Emerging role of high-density lipoprotein in the prevention of cardiovascular disease

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In major statin trials, the relative risk reduction is typically in the range 25–35%, thus indicating that the majority of cardiac events continues to occur despite statin therapy. Hence, there is a considerable interest in identifying novel therapies capable of further reducing cardiovascular disease risk. One such potential therapeutic target is a low level of high-density lipoprotein (HDL) cholesterol. Emerging targets involved in HDL metabolism are: (i) liver X receptor and peroxisome proliferator-activated receptor agonists; (ii) cholesteryl ester transfer protein inhibitors; (iii) HDL mimetics (ETC-216); (iv) apolipoprotein A-I synthetic peptides; and (v) HDL delipidation and reinfusion. Although they are at various stages of development, each of these therapies has promise for the treatment of cardiovascular disease in humans.

High-density lipoprotein (HDL) cholesterol (HDL-C) concentrations are inversely associated with cardiovascular disease (CVD) risk [1]. HDL and its major protein constituent, apolipoprotein (apo) A-I, are important mediators of reverse cholesterol transport (RCT), a process by which free cholesterol is removed from the peripheral tissues of the body, transferred back to the liver and, ultimately, excreted into the bile [2]. In addition, HDL protects low-density lipoproteins (LDLs) against oxidative modification [3] and has direct anti-inflammatory, antithrombotic and profibrinolytic effects [4–6]. Thus, there are several mechanisms by which HDL could provide protection against CVD.

The current guidelines established by the third Adult Treatment Panel of the National Cholesterol Education Program define a low HDL-C level as <40 mg/dl [7]. Low HDL-C is the most common lipid abnormality observed in patients with known coronary heart disease (CHD), with about a half of CHD patients having this as their primary lipid abnormality [8]. Despite data from large-scale clinical trials indicating that even modest increases in HDL-C concentrations can significantly reduce CHD risk [9,10], well-tolerated drugs with significant HDL-raising potential are still lacking.

Current therapies for increasing HDL-C concentrations

Therapies currently available for increasing HDL-C include fibric acid derivatives, or fibrates, niacin and statins. Fibrates, such as gemfibrozil and fenofibrate, have only modest effects on HDL-C levels, raising them on average by 10–15% [11]. Although niacin can increase HDL-C levels in the order of 15–30%, the doses of niacin required to achieve such increases are often not well tolerated [11]. Statins, the most widely used class of drugs in the treatment of dyslipidemias, are only capable of increasing HDL-C by 5–12% [11,12].

Emerging therapies for reducing cardiovascular disease risk via HDL

ATP-binding cassette transporter A1

A major mechanism by which HDL might protect against the development of atherosclerosis is through

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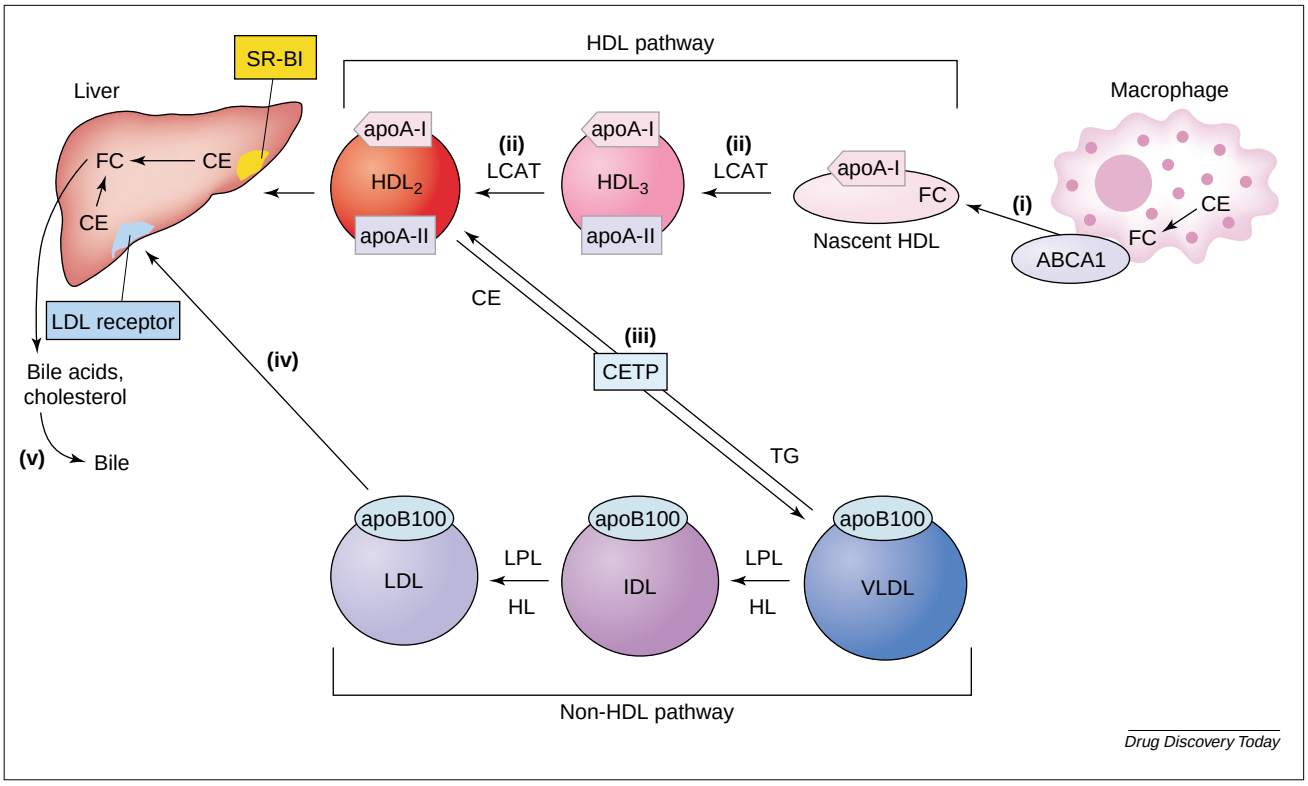

FIGURE 1

Major steps in the reverse cholesterol transport pathway. RCT is a complex process that involves transport proteins, modifying enzymes and cell surface receptors. RCT consists of five main steps: (i) cholesterol efflux from peripheral cells; (ii) cholesterol esterification; (iii) cholesteryl ester transfer; (iv) hepatic cholesterol uptake; and (v) hepatic excretion of cholesterol. In the first step of RCT, ABCA1 mediates the apolipoprotein-stimulated pathway of cholesterol efflux from peripheral cells to lipid-poor HDL. Next, the FC on nascent HDL is esterified by LCAT, generating a spherical HDL particle. LCAT facilitates RCT by decreasing the amount of free cholesterol on HDL, thus maintaining a concentration gradient of cholesterol between HDL and peripheral cells. The third step in RCT is the cholesteryl ester transfer, which is mediated by CETP. This protein promotes the exchange of CE from the HDL pathway to apoB-containing pathway, providing an avenue for uptake of cholesteryl esters by hepatic receptors. The uptake of cholesteryl esters by receptors is the fourth step in RCT. SR-BI is primarily involved in the selective uptake of cholesteryl esters from HDL, whereas the LDL receptor pathway clears those within apoB-containing lipoproteins. The final step in RCT is the excretion of cholesterol by the liver. This can occur directly via the secretion of cholesterol into bile or indirectly by secretion of cholesterol after conversion to bile salts. Abbreviations: CE, cholesteryl esters; FC, free cholesterol; HL, hepatic lipase; IDL, intermediate density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LPL, lipoprotein lipase; SR-BI, scavenger receptor class BI ; VLDL, very low-density lipoprotein.

its role in RCT (Figure 1). A significant advance in our understanding of this pathway occurred with the identification of mutations in the gene encoding ATP-binding cassette transporter A1 (ABCA1) as the cause of Tangier disease [13–15], a rare genetic disorder characterized by extremely low levels of HDL-C in the plasma and aberrant apolipoprotein-mediated cellular cholesterol efflux [16]. Many studies have now established that ABCA1 mediates the efflux of phospholipids and cholesterol to apolipoprotein acceptors, the initial step in the RCT pathway, and, thus, plays a crucial role in HDL metabolism. This discovery generated great interest in the development of therapies to increase ABCA1 expression.

To date, no therapeutic agents designed to increase ABCA1 expression have been tested in humans. However, evidence from animal studies supports the hypothesis that upregulation of ABCA1 expression might be beneficial in the prevention of atherosclerosis. When fed on an atherogenic diet, C57BL/6 mice that overexpressed human ABCA1 primarily in the liver and in macrophages had significantly (P <0.0005) higher levels of HDL-C (+183%) and apoA-I (+151%) but significantly (P <0.005) lower levels of non-HDL-C (–47%) and apoB (–36%) compared with their nontransgenic littermates [17]. These changes translated into a 65% reduction in mean aortic lesion area in human ABCA1 transgenic C57BL/6 mice relative to controls. By contrast, overexpression of human ABCA1 in an animal model of spontaneous atherosclerosis (the apoE-deficient mouse) had minimal effects on plasma lipoproteins and led to a significant increase in lesion development in male (+160%, P = 0.002) and female (+100%, P <0.001) apoE$^{-/-}$ mice, compared with age- and gender-matched littermates. The results of this study not only indicate that upregulation of ABCA1 might reduce atherogenic risk in humans but also emphasize the important role of apoE in cholesterol metabolism and atherogenesis.

Additional insight into ABCA1 function has been provided by the identification of response elements in the promoter region of the gene [18]. This includes the liver X receptor (LXR)–retinoid X receptor (RXR) promoter

TABLE 1

Effects of CETP inhibition on the development of atherosclerosis in animals

Animal model	CETP inhibitor	Change (%)		Aortic lesion area	Comments	Refs
		HDL-C	LDL-C			
Japanese white male rabbits	Antisense oligo-deoxynucleotides	+32[a]	−24	Decrease	Significant increase in LDL receptor mRNA and 36% decrease in aortic lesion surface were seen in treated versus untreated animals	[25]
New Zealand white rabbits	CETi-1	+42[b]	−24	Decrease	Aortic surface with atherosclerotic lesions was 40% smaller in vaccinated versus control rabbits	[26]
Japanese white male rabbits	JTT-705	+94[c]	−40–50	Decrease	Lesion area in aortic arch was 30.3% in control rabbits compared with 9.2% in treated rabbits	[27]
New Zealand white rabbits	Torcetrapib	+363[d]	Stable[e]	Decrease	Aortic lesion surface was reduced by 60% in torcetrapib-treated (16.4 ± 3.4%) versus control (39.8 ± 5.4%) rabbits (P <0.001)	[28]

[a]At 11 weeks, the fraction of cholesterol in HDL, isolated by HPLC, was higher in animals treated with antisense oligodeoxynucleotides (18.6%) versus control animals (13.1%), whereas the fraction of cholesterol in LDL was lower (14.5% versus 19.1%, respectively).

[b]When the average plasma HDL-C values for the vaccinated and control groups were plotted as a function of time and the areas under the curves for weeks 19 and 32 determined, the vaccinated group had more HDL-C (P <0.07) than the control group.

[c]After 3 months on a 0.2% cholesterol diet, JTT-705 treated animals had significantly higher HDL-C levels than control animals (33 versus 17 mg/dl, P <0.01).

[d]After 16 weeks on a 0.2% cholesterol diet, torcetrapib-treated animals had significantly higher HDL-C levels than control animals (207 ± 32 mg/dl versus 57 ± 6 mg/dl).

[e]After 16 weeks on a 0.2% cholesterol diet, non-HDL-C levels were similar in control (645 ± 105 mg/dl) and torcetrapib-treated (690 ± 104 mg/dl) rabbits.

Abbreviation: LDL-C, low-density lipoprotein cholesterol.

element, which is activated by oxysterols. Activation of LXR–RXR, in turn, enhances the transcription of ABCA1. Several laboratories are in the process of evaluating endogenous and synthetic compounds for their ability to activate LXR and, thus, upregulate ABCA1 expression. One of the limitations of this strategy is that, in addition to activating transcription of genes involved in the RCT pathway, LXR agonists also induce genes that stimulate lipogenesis, including the sterol response element binding protein and fatty acid synthetase [19]. Induction of the fatty acid synthetase genes can lead to increased hepatic triglyceride (TG) synthesis and hypertriglyceridemia. To circumvent this problem, Miao *et al.* [20] have developed the selective LXR modulator GW3965. This compound is a weak LXR activator compared with its predecessor T0901317. In mice, GW3965 increased plasma HDL-C levels by 18% and 20% at doses of 60 and 100 mg/kg, respectively, without increasing hepatic or plasma TG concentrations. In comparison, although 50 mg/kg of T0901317 increased HDL-C levels by 53%, it also increased liver TG content tenfold.

Peroxisome proliferator-activated receptor α (PPARα) agonists induce peroxisomal fatty acid β-oxidation and also mediate HDL metabolism [21]. An alternative approach that involves simultaneous activation of PPARα and LXR, which could lead to additive effects on HDL-C elevation and attenuation of TG accumulation, is currently being explored by Beyer *et al.* [22]. Coadministration of T0901317 (50 mg/kg) and the specific PPARα agonist Wy14643 (10 mg/kg) for 7 days in C57B6 mice led to a synergistic increase in HDL-C levels, without altering liver TG concentrations [22]. These results suggest that the development of LXRα–PPARα dual agonists might have potential utility in modulating lipid homoeostasis in humans. In addition, several new PPARα agonists that raise HDL-C and lower TG to a greater extent than currently available PPARα activators (i.e. fibrates) are currently in development. These include LY-518674 (Lilly), GW-590735 (GlaxoSmithKline) and K111 (Roche).

Cholesteryl ester transfer protein inhibition

Among the emerging therapies targeting HDL metabolism, cholesteryl ester transfer protein (CETP) inhibitors are in an advanced stage of clinical development. CETP plays a crucial role in HDL metabolism by mediating the exchange of cholesteryl esters from HDL for triglycerides in apoB-containing lipoproteins [23]. Because humans with CETP deficiency due to molecular defect(s) in the CETP gene have markedly high plasma levels of HDL-C and apoA-I [24], it has been proposed that CETP inhibition would, likewise, increase HDL-C levels. Partial inhibition of CETP activity by antisense oligonucleotides [25], vaccine-induced antibodies [26] or small molecules [27,28] has been shown to increase HDL-C levels significantly and reduce the development of diet-induced atherosclerosis in animal models (Table 1). To date, three different CETP inhibitors, JTT-705 (Japan Tobacco), torcetrapib (Pfizer), and CETi-1 (Avant Immunotherapeutics), have been tested for their effects on plasma lipids in humans [29–33] (Table 2).

De Grooth *et al.* [29] were the first to report the effects of pharmacological CETP inhibition in humans (Table 2). In a randomized, double-blind, placebo-controlled study, researchers evaluated the safety and efficacy of JTT-705 in 198 healthy subjects with mild hyperlipidemia. Enrollment criteria included an age of 18 to 65 years, an HDL-C level of ≤62 mg/dl (1.6 mM) and a TG level of ≤400 mg/dl (4.5 mM). There were no exclusion criteria for LDL cholesterol. A total of 134 men and 64 women were randomized to

TABLE 2

Effects of CETP inhibition on plasma lipids in humans

Study subjects	CETP inhibitor (dose)	Baseline HDL-C (mg/dl)	Change (%)[a]		Comments	Refs
			HDL-C	LDL-C		
134 M and 64 F with mild hyperlipidemia and normal HDL-C	**JTT-705**				The 900 mg/d dose of JTT-705 was associated with a higher frequency of gastrointestinal complaints ($P = 0.058$)	[29]
	(300 mg/d, $n = 48$)	45	+15	−4		
	(600 mg/d, $n = 48$)	47	+26	−6		
	(900 mg/d, $n = 50$)	45	+37	−7		
120 M and 35 F with Type II dyslipidemia	**JTT-705**				Combination therapy of JTT-705 600 mg/dl and pravastatin 40 mg/dl effectively increased HDL-C levels and was well tolerated up to 4 weeks	[30]
	(placebo + pravastatin, $n = 52$)	48	0	+2		
	(300 mg/d + pravastatin, $n = 53$)	49	+13	+1		
	(600 mg/d + pravastatin, $n = 47$)	48	+28	−5		
40 healthy M and F	**Torcetrapib**				In normolipidemic subjects, torcetrapib was well tolerated and increased HDL-C levels in a dose-dependent manner	[31]
	(10 mg/d, $n = 6$)	60	+16	+9		
	(30 mg/d, $n = 6$)	48	+28	−14		
	(60 mg/d, $n = 6$)	53	+62	−11		
	(120 mg/d, $n = 6$)	49	+73	−21		
	(240 mg/d, $n = 6$)	54	+91	−42		
17 M and 2 F with HDL-C <40 mg/dl and LDL-C <160 mg/dl[b]	**Torcetrapib**				In patients with low HDL-C, torcetrapib markedly increased HDL-C levels and decreased LDL-C levels when given alone or in combination with a statin	[32]
	(120 mg/d + atorvastatin, $n = 9$)	29	+61	−17		
	(120 mg/d, $n = 10$)	32	+46	−8		
	(120 mg twice daily, $n = 6$)	34	+106	−17		
48 healthy adults with HDL-C <60 mg/dl	**CETi-1**				At the doses used in this study, CETi-1 did not significantly alter CETP activity or HDL-C levels	[33]
	(10 μg, $n = 9$)	32	+22[c]	ND		
	(25 μg, $n = 8$)	42	+2	ND		
	(100 μg, $n = 9$)	44	+4	ND		
	(250 μg, $n = 9$)	39	+2	ND		

[a]Percentage change versus baseline or placebo.

[b]Subjects having LDL-C level of >160 mg/dl were considered for the atorvastatin arm of the study provided that they met all other criteria, including that of having a LDL-C level of <160 mg/dl once stabilized on atorvastatin 20 mg.

[c]After 25 weeks of treatment, no significant changes were noted in HDL-C levels relative to baseline.

Abbreviations: F, female; M, male; ND, no data.

placebo or 300 mg (low dose), 600 mg (intermediate dose) or 900 mg (high dose) JTT-705 per day for 4 weeks. Each treatment group consisted of a similar ratio of males to females, with ratios of 35:15, 35:13, 29:19 and 35:17 for the placebo, low, intermediate and high groups, respectively. After 4 weeks of treatment with JTT-705, dose-dependent decreases in CETP activity were observed among the groups, with the greatest reduction (–37%, $P < 0.0001$) noted in the high-dose group. In turn, dose-dependent increases were seen in plasma HDL-C concentrations. Mean increases of 15%, 26%, and 34% were noted for the low-, intermediate-, and high-dose groups, respectively. A 7% reduction in LDL cholesterol ($P < 0.02$) was also observed in the high-dose group. Dosages up to 900 mg/d of JTT-705 were well tolerated and exhibited a clean safety profile. Although not statistically significant ($P = 0.058$), the 900 mg dose was associated with a higher frequency of gastrointestinal complaints; however, no patient withdrawals because of this side effect were reported.

Recently, the results of another Phase II trial of JTT-705 were reported by Kuivenhoven *et al.* [30] (Table 2). In this trial, 155 subjects having an LDL cholesterol level of >160 mg/dl were treated with pravastatin 40 mg/d coadministered with placebo or JTT-705 300 mg/d or JTT-705600 mg/d. After 4 weeks, the combined therapy of pravastatin and JTT-705 600 mg was associated with a 30% decrease ($P < 0.001$) in CETP activity, a 28% increase ($P < 0.001$) in HDL-C and a 5.5% reduction ($P < 0.03$) in LDL cholesterol relative to placebo. Thus, based on the available data, JTT-705 appears to be a safe and reasonably efficacious agent for HDL-raising in humans. However, whether the effects of JTT-705 on plasma lipid concentrations will translate into reduced susceptibility to atherosclerosis in humans as it did in rabbits remains to be determined [27].

To date, the most potent CETP inhibitor is torcetrapib. The effects of torcetrapib on plasma lipoproteins in normolipidemic subjects [31] and in patients with low HDL-C (<40 mg/dl) [32] have been reported (Table 2). In a study by Clark *et al.* [31], 40 healthy males and females between the ages of 18 and 55 years were randomized to placebo or torcetrapib at doses of 10, 30, 60 and 120 mg daily, or 120 mg twice daily, for 14 days. Relative to baseline, increases of 28%, 62%, 73% and 91% in HDL-C were seen for the 30, 60, 120 mg daily and 120 mg twice-daily groups ($P < 0.001$ for all comparisons), respectively. Additionally, LDL cholesterol levels were significantly reduced in the

120 mg once- (–21%, P <0.05) and twice-daily (–42%, P <0.001) groups. Torcetrapib was well tolerated at all doses, with all treated subjects completing the study.

In 2004, the effects of CETP inhibition with torcetrapib on plasma lipoproteins in subjects with low baseline levels of HDL-C were reported [32] (Table 2). Nineteen subjects with low HDL-C (<40 mg/dl), nine of whom were also treated with atorvastatin 20 mg, received placebo for 4 weeks, followed by torcetrapib 120 mg daily for an additional 4 weeks. Six subjects in the non-atorvastatin cohort also participated in a third phase, in which they received torcetrapib 120 mg twice daily for 4 weeks. Torcetrapib 120 mg daily increased plasma concentrations of HDL-C by 61% (P <0.001) and 46% (P = 0.001) in the atorvastatin and non-atorvastatin cohorts, respectively, whereas the 120 mg twice-daily dose increased HDL-C by 106% (P <0.001). Additionally, torcetrapib further reduced LDL cholesterol levels by 17% in the atorvastatin cohort (P = 0.02). Torcetrapib also significantly altered the distribution of cholesterol among HDL and LDL subclasses, resulting in increased mean HDL and LDL particle size in each of the cohorts. Torcetrapib was well tolerated without clinically important drug-related adverse events or withdrawals. Thus, in subjects with low HDL-C, CETP inhibition with torcetrapib markedly increased HDL-C levels and also decreased LDL cholesterol levels, when either administered as monotherapy or in combination with a statin.

Recently, the results of a Phase I clinical trial of CETi-1 in humans were reported by Davidson *et al.* [33] (Table 2). Forty-eight healthy adults with an HDL-C level of <60 mg/dl were randomized to placebo or 10, 25, 100 or 250 μg CETi-1. Neither significant changes in CETP function nor HDL-C levels were observed among the groups in this study. These findings were not unexpected because of the use of a sub-optimal dosing regimen to obtain a full complement of safety data. Additional trials designed to assess the effects of CETi-1 on plasma lipid concentrations in patients with low HDL-C are now underway [33].

Human apoA-I and apoA-I mimetic peptides

In 2003, a report by Nissen *et al.* [34] sparked great interest in the potential therapeutic use of apoA-I. In this study, intravenous infusion of recombinant apoA-I_{Milano}-phospholipid complexes (ETC-216) into humans had striking effects on atheroma burden, as measured by intravascular ultrasound, in patients with acute coronary syndromes. ApoA-I_{Milano} is thought to have atheroprotective properties because individuals with this apoA-I variant have much less atherosclerosis than expected from their HDL-C levels (10–30 mg/dl) [35]. A total of 104 patients were randomized to placebo or low- (15 mg/kg) or high-dose (45 mg/kg) ETC-216, which was administered intravenously at weekly intervals for five doses. An absolute reduction from baseline of –14.1 mm^3 in atheroma volume, or 4.2% (P <0.001), was seen for the combined low- and high-dose groups. For the placebo group, the corresponding change was –2.9 mm^3. Although promising, these results require confirmation in larger clinical trials with morbidity and mortality endpoints. Future studies should also address the issue of whether native apoA-I would behave similarly to apoA-I_{Milano} in terms of its effect on atheroma burden.

Another emerging strategy for CVD prevention is the development of apoA-I mimetic peptides. This strategy is based on the hypothesis that apoA-I mimetic peptides could be capable of stimulating the first step in the reverse cholesterol transport pathway – cellular cholesterol efflux (Figure 1) – in a manner similar to native apoA-I, which is 243 amino acid residues in length. In comparison, the apoA-I mimetic peptide D-4F comprises only 18 amino acids.

D-4F was designed to contain a class A amphipathic helix, with a polar and a nonpolar face [36]. The amphipathic helix structure plays an important role in lipid binding and, in turn, cellular cholesterol efflux [37]. The physical characteristics of D-4F, its interaction with model membranes and the importance of the hydrophobic face in determining biological activity were recently described [38,39]. In mice, oral administration of D-4F reduces atherosclerosis, independently of plasma cholesterol levels [40]. Further studies designed to address the mechanism(s) by which D-4F might reduce atherosclerosis have shown that 500 μg of D-4F administered orally to mice led to the formation of pre-β HDL, increased cholesterol efflux from macrophages and enrichment of HDL with apoA-I and paraoxonase [41]. Similarly, when added to human plasma at nanomolar concentrations in an *in vitro* system, D-4F caused the movement of apoA-I to smaller particles, reduction of lipid hydroperoxides, increase of paraoxonase activity and conversion of proinflammatory HDL to anti-inflammatory HDL [42]. To date, *in vivo* effects of D-4F in humans have not been reported.

Another mimetic peptide currently under investigation is 37pA [43,44]. The 37pA peptide [45], which contains two identical class A amphipathic helices linked by a proline, has been shown to stimulate cholesterol and phospholipid efflux from cells [46,47]. Furthermore, the 37pA peptide promotes lipid efflux through ABCA1-dependent and -independent pathways [43], with the ABCA1-independent pathway involving a cytotoxic membrane microsolubilization process. To address this limitation, Remaley *et al.* [44] recently reported the generation of 5A-37pA, a modified version of 37pA, which consists of five hydrophobic residues (Leu3, Phe6, Val10, Leu14 and Phe18) substituted for alanine in the C-terminal helix. *In vitro* experiments revealed that the 5A-37pA peptide had greater ability to efflux cholesterol through the ABCA1-mediated pathway than 37pA and, importantly, was less cytotoxic. Additional studies are required to determine the therapeutic potential of apoA-I mimetic peptides in humans.

HDL delipidation and reinfusion

The first step in RCT involves the efflux of cholesterol from peripheral cells to HDL. The concept that lipid-poor pre-β

HDL are better acceptors of cholesterol than mature α HDL has led to the generation of LSI-S955 (Lipid Sciences), a delipidation agent designed to remove lipids selectively from HDL in plasma without substantially affecting the composition of other lipoproteins. In 2004, Sacks *et al.* [48] reported the results of a preliminary study conducted on plasma samples from 32 subjects. Using Lipid Sciences' proprietary selective delipidation process, HDL-C was reduced by 76 ± 11% in delipidated versus undelipidated plasma. By contrast, a modest 12% increase in LDL cholesterol was observed in delipidated plasma, relative to undelipidated plasma. Analysis of HDL subpopulations by 2D gel electrophoresis demonstrated a marked increase in pre-β subpopulation of HDL and a decrease in α HDL. Consistent with the increase in pre-β HDL, *in vitro* experiments revealed that cholesterol efflux to delipidated plasma was increased 18–20 fold, relative to control plasma. Thus far, delipidated plasma has been administered to mice, pigs and monkeys with no apparent adverse side effects. Similar studies in humans are still to be conducted.

Conclusions

Based on the available data, CETP inhibitors and ETC-216 appear to have great promise for the treatment of CVD, whereas the therapeutic potential of selective LXRα agonists, dual LXRα–PPARα agonists, apoA-I mimetic peptides and HDL delipidation-reinfusion remains to be determined. In the case of CETP inhibitors, additional clinical trials are required to establish whether the increases in HDL-C will translate into a reduced risk for atherosclerotic CVD in humans. Although ETC-216 causes regression of atherosclerosis in humans, larger clinical trials are needed to determine whether short-term therapy with ETC-216 provides protection against cardiovascular events.

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