the corresponding *S*-enantiomer was found to be significantly less active, and all of its activity could be attributed to minor contamination by the highly potent eutomer **24**.[65,94] A chiral synthesis of **24** was also reported[93,96] starting from the prerequisite known chiral epoxide *R*-(+)-2-trifluoromethyloxirane,[97] providing an independent synthesis of **24** that was comparable in all respects to the same material isolated by chiral chromatography from **23b** and providing sufficient material for more extensive biological characterization.[94]

Further modification of the 3-phenoxy moieties in **23b** and **24** led to the identification of an unusual 4-choro-3-ethyl derivative **21** (IC_{50} = 0.003 μM, buffer; IC_{50} = 0.059 μM, human plasma) as an exceptionally potent CETP inhibitor in vitro exhibiting approximately 10-fold higher activity than **24**. In general, the addition of small lipophilic alkyl, haloalkyl, haloalkoxy, or halogen moieties to the phenoxy ring increased potency relative to **23b**, while analogues containing electron donating or hydrogen bond accepting groups exhibited lower potency. Compounds with polar or strong electron-withdrawing groups also displayed lower potency. Related analogues containing either an individual 3-ethyl or 3-trifluoromethoxy group on

the phenoxy ring displayed nearly equal low nanomolar potency compared to **21**. Replacement of the phenoxy ring in **23b** with either simple aliphatic or cycloalkyl ethers as well as basic heteroaryloxy groups led to reduced potency. As observed in all the other SAR studies of this system, replacing the 3-tetrafluoroethoxy moiety in **24** with a shortened trifluoromethoxy group, trifluoromethyl, or pentafluoroethyl functionalities decreased the potency in both buffer and human plasma by approximately 10-fold.

Extensive biochemical characterization studies have also been reported for **21**, **23b**, and **24**.[65,93,94] Since the IC_{50} of **21** approached the CETP protein concentration employed in the buffered assay, assay conditions were modified to allow lower protein concentrations to be used. As a result, **21** was identified under these modified buffered conditions as the first picomolar CETP inhibitor reported in the literature (IC_{50} = 0.0008 μM, buffer), and the activity of **21** therefore represents a startling 50000-fold improvement over the initial screening hit **22a**. However, it is also likely that other low-nanomolar inhibitors reported by Bayer and Pfizer would show similar picomolar potency under these modified conditions.

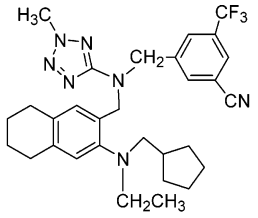
It is extremely interesting that such a very high fluorine content is not only present but required for activity in all three of the chemical series that represent potent, reversible CETP inhibitors. While each of Bayer's clinical candidates **10a** and **13a** contains five fluorine atoms, the Searle/Pharmacia amino alcohol leads **21** and **24** each contain seven fluorines, and Pfizer's torcetrapib **14** has a remarkable nine fluorine atoms. The basis for this unusual and likely expensive requirement is not understood at this time.

More detailed biochemical studies clearly demonstrate that like **24**, compound **21** is a potent, reversible inhibitor of CETP-mediated CE and TG transfer. There was no additional time-dependent effect on the inhibitory activity, since plots of activity versus the concentrations of either inhibitor were virtually superimposable in the presence or absence of preincubation. These results for **24** and **21** are consistent with a reversible mode of action and stand in sharp contrast to the covalent disulfide and thiol-based modifiers of CETP.[67,68] The CETP inhibitory activity of **21** and **24** was not only specific but also highly selective because neither **21** nor **24** blocked PLTP-mediated transfer (PLTP, IC_{50} > 100 μM) or LCAT (IC_{50} > 100 μM) at concentrations more than 100000-fold higher than their respective IC_{50} values for CETP. [65,93,94]

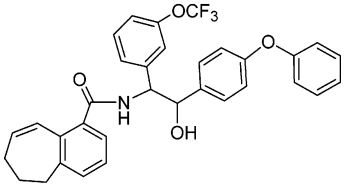
When radiolabeled tritium derivatives were used, **21** and **24** were shown to associate with LDL and HDL, but this association did not disrupt the overall structural integrity of these lipoprotein particles. At concentrations up to 1000-fold higher than their CETP serum IC_{50}, neither **24** nor **21** had an effect on the overall structural integrity of either LDL or HDL. Neither of these compounds, **21** and **24**, interfered with the ability of CETP to bind to HDL disk particles. Competition experiments using size-exclusion chromatography also demonstrated that both **21** and **24** completely blocked the binding of [^{3}H]CE to purified recombinant human CETP. On the basis of the relative concentrations of inhibitor and lipid used in these competition experiments, it has been estimated that **24** binds approximately 5000-fold more efficiently to CETP than the natural CE ligand. These results are again consistent with a reversible and competitive biochemical mode of action across this acyclic trifluoro-3-amino-2-propanol series.[65,93,94]

Because of their easy synthesis, more than 800 examples of related achiral[98] and chiral[96,99] trifluoro-3-amino-2-propanol derivatives have appeared in issued U.S. patents from the Searle/Pharmacia group, and an extensive SAR has been developed. More than 30 issued U.S. patents have appeared since 2002 containing compound per se and method claims for chain-shortened as well as chain-lengthened variations of this series along with a number of other closely related classes.

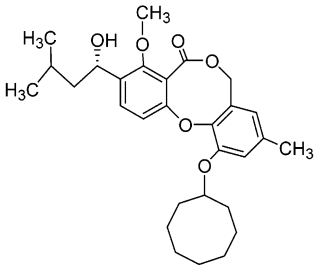
New Exploratory Classes. Early reports in various patent applications indicate that there is a continued strong interest in identifying new structural motifs for CETP inhibitors, although it is still too soon to assess their overall pharmacological significance or clinical potential. In contrast to its first-generation candidate **3a**, a covalent cysteine modifier of CETP, Japan Tobacco is exploring an alternative acyclic series with likely reversible binding behavior based on novel N,N-disubstituted aminotetrazoles, as represented by **25** (IC_{50} = 0.08 μM, human plasma) with excellent leadlike activity in human plasma.[100] Acyclic acylated amino alcohols containing multiple aromatic rings have also been reported by Takeda with potent CETP inhibition properties, as represented by compound **26** (IC_{50} = 0.008 μM, buffer; IC_{50} = 0.08 μM, human plasma), in a patent application containing nearly 400 examples marking Takeda's first entry into this field.[101] Recent patent applications from the Bayer research group disclosed an interest in the chiral highly oxygenated dibenzodioxocin-5-one derivatives represented by **27** (IC_{50} = 0.8 μM, buffer) as a completely new template for CETP inhibition.[102]



25: IC_{50} = 0.08 μM, human plasma



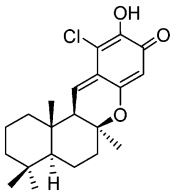
26: IC_{50} = 0.008 μM, buffer



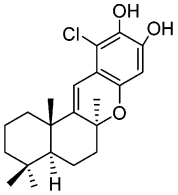
27: IC_{50} = 0.8 μM, buffer

Various natural products have activity as CETP inhibitors, and interest in this area continues.[2] New total syntheses of the marine natural products (+)-chloropuupehenone **28** (IC_{50} = 0.3 μM, buffer) and the related (+)-chloropuupehenol **29** (IC_{50} = 31 μM, buffer) have recently been reported,[103] and their individual CETP in vitro inhibition properties have been determined. However, on the basis of their structural complexity, these compounds represent relatively weak inhibitors under

buffered conditions compared to many of the other classes described above. No data in the presence of human serum have been reported for either of these compounds.



28: IC_{50} = 0.3 μM, buffer



29: IC_{50} = 31 μM, buffer

Preclinical Pharmacology of CETP Inhibitors

CETP protein is not expressed in the typical rodent species (mouse, rat) used in early pharmacological studies.[50] However, transgenic mice have been created expressing human or simian CETP that can be used to monitor the modulation of CETP activity in vivo after single dose oral or parenteral administration of a potential inhibitor. To evaluate an inhibitor's potential to raise HDLc in animals, multiple dosing for several days up to 2 weeks must be conducted. These multidose studies can be performed in transgenic CETP mice or alternatively have also been frequently reported in hamsters that naturally express low levels of CETP protein. The ability of a CETP inhibitor to limit the progression of atherosclerotic lesions has usually been evaluated in chronic studies requiring up to 6 months of dosing in rabbits that naturally express CETP and are particularly prone to diet-induced atherosclerosis.

Pharmacology of Disulfide-Based Inhibitors. Although the disulfide-based inhibitors **1a** (Searle/Pharmacia) and **2b** (Japan Tobacco Co.) exhibited poor oral exposure in animals, iv administration of **1a** as a single dose to hamsters reportedly reduced CETP activity by more than 50% in plasma ex vivo. The continuous infusion of **1a** to male hamsters maintained on a normal chow diet at 9.2 (mg/kg)/day for 8 days produced a 30% reduction in LDLc and a 26% increase in HDLc.[104] These results compared favorably with those described earlier for the CETP-neutralizing TP-2 antibody, which after iv administration to hamsters produced a 60% reduction in CETP activity with a concomitant 30−40% elevation of HDL in both normal and hypercholesterolemic animals.[55]

Pharmacology of 3a. The most extensive pharmacological studies reported in the literature for any CETP inhibitor have been performed with **3a** under both acute and chronic dosing conditions, and several reviews have appeared.[105] The thioester **3a** not only was shown to have low micromolar activity in human plasma (IC_{50} = 5.5−6 μM, human plasma) as described previously but was equally effective at inhibiting CETP-mediated transfer in plasma taken from multiple normolipidemic animal species including rabbits (IC_{50} = 1.0 μM), cynomolgus monkeys (IC_{50} = 2.4 μM), marmosets (IC_{50} = 6.3 μM), and hamsters (IC_{50} = 11.7 μM).[106] The activity of the corresponding free thiol derivative **3b** was about twice as potent as **3a** under similar conditions in most cases: human plasma (IC_{50} = 2.8 μM), rabbits (IC_{50} = 0.44 μM), cynomolgus monkeys (IC_{50} = 1.3 μM), marmosets (IC_{50} = 1.1 μM), and hamsters (IC_{50} = 0.52 μM).[106] Compared to **3a** (IC_{50} = 11.7 μM) however, the activity of **3b** (IC_{50} = 0.52 μM) was more than 20-fold more potent in hamster plasma. These data suggest that the conversion of **3a** to the active free thiol **3b** may vary significantly in plasma derived from different species, particularly in hamsters.

Initial acute studies involved administration of **3a** orally in the diet to Japanese white rabbits. Under these conditions, **3a** produced a 95% inhibition of CETP activity ex vivo 2 h after a single oral dose of 30 mg/kg was administered, and the effect persisted for up to 9 h.[68,69] Oral administration of **3a** as a 0.5% methylcellulose suspension once a day at 10, 30, 100, and 300 (mg/kg)/day to Japanese white rabbits for 3 consecutive days reduced plasma CETP activity significantly ex vivo at both day 1 and day 3 with doses of 30 (mg/kg)/day or higher and produced dose-dependent HDLc elevations of 16%, 27%, 54%, and 59%, respectively.[68] In this study, HDL-associated TG levels were reduced by 29% and 41% in the two higher doses of 100 and 300 (mg/kg)/day, respectively.

When **3a** was administered in the diet to rabbits maintained on normal chow for 7 days, a dose-dependent inhibition of CETP and a dose-dependent elevation of HDLc were observed. The percentage change in HDLc was inversely correlated with CETP activity. Plasma CETP activity was reduced by 66%, 87%, and 87% ex vivo 2 h after the first dosing at 50, 100, and 200 (mg/kg)/day, respectively. On day 7 of this study, a concomitant dose-dependent percentage increase in HDLc was observed 7 h after the final dosing at 50 (27%), 100 (41%), and 200 (31%) (mg/kg)/day, respectively.[106] In a separate study, the HDL obtained from the plasma of rabbits treated with **3a** effectively reduced the CE concentration in macrophages in vitro as efficiently as the HDL obtained from control rabbits, thus demonstrating that **3a** did not alter the HDL functionality for cholesterol eflux.[107] As expected, there was no change in the HDLc when **3a** was administered orally to rats, which do not express CETP protein.[69]

Oral administration of **3a** as a 0.5% methylcellulose suspension has also been shown to reduce plasma CETP activity, raise HDLc, and lower the overall atherogenic index (i.e., the non-HDLc/HDLc ratio) in normolipidemic hamsters and marmosets. Administering **3a** orally to hamsters at 50, 100, and 200 (mg/kg)/day for 7 days reduced plasma CETP activity by 66−87% ex vivo when measured 2 h after the first dose. A concomitant increase in HDLc was also observed on day 7: 27% at 50 (mg/kg)/day, 40% at 100 (mg/kg)/day, and 31% at 200 (mg/kg)/day. More consistent dose-dependent elevations in HDLc were observed following chronic dosing in marmosets. Administering **3a** orally to marmosets at 30, 60, and 90 (mg/kg)/day for up to 28 days, reduced plasma CETP activity by 34−71% ex vivo when measured 2 h after the first dose. A concomitant dose-dependent increase in HDLc was also observed on day 28: 31% at 30 (mg/kg)/day, 45% at 60 (mg/kg)/day, and 81% at 90 (mg/kg)/day.[106] Thus, oral dosing of **3a** raised HDLc in all three species, and as a result, the overall atherogenic index as defined by the non-HDLc/HDLc ratio was also reduced in all three species.

Compound **3a** was also administered orally in the chow to Japanese white rabbits maintained on a 0.2% cholesterol-based diet for up to 6 months of dosing at a mean daily dose of 255 (mg/kg)/day.[69] Under these conditions, **3a** produced a 90% increase in HDLc and decreased VLDLc and LDLc by 40−50%. Analysis of the lipoprotein subfractions indicated that **3a** significantly increased the larger HDL particles, both HDL_2 (170%) and HDL_3 (59%), as well as serum apo-AI levels (78%). After 6 months of dosing, the progression of atherosclerosis measured at the aortic arch in these animals was reduced by an impressive 70% following treatment with **3a** and was nearly identical to the 80% reduction observed in an oral simvistatin control group.[69] In addition, there was no obvious overt in vivo toxicity observed in the rabbits used in this study as assessed by body weight, liver weight, liver function, and renal function tests.

Japanese white rabbits that were maintained on a slightly higher (0.25%) cholesterol diet as a model for moderate hypercholesterolemia have also been treated orally with **3a** in the feed at doses of 100 or 300 (mg/kg)/day for up to 8 weeks.[108] In this study, the 300 (mg/kg)/day dose effectively raised HDLc in these animals by over 130%, yet there was no significant difference in the aortic atherosclerotic lesion area between the 300 (mg/kg)/day treatment group and the vehicle-treated controls. Thus, **3a** had no effect on the progression of atherosclerosis in this shorter hypercholesterolemic model even though it raised HDLc, and the data suggest that the overall lipidemic state may contribute to its overall effectiveness. In particular the non-HDLc levels obtained in this study were very high, and treatment with **3a** produced only a minor decrease in these lipoproteins of approximately 25%. This result implies that using CETP inhibitors as monotherapy under conditions of very high VLDLc and LDLc may offer insufficient protection from atherosclerosis.

In a second longer term study, 600 (mg/kg)/day of **3a** was administered orally to Japanese white rabbits maintained on a normal diet for up to 7 months.[109,110] In this study, treatment with **3a** dramatically inhibited plasma CETP activity by 81% versus controls at the 5-month time point and significantly increased serum HDLc as well as serum apo-AI, apo-E, and HDL_2c levels after 7 months of treatment. As observed under acute dosing conditions, treatment with **3a** again increased the phospholipid content of HDL particles and overall serum phospholipid levels. Interestingly, in this study, less overall weight gain was observed in those animals receiving treatment with **3a**, suggesting that CETP may play some role in energy metabolism and that CETP inhibitors thus may also provide an extra therapeutic benefit in obese patients.

The pharmacological studies to date reported for **3a** provide little insight into the formulations that might be used in a clinical setting, although one patent application has appeared describing a conventional 550 mg tablet formulation containing 100 mg of **3a**.[111] On the other hand, the lipophilic nature of **3a** combined with its proven efficacy in rabbit atherosclerosis models under fed conditions suggests that **3a** might exhibit a significant food effect for maximal bioavailability in humans. To address a potential food effect in humans, investigators at Japan Tobacco Co. have recently designed a special kit for **3a** to provide patients with specific instructions to ingest it with a meal because its absorption is improved in the presence of food as described in another recently published patent application.[112]

Pharmacology of 5,6,7,8-Tetrahydroquinolines. Limited information on the pharmacological efficacy and overall SAR trends for the Bayer tetrahydroquinoline series has been presented.[73,113] Data on individual compounds taken from a number of Bayer patents indicate that many representatives from its early leads, including **5a**,**b** as well as its different classes of bicyclic inhibitors, modulate CETP activity in animals and raise HDLc in both CETP transgenic mice and hamsters. For example, iv administration of a racemic mixture of **5a** ($IC_{50} = 0.17\ \mu M$) to hamsters at 20 mg/kg decreased CETP activity in plasma by 50% ex vivo after 2 h and increased HDLc by 30% after 24 h versus the vehicle control group. Oral dosing of a racemic mixture of **5a** at 100 mg/kg in hamsters produced a comparable 20% increase in HDLc after 24 h. Similarly, oral dosing of the single enantiomer **5a** in hamsters b.i.d. at 45 mg/kg (90 (mg/kg)/day) for 24 h produced a 20% increase in HDLc.[74] Comparable results were reported for the early bicyclic 1,2,3,4-tetrahydro[1,8]naphthyridine lead, **7**. Oral administration of **7** at 10 mg/kg inhibited 50% of CETP-mediated transfer activity ex vivo in hamsters. When dosed orally in feed at 80 ppm for 1 week, compound **7** raised HDLc by 14% in transgenic mice expressing human CETP protein.[77]

Much more impressive biological results were reported for the first Bayer clinical candidate **10a**.[73,113] Oral dosing of **10a** at 5 and 10 (mg/kg)/day in human CETP transgenic mice for only 3 days produced dose-dependent HDLc elevations of 35% and 50%, respectively. In this study, oral dosing of **10a** at 10 (mg/kg)/day for 3 days lowered non-HDLc by ~20% and also lowered triglycerides by about 30%. In hamsters, oral administration of **10a** at 3 and 10 (mg/kg)/day b.i.d. for 11 days produced a dose-dependent increase in HDLc and markedly lowered total cholesterol and triglycerides. After 11 days, HDLc increased by about 18%, and triglycerides were reduced by about 45% in the 10 (mg/kg)/day group. More importantly, like **3a**, **10a** was found to be effective in a chronic rabbit model of atherosclerosis. Oral administration of **10a** in the feed to New Zealand white rabbits for 3 months at a daily dose of either 50 or 150 mg/kg reduced the atherosclerotic plaque areas in a dose-dependent manner by 40% and 70%, respectively, and reduced CE levels in the atherosclerotic fatty streak.[73,113] In this chronic rabbit study, **10a** also dose-dependently increased HDLc, which reached a maximal 4-fold increase in the higher dose group, although no changes in LDLc were observed.

Pharmacology of Tetrahydronaphthalenes. The second-generation tetrahydronaphthalene derivatives from Bayer were shown to be significantly more potent than **10a** in animal studies.[73,113] For example, oral administration of the chiral 2-cyclopentylspirocyclobutyltetrahydronaphthalene **11b** to hamsters at 3 mg/kg inhibited CETP-mediated transfer by 72% after 24 h, and its ex vivo ED_{50} for this inhibition was reportedly less than 0.3 mg/kg. After only two oral doses at 0.3 mg/kg to hamsters, **11b** reportedly raised HDLc by 19% after 24 h. After being dosed in feed at 30 ppm for 1 week, **11b** also raised HDLc by 60% and lowered LDLc by 18% in transgenic mice expressing human CETP.[81a] The corresponding 2-isopropylspirocyclobutyltetrahydronaphthalene analogue **11a** was even more effective at raising HDLc in either transgenic mice or hamsters after prolonged treatment. For example, oral administration of **11a** to hamsters at 3 mg/kg inhibited CETP-mediated transfer by 83% after 24 h, and its ex vivo ED_{50} for this inhibition was again less than 0.3 mg/kg.[81a] After only two oral doses at 1 mg/kg to hamsters, **11a** reportedly raised HDLc by 15% after 24 h. Oral administration of **11a** to hamsters for 4 days at 1 and 3 (mg/kg)/day produced relatively small HDLc increases of 11% and 28%, respectively. However, oral administration of **11a** in the feed at 0.6 mg/kg for 7 days produced an impressive 57% increase in HDLc and nearly a 20% reduction in LDLc in transgenic mice expressing the human CETP protein.[73] These results compared very favorably with the HDLc elevations of 30−40% reported previously using continuous iv administration of the TP-2 antibody.[55]

Pharmacology of Torcetrapib. Detailed pharmacological studies with **14** have not yet appeared in the literature, although some initial results have been presented.[82,114] These initial reports indicated that torcetrapib effectively inhibited CETP-mediated transfer activity in animals and dose-dependently increased HDLc and reduced LDLc. Interestingly, the LDLc lowering with torcetrapib was also found to be additive in animals when atorvastatin was dosed simultaneously. The effects observed in animals on LDLc lowering using the torcetrapib/atorvastatin combination were greater than those observed with atorvastatin alone. These data suggest that combinations of torcetrapib and atorvastatin may have clinical utility, and this

hypothesis is being tested clinically. Nevertheless, these initial results are the first time that additive preclinical efficacy for lowering non-HDLc has been demonstrated using a potent CETP inhibitor with a statin.

In a recent report, torcetrapib was dosed orally in the feed (0.15% torcetrapib in chow) to New Zealand white rabbits maintained on a diet of 0.2% cholesterol and coconut oil for sixteen weeks.[114] Animals were bled periodically to monitor for changes in cholesterol and CETP activity over the course of the study. Torcetrapib treatment inhibited CETP-mediated CE transfer immediately by 70−80%, which was maintained throughout the study. A concomitant nearly 4-fold elevation in HDLc levels was also observed in the torcetrapib-treated animals versus vehicle controls both at week 1 (200 ± 15 vs 57 ± 4 mg/dL) and at week 16 (207 ± 32 vs 57 ± 6 mg/dL). As a result of the proatherogenic diet, non-HDLc levels increased gradually over time, and at 16 weeks, the levels were virtually identical in both the control and torcetrapib-treated groups, suggesting that torcetrapib alone in rabbits has little effect on LDLc levels. At the end of the study, the aortic atherosclerotic lesion area was reduced by nearly 60% in the torcetrapib-treated group versus vehicle controls. The reduction in aortic lesion area was associated with the observed elevated HDLc levels but was not associated with a reduction in non-HDLc.

The observation that non-HDLc levels were unchanged in torcetrapib-treated rabbits has increased interest in clinically evaluating statin combinations with torcetrapib. As a result, several recent Pfizer patent applications have described the therapeutic utility of various torcetrapib combinations with statins, such as atorvastatin.[115] Several applications have also appeared claiming oral dosing formulations containing these combinations,[116] including a fixed-dosed combined tablet formulation having a different time-release for each of the individual components.[117]

Pharmacology of N,N-Disubstituted Trifluoro-3-amino-2-propanol Inhibitors. Early proof of concept efficacy data have been reported in acute animal studies for this trifluoroalkyl-tertiary-amino alcohol class. However, neither chronic dosing studies nor efficacy in models of atherosclerosis have been described for this series. For example, iv administration of a single dose of **23b** at 30 mg/kg inhibited CETP-mediated transfer ex vivo by 50% after 30 min in human CETP transgenic mice and by 30% after 3 h in cholesterol-fed hamsters. Compound **23b** showed similar, but slightly lower, potency for inhibiting CETP-mediated transfer in cholesterol-fed hamster serum ($IC_{50} = 12\ \mu M$) compared to its activity in human serum ($IC_{50} = 5.6\ \mu M$). Once a day oral dosing of **23b** for 5 days at 30 mg/kg raised HDLc by 20% in transgenic mice expressing the human CETP protein.[94]

By comparison, **24a** was nearly 20-fold more potent in hamster serum ($IC_{50} = 0.6\ \mu M$) and 10-fold more potent in human serum ($IC_{50} = 0.6\ \mu M$) than **23b**. Surprisingly, iv administration of a single dose of **24a** at 33 mg/kg inhibited CETP-mediated transfer by only 30% ex vivo after 4 h in cholesterol-fed hamsters, even though the plasma levels for **24a** at 4 h exceeded the hamster serum IC_{50} by more than 10-fold. Thus, the additional in vitro potency of **24a** in serum did not translate into better ex vivo activity.[94]

Similarly, the improved compound **21** was nearly 10-fold more potent in human serum ($IC_{50} = 0.06\ \mu M$) than **24a** ($IC_{50} = 0.6\ \mu M$) and about 100-fold more potent than **23b** ($IC_{50} = 5.6\ \mu M$). As observed previously for **24a**, administration of **21** as a single oral dose at 30 mg/kg to hamsters inhibited the CETP-mediated transfer of [^{3}H-CE]HDL to LDL after 4 h by about 37%. In transgenic mice expressing human CETP, a single oral dose of **21** at 30 mg/kg inhibited 38% of the CETP-mediated transfer after 30 min. Thus, **21** reduced CETP activity ex vivo to about the same extent as observed previously for **23b** and **24a**, and the 10-fold improvement in potency of **21** over **24a** in the human serum assay did not translate into improved activity ex vivo for either transgenic CETP mice or hamsters.[93]

Multiple oral dosingof **21** once a day at 30 mg/kg for 5 days in transgenic mice expressing human CETP produced a statistically significant 12% elevation of HDLc, a 12% reduction in LDLc, and a 22% reduction in VLDLc. Plasma levels taken 4 h after the final oral dose were about 100-fold above the human serum IC_{50}. Oral once a day dosing of **21** at 3 and 30 mg/kg for 5 days in hamsters produced a small elevation of HDL (6.2% and 5.4%, respectively), and no significant change in LDLc or VLDLc was observed. Thus, the efficacy of **21** for raising HDLc and lowering LDLc occurs at levels similar to that observed previously for **23b**, and the 100-fold improvement in potency of **21** over **23b** in the human serum assay did not translate into significantly improved efficacy in animals. This may be partially due to the known tight affinity that members of this series have exhibited for other plasma proteins, particularly serum albumin, or due to other factors present in the animal serum. The strong association of **21** with human serum albumin ($K_d \leq 0.01\ \mu M$) was reportedly confirmed independently using ^{19}F NMR methods. Thus, the unfavorable interaction of **21** with other plasma proteins may limit its overall efficacy in vivo.[93] However, longer term studies in other species such as those conducted in rabbits for **3a** and torcetrapib have not been reported for this series. Nevertheless, these agents appear to hold promise as an alternative highly potent class for further in vivo optimization that could complement the bicyclic heterocycles identified by Bayer and Pfizer.

As described above for torcetrapib, the relatively small impact that these trifluoro-3-tertiaryamino-2-propanols display in vivo for modulating non-HDLc levels suggests that concurrent therapy with other lipid-lowering agents may be necessary. Accordingly, several recent separate patents have appeared describing combinations of these compounds with other lipid modulating agents including ileal acid transport inhibitors,[118] fibric acid derivatives,[119] bile acid sequestering agents,[120] HMG Co-A reductase inhibitors,[121] and nicotinic acid derivatives.[122] However, no representative animal data were disclosed for these various combinations.

As indicated previously, no animal efficacy data have yet been reported for any of the representative compounds from the newer exploratory classes.

Clinical Evaluation of CETP Inhibitors

Clinical Studies with 3a. Detailed phase I clinical safety and tolerability studies of **3a** have not been reported, but they have been briefly summarized.[7] Thioester **3a** has been evaluated in healthy male volunteers in three separate phase I studies. Escalating single dose studies demonstrated that **3a** was well tolerated in single oral doses from 100 to 1800 mg/day, and there were no overt signs of significant toxicity. In a crossover bioavailability study, **3a** appeared to more effectively inhibit CETP activity in human plasma in the postprandial phase compared with the fasted state. After multiple dosing for 14 days, daily administration of both 600 and 900 mg/day of **3a** raised HDLc levels and reduced LDLc compared to placebo.

Proof of concept data for clinical efficacy with **3a** have been reported in a placebo-controlled phase II study with nearly 200

healthy subjects with mild hyperlipidemia.[7] In this study, daily oral doses of 300, 600, and 900 mg of **3a** dosed over 4 weeks were compared with placebo, and **3a** was well tolerated in multiple oral doses up to 900 mg/day. However, some mild gastrointestinal side effects (diarrhea, nausea, etc.) were apparent that appeared to be associated more with the highest dose group. Treated subjects exhibited a clear, dose-dependent inhibition of CETP activity after 1 week of treatment, which reached a maximal statistically significant 37% inhibition after 4 weeks in the 900 mg group. A statistically significant and dose-dependent increase in CETP protein concentration accompanied the inhibition of CETP activity that reached a maximal ~67% increase in the 900 mg group. Whether this increased CETP mass occurred because of an enhanced induction of the protein following treatment or as a result of decreased catabolism of the inhibitor−protein complex was not discussed. At the same time, there was no apparent effect among any of the treatment groups on either LCAT or PLTP activity, demonstrating the very selective inhibition of CETP-mediated transfer in humans with **3a**.

A dose-dependent increase in HDLc was also observed that reached a maximum after 1 week of treatment. However, the added benefit on HDLc levels from the 900 mg dose over the 600 mg dose became more apparent after 4 weeks of treatment. Treatment with 900 mg of **3a** produced a maximal 34% increase in HDLc levels after 4 weeks, whereas the 600 mg (26%) and 300 mg (15%) groups achieved smaller increases in HDLc. Each of the treatment groups also had significant increases in HDL_2 and HDL_3 subfractions accompanied by increased apo-AI and apo-AII levels. Whereas increases in HDL_3 appeared to plateau in the 300 mg group, increases in the HDL_2 subfraction was dose-dependent and continued to increase across the dosing range. Accompanying these changes in HDLc, a dose-dependent decrease in LDLc was also observed that reached statistical significance with a maximal 7.4% decrease in LDLc in the 900 mg group. No significant changes were observed in other markers including apo-B, apo-E, total cholesterol, and triglyceride levels in the **3a** treatment groups at doses up to 900 mg/day. Nevertheless, the 34% increase in HDLc observed with the 900 mg treatment group compared very favorably with the 25−30% increase frequently observed with multigram daily doses of niacin.[7]

Recently, the initial results from a separate 4-week phase II study were reported that compared 300 and 600 mg oral daily doses of **3a** in combination with 40 mg daily doses of pravastatin for 4 weeks in over 150 patients with LDLc above 160 mg/dL, HDLc less than 60 mg/dL, and triglycerides less than 400 mg/dL.[123] In this study, the combinations were well tolerated across all treatment groups. The 600 mg of **3a** plus 40 mg of pravastatin group produced a statistically significant 30% inhibition of CETP activity after 4 weeks that was accompanied by a concomitant statistically significant 28% increase in HDLc. Once again, a statistically significant, dramatic increase in CETP protein concentration over baseline levels was also observed that reached a maximal ~103% increase in the 600 mg group. An explanation for the increased CETP protein levels following treatment was not reported. Concurrently, a lower but significant 5% reduction in LDLc was achieved with the 600 mg group. While the overall effect on LDLc lowering was not dramatically different in this study using the pravastatin combination compared to the first phase II study with **3a** alone in healthy volunteers, it is important to recognize the difference in patient populations between the two studies. These combined results suggest that **3a** in combination with a statin may hold promise as a clinically beneficial therapeutic in longer term trials.

A recent patent application from Japan Tobacco described a therapeutically useful combination of **3a** with a statin that decreased the atherogenic index in rabbits.[124] In October 2004, Japan Tobacco announced a joint phase III clinical development program for **3a** in partnership with Roche to evaluate its therapeutic utility in reducing cardiovascular events.

Clinical Studies of 5,6,7,8-Tetrahydroquinolines. Very limited information on the overall clinical efficacy for Bayer's first-generation 5,6,7,8-tetrahydroquinoline candidate **10a** has been presented.[73,113] Phase I testing of **10a** orally at 50 and 100 mg has been completed in healthy human volunteers. Compound **10a** reportedly inhibited CETP-mediated transfer, and the level of inhibition could be correlated with plasma drug levels. Essentially no details of the clinical testing of the second-generation tetrahydronaphthalene candidate **13a** have been released. Nevertheless, Bayer has announced that the clinical development of both of these candidates has been discontinued.[125] No reasons have been cited that explain the termination of clinical testing for these two compounds, although Bayer reportedly has brought a third-generation compound into preclinical development.

Clinical Studies with Torcetrapib. The initial phase I multidose placebo-controlled efficacy results using **14** in healthy human volunteers have been reported.[8a] Torcetrapib was dosed orally under fed conditions at doses of 10, 30, 60, and 120 mg once daily and at 120 mg b.i.d for 14 days. Overall, torcetrapib was well tolerated, and all the subjects receiving active drug completed the study. The exposure of torcetrapib was dose-dependent in humans with an observed C_{max} occurring within 2−6 h of the initial dose. The dose-dependent inhibition of CETP activity was observed immediately with the first dose, correlated with drug exposure, reached a maximum in the same 2−6 h time frame that drug exposure peaked, and declined as the drug cleared. The resulting first dose EC_{50} was determined in vivo to be 43 nM and corresponded quite closely to an IC_{50} of 50 nM measured in human plasma under in vitro conditions. The level of CETP inhibition was maintained above 90% for all subjects in the 120 mg b.i.d. group. Mean inhibition of CETP activity increased with dose and, as determined immediately before the administration of the last dose, was reported to be 12%, 35%, 53%, and 80% for the 30, 60, and 120 mg daily and 240 mg (120 mg b.i.d.) groups, respectively. While the plasma CETP protein concentration was essentially the same between treated and placebo subjects, a dose- and time-dependent increase in the mass of plasma CETP protein was observed in all treated subjects. This increase in CETP mass was attributed to the favorable shift in HDL-bound CETP in the presence of torcetrapib.

After multiple dosing for 14 days, torcetrapib dose-dependently raised HDLc levels and reduced LDLc compared to placebo, while there was no significant overall change in total cholesterol or TG. Thus, daily doses of torcetrapib at 10, 30, 60, and 120 mg daily and 120 mg twice daily produced remarkable increases in HDLc of 16%, 28%, 62%, 73%, and 91%, respectively. These were accompanied by a corresponding shift to larger HDL particle size and dose-dependent reductions in LDLc of −9%, 14%, 11%, 21%, and 42%, respectively. A concomitant dose-dependent increase in apo-AI and apo-E lipoproteins along with a reduction in apoB lipoproteins was also observed in the torcetrapib-treated groups. For the highest dose group receiving 120 mg b.i.d., these reached maximal increases of 27% and 66% for apo-AI and apo-E, respectively,

along with a 26% reduction in apo-B. Thus, treatment of healthy subjects with torcetrapib inhibited CETP activity raised HDLc and lowered LDLc and produced concomitant increases in apo-A and apo-E and reductions in apo-B lipoproteins.[8a]

Very impressive changes in lipoprotein profiles were also observed in the highest dose group of 240 mg (120 mg, b.i.d), which after 14 days inhibited CETP activity by more than 80%, raised HDLc by 91%, and lowered LDLc by 42%, although total cholesterol levels were unaffected. These changes are among the largest increases in HDLc observed following pharmacological intervention in human subjects. The maximal HDLc elevations reported for torcetrapib do not reach the 3-fold or higher increases in HDLc observed in subjects with complete CETP deficiency. Similarly, total cholesterol was unchanged following torcetrapib treatment unlike the large increases in total cholesterol that were observed in subjects with complete CETP deficiency. Thus, exposure to torcetrapib apparently induced a lipoprotein profile in healthy humans more consistent with partial CETP deficiency.[8a]

A small phase II clinical proof of concept study has also been conducted comparing 120 mg of torcetrapib once or twice daily for 4 weeks to placebo in subjects having low HDLc (<40 mg/dL) either as monotherapy or with a statin.[8b] One arm of this study included a small group of patients jointly treated for 4 weeks once daily with both 120 mg of torcetrapib and 20 mg of atorvastatin. Treatment with 120 mg of torcetrapib alone inhibited plasma CETP activity by 28% when given q.d. and by 65% b.i.d. After 4 weeks, 120 mg of torcetrapib alone produced impressive increases in HDLc of 46% (q.d.) and 106% (b.i.d.), compared to placebo. Torcetrapib also produced significant increases in both the HDL_2 and HDL_3 subfractions, and these changes were translated into a significant increase in HDL particle size in the torcetrapib-treatment groups. In contrast to the phase I study, however, the reductions observed in LDLc in this study were smaller and did not reach statistical significance, even though total cholesterol levels remained unchanged. After 4 weeks, treatment with 120 mg of torcetrapib alone produced nonsignificant reductions in LDLc of 8% (q.d.) and 17% (b.i.d.). Even though these LDLc changes did not reach statistical significance, the LDLc reductions observed with torcetrapib alone exceeded the 7% lowering in LDLc observed clinically with **3a** at 900 mg/day.[7] At the same time, the overall LDL particle size increased in torcetrapib-treated patients, while the levels of proatherogenic small LDL particles were reduced.

The lipoprotein particle sizes associated with both HDL and LDL are important contributors to CAD. Patients with CAD tend to have higher levels of small, dense LDL particles[126] and lower levels of large HDL particles.[127] Treatment with 120 mg of torcetrapib for 4 weeks in patients having low HDLc raised the levels of larger HDL particles to levels observed in normolipidemic age- and sex-matched control subjects. Torcetrapib treatment also increased the mean particle size of LDL and reduced the levels of small, dense proatherogenic LDL particles.

Similarly impressive changes were also observed in subjects treated daily with both 120 mg of torcetrapib and 20 mg of atorvastatin. After 4 weeks, subjects' plasma CETP activity was inhibited by 38%, HDLc increased by 61%, and LDLc was reduced by a statistically significant 17% beyond that achieved with atorvastatin alone. Joint treatment with torcetrapib and atorvastatin raised apo-A by 13% and reduced apo-B plasma levels by 14%. In a follow-up report, more details have been described for the effect of torcetrapib treatment on HDL subspecies and apo-AI metabolism.[128] The increase in apo-AI observed following partial CETP inhibition with torcetrapib treatment was attributed to a reduction in apo-AI catabolism. This combination also produced significant increases in both the HDL_2 and HDL_3 subfractions and significantly increased HDL particle size.[8b] The overall LDL particle size also increased, and the level of proatherogenic small LDL particles was reduced.

The relationship between CETP protein concentration and the potential response to statin therapy in men with CAD has recently been investigated in placebo-controlled clinical trials with pravastatin.[129] In this REGRESS trial, after 2 years of treatment, higher CETP plasma protein concentrations were associated with a faster progression of atherosclerosis. Similarly, the highest improvement in lipoprotein profile and angiographic parameters after 2 years occurred with pravastatin treatment in those subjects having the highest baseline levels of CETP. Thus, CETP plasma protein levels appear to be an important determinant of a patient's potential response to statin therapy. Similarly, in the CARE trial, patients with familial hypercholesterolemia were treated for 2 years with two different statins, and plasma protein levels of CETP were associated with a more atherogenic lipid profile and increased progression of atherosclerosis.[30] Whereas statin treatment helped to improve the lipoprotein profiles in these patients, their overall effectiveness was not as high in patients having high plasma CETP levels. These results suggest that statins cannot completely correct lipoprotein abnormalities in patients with high plasma CETP protein levels.[30] On the basis of these data, a CETP inhibitor may be particularly effective in improving the atherogenic lipoprotein profile in subjects with high CETP plasma levels.

Overall, these exciting initial phase II results with torcetrapib were very encouraging and compared quite favorably with the clinical data presented above for **3a**. Substantially higher increases in HDLc were achieved with the 120 mg dose of torcetrapib alone either q.d. (46%) or b.i.d. (106%) in patients with low HDLc than were observed with the highest 900 mg dose of **3a** (34%) after 4 weeks in patients with mild hyperlipidemia.[7] Similarly, greater reductions in LDLc were also observed with torcetrapib treatment than were obtained with the highest dose of **3a** after 4 weeks. However, it is important to keep in mind that both the level of CETP inhibition and the total amount of increased HDLc that is required to influence cardiovascular events in patients with low HDLc are still unknown. Nevertheless, on the basis of these combined data, torcetrapib appears to have potential clinical benefit both as monotherapy and in combination with statins.

Conclusions and Future Prospects

The clinical changes observed in lipoprotein profiles with both **3a** and torcetrapib in these small phase II trials are encouraging, particularly when compared to current therapies. The percentage increases in HDLc observed with torcetrapib and **3a** are substantially higher than those observed clinically following statin (5−10%) or fibrate (6−8%) treatment. The changes observed in HDLc with the 120 mg dose of torcetrapib also readily exceed the 25−30% increases in HDLc commonly observed with niacin. Nevertheless, it will be especially important to determine whether these agents perform similarly across a broader, more diverse patient population without disrupting normal lipoprotein balance and function.

At the same time, there is still a clear need to demonstrate the direct benefit of these CETP inhibitors on reducing morbidity and mortality in phase III trials with CAD patients. Such trials are extremely expensive, require large patient populations, and

will take several years to complete. Both Pfizer and Japan Tobacco have announced aggressive phase III development programs for their respective compounds, and the results from these trials are anxiously awaited. These trials should also help address several important unanswered questions: (a) How much CETP inhibition is required for clinical efficacy? (b) Is CETP inhibition restricted to a certain range of baseline CETP protein plasma levels? (c) How important is the patient's diet or underlying lipidemic and metabolic state to the overall clinical effectiveness of CETP inhibitors? (d) What effect does CETP inhibition have on HDL functionality and catabolism?[130]

Recently, a shorter 18-month trial has been reported in which pravastatin and atorvastatin were compared for their ability to reduce the progression of atherosclerotic lesions in CAD patients via surrogate endpoints including intima media thickness or coronary atheroma volume using intravascular ultrasonography (IVUS).[131] It will be very interesting to see if similar data can be generated using IVUS techniques for either **3a** or torcetrapib. Pfizer recently announced that its clinical development program for torcetrapib included trials using these IVUS imaging techniques and expects their first initial results to be reported in late 2006.[132]

If successful, such trials not only should dramatically increase the interest in identifying other classes of CETP inhibitors that could potentially lead to a wider variety of new clinical compounds but also could increase interest in other approaches to raising HDLc. Moreover, the success of CETP inhibitors in phase III trials with CAD patients having low HDLc may also extend their clinical use into other diseases that are characterized by low HDLc levels, such as metabolic syndrome, stroke, peripheral vascular disease, obesity, and diabetes. For example, the proatherogenic role played by CETP in diabetic patients has been the subject of ongoing studies.[133] Similarly, both obese children and morbidly obese women have increased plasma CETP plasma protein levels, and weight reduction in morbidly obese women produced a dramatic 37% reduction in CETP activity and a correspondingly improved lipoprotein profile.[134,135] Just as the early statins revolutionized the treatment of hyperlipidemic CAD patients, these CETP inhibitors, if successful in phase III trials, have the potential to dramatically impact the course of the disease, particularly in those patients that are unresponsive to statin therapy.

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Biography

James A. Sikorski received a B.S. degree with honors in chemistry at Northeast Louisiana State University in 1970. He attended Purdue University and received his M.S. and Ph.D. degrees in organic chemistry, under the direction of Nobel laureate, Professor Herbert C. Brown. He joined Monsanto Agricultural Company in 1976, advancing to Science Fellow. In 1988, he moved to drug discovery research first as Science Fellow in Medicinal Chemistry at Monsanto Corporate Research, then at Searle and Pharmacia where he contributed to several anti-infective, anti-inflammatory, and cardiovascular projects. Dr. Sikorski has received both the 1994 St. Louis ACS Award and the 1999 Kenneth A. Spencer Award. In 2001 he joined AtheroGenics, Inc. and is currently Senior Director of Medicinal Chemistry where his research is focused on the discovery of novel agents to treat chronic inflammatory diseases.

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