High-Density Lipoprotein-Targeted Therapy and Apolipoprotein A-I Mimetic Peptides

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Numerous randomized clinical trials have established statins as the major standard therapy for atherosclerotic diseases because these molecules decrease the plasma level of low-density lipoprotein (LDL) cholesterol and moderately increase that of plasma high-density lipoprotein (HDL) cholesterol. The reverse cholesterol transport pathway, mediated by HDL particles, has a relevant antiatherogenic potential. An important approach to HDL-targeted therapy is optimization of the HDL-cholesterol level and enhanced removal of plasma cholesterol, together with the prevention and mitigation of inflammation related to atherosclerosis. Small-molecule inhibitors of cholesteryl ester transfer protein (CETP) increase the HDL-cholesterol level in subjects with normal or low HDL-cholesterol. However, CETP inhibitors do not seem to reduce the risk of atherosclerotic diseases. HDL therapies using reconstituted HDL, including apolipoprotein (Apo) A-I Milano, ApoA-I mimetics, or full-length ApoA-I, are dramatically effective in animal models. Of those, the ApoA-I-mimetic peptide called FAMP effectively removes cholesterol via the ABCA1 transporter and acts as an antiatherosclerotic agent by enhancing the biological functions of HDL without elevating the HDL-cholesterol level. Our review of the literature leads us to conclude that HDL-targeted therapies have significant atheroprotective potential and thus may effectively treat patients with cardiovascular diseases. (*Circ J* 2015; **79**: 2523–2528)

Key Words: Apolipoprotein; Atherosclerosis; Cardiovascular disease; Dyslipidemia; Reverse cholesterol transport

Dyslipidemia is one of the more relevant risk factors for coronary artery disease. Previous epidemiological studies and clinical trials demonstrated an inverse association between the serum level of high-density lipoprotein (HDL) cholesterol and the risk of cardiovascular events and that HDL-cholesterol exerts many potential antiatherogenic effects.¹ For example, HDL particles transport cholesterol from peripheral cells to the liver and steroidogenic organs that use cholesterol for the synthesis of lipoproteins, bile acids, and steroid hormones.¹ Low-density lipoprotein (LDL) cholesterol-lowering therapy moderately increases the plasma level of HDL cholesterol^{2–5} and intensive statin therapy reduces the volume of coronary plaque in patients with acute coronary syndrome (ACS). These results were not predicted by any of the indices in the patients' baseline lipid profiles or by the LDL-cholesterol levels at follow-up, but they did correlate with the LDL-/HDL-cholesterol ratio at follow-up.⁶ HDL plays a major role in reverse cholesterol transport (RCT), and the RCT system has various potential antiatherogenic properties.^{1,2}

HDL Metabolism and RCT

HDL is isolated using ultracentrifugation at a density range

of 1.063–1.21 g/ml (HDL₂, 1.063–1.125 g/ml; HDL₃, 1.125–1.21 g/ml).⁷ However, HDL comprises a heterogeneous group of particles that differ in size, density, composition of lipids and apolipoproteins (Apo), and electrophoretic mobility. Gel electrophoresis separates HDL into a major subfraction that identically migrates as alpha HDL and other subfractions with mobilities similar to that of pre- β HDL. Alpha HDL is the most abundant HDL particle in human plasma, and pre- β HDL represents only 2–14% of the ApoA-I population.^{8,9}

HDL metabolism is associated with several HDL-related genes, and its synthesis involves a complex pathway. Although the underlying genetic defects in many patients with primary low-HDL cholesterolemia are not clearly understood, defects in 3 pivotal molecules are associated with low plasma HDL-cholesterol levels: ApoA-I, lecithin cholesterol acyltransferase, and ATP-binding cassette (ABC) A1.¹⁰ HDL metabolism involves at least 3 important steps. First, lipid-free or lipid-poor ApoA-I removes free cholesterol from peripheral cells via ABCA1 to form nascent HDL. Second, the nascent HDL transported by ABCA1 is lipidated to generate mature HDL. Third, the mature HDL interacts with other ApoB-containing lipoproteins, such as VLDL, IDL, and LDL. ABCA1 is a key molecule for generating HDL particles de novo, which is the

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initial step of RCT, because ABCA1 proteins transport cholesterol and phospholipids from the inner to the outer leaflet of the membrane. Lipid-poor ApoA-I subsequently removes these transported cholesterol and phospholipid molecules to form nascent HDL.¹¹

HDL-Targeted Therapy for Atherosclerosis

Inhibition of Cholesteryl Ester Transfer Protein (CETP)

CETP regulates the cholesterol level of the HDL particles and mediates the transfer of the cholesteryl esters of HDL to ApoB-containing lipoproteins, as well as those of triglycerides from triglyceride-enriched lipoproteins to HDL.¹² Patients with mutations in the gene encoding CETP produce high levels of HDL-cholesterol.¹³ Inhibitors of CETP activity, such as torcetrapib, dalcetrapib, anacetrapib, and evacetrapib, benefit subjects with normal or low levels of HDL-cholesterol by increasing its level.^{14,15} Furthermore, CETP deficiency markedly reduces the rate of ApoA-I turnover, accounting for the high HDL-cholesterol levels in humans with inherited hypercholesterolemia.^{16–18} Moreover, inhibition of CETP activity in subjects with low HDL cholesterol levels increases the cholesterol percentage in the HDL₂ particle compared with that in the HDL₃ particle and increases the cholesterol levels of large

HDL particles.¹⁵ Therefore, inhibiting CETP activity is a pharmacological approach to raising the HDL-cholesterol level and potentially reducing the risk of cardiovascular disease. Small-molecule CETP inhibitors such as torcetrapib,^{19–22} dalcetrapib,^{23–25} anacetrapib,^{26,27} and evacetrapib,^{28,29} significantly increase HDL-cholesterol levels in humans. However, this pharmacological approach does not seem to be beneficial for atherosclerotic disease. For example, the ILLUSTRATE study failed to show any slowing in atherosclerosis progression and determined a significant increase in the number of deaths from cardiovascular and noncardiovascular causes in the torcetrapib-treated group despite a 61% increase in HDL-cholesterol concentration and a 20% decrease in the level of LDL-cholesterol.¹⁹ Moreover, a multicenter, randomized, double-blind, placebo-controlled clinical trial, dal-OUTCOMES, was designed to test the hypothesis that dalcetrapib reduces cardiovascular morbidity and mortality in patients with recent ACS. However, dalcetrapib increased HDL-cholesterol levels, but did not reduce the risk of recurrent cardiovascular events. This trial was prematurely terminated because of a lack of benefit of dalcetrapib for patients with recent ACS.^{25,30}

Reconstituted HDL and ApoA-I Mimetics

HDL comprises heterogeneous particles with various densities

and sizes, and ApoA-I is a common major protein of the HDL particle. ApoA-I-deficient and LDL receptor-deficient mice exhibit significant progression of atherosclerosis, approximately 5-fold increase than in LDL receptor-deficient mice, and this result demonstrates that ApoA-I deficiency is associated with a loss of protection from atherosclerotic development in a similar model of familial hypercholesterolemia.³¹ In contrast, high levels of human ApoA-I lead to a significant increase in HDL-cholesterol levels and a decrease in the development of atherosclerotic plaque lesions in *ApoE* knock-out mice.^{32,33}

Numerous researchers are working to increase the quantity of cholesterol in the HDL particle and enhance the biochemical function of HDL as an approach to therapy.^{34,35} HDL-targeted therapies that inject full-length ApoA-I, reconstituted HDL (eg, the ApoA-I-phospholipid complex), or an ApoA-I mimetic peptide-lipid complex are remarkably effective (Figure 1). Reconstituted HDL must be disc-shaped and may be effective for treating patients with atherosclerosis.

ABCA1 plays a pivotal role in mediating phospholipid and cholesterol efflux of lipid-free ApoA-I as described earlier and is involved in the formation of the discoidal HDL precursor. Mature HDL particles, which are spherical, incorporate cellular cholesterol through sterol efflux via other ABC transporters such as ABCG1 and ABCG4.³⁶ Rye et al prepared discoidal reconstituted HDL, which is complexed with human serum-derived ApoA-I containing 1-palmitoyl-2-oleoylphosphatidylcholine (POPC).³⁷ The POPC/ApoA-I disc incorporates cholesterol derived from macrophages and is active in ABCA1-deficient Tangier disease patients (see later) as well as in normal subjects.³⁸

ETC-642 is a form of reconstituted HDL with a predicted single amphipathic helix of 22 amino acid residues that mediates the formation of complexes of HDL with phospholipids.³⁹ The characteristics of this reconstituted HDL are similar to those of the POPC/ApoA-I disc. The results of experiments using these reconstituted HDLs indicate that the ApoA-I-phospholipid complexes, but not the delipidated ApoA-I, mediate cholesterol efflux through ABCA1-dependent and nonspecific ABCA1-independent pathways. Thus, the main role of the ABCA1 transporter is to mediate the formation of the ApoA-I-phospholipid complex, and the artificial ApoA-I-phospholipid complex has the potential to incorporate cholesterol from cells via an ABCA1-independent pathway through, for example, the ABCG1 transporter. However, it is unclear whether ABCG1 and scavenger receptor class B member 1 mediate the efflux of cholesterol to reconstituted HDL independently or through the same pathway as the ApoA-I-phospholipid complex.

ApoA-I Milano

ApoA-I Milano is a mutant form of human ApoA-I that is generated by a point mutation that replaces an arginine with a cysteine residue at position 173. The levels of HDL-cholesterol and ApoA-I are markedly reduced in patients with this mutation.^{40,41} However, these patients are not at higher risk for cardiovascular disease. The higher levels of cellular cholesterol efflux mediated by ApoA-I Milano in cognate patients compared with subjects with ApoA-I are explained by the formation of ApoA-I Milano dimers linked by disulfide bonds between C173 residues.^{42,43}

Chiesa et al conducted intravascular ultrasound (IVUS) and magnetic resonance imaging studies of rabbits fed a high-cholesterol diet and found that ETC-216 [new code name: MDCO-216 (The Medicines Company)], an ApoA-I Milano-

Figure 2. Intravascular ultrasound (IVUS) analysis of the effects of coronary plaque regression of treatment using a statin and ETC-216 [new code name: MDCO-216 (The Medicines Company)]. Data from previous 3 clinical trials.^{45,47,48}

phospholipid complex (recombinant ApoA-I Milano complexed with POPC), significantly decreased the volume of carotid artery plaques.⁴⁴ Nissen et al found that 5 weekly intravenous administrations of ETC-216 to humans lead to a regression of coronary atherosclerotic plaques.⁴⁵ Furthermore, IVUS analysis revealed that after infusing ETC-216, regression of coronary atherosclerosis is accompanied by reverse remodeling of the external elastic membrane without changing luminal dimensions.⁴⁶ Nissen et al conducted randomized, controlled clinical trials (REVERSAL⁴⁷ and ASTEROID⁴⁸) and used IVUS to investigate the effects of statins on the regression of coronary plaques. Comparison of the results of the 3 trials demonstrates that subacute ETC-216 treatment reduces coronary atheroma volume to the same extent as 18–24 months of intensive statin therapy (Figure 2).

Fukuoka University APOA-I Mimetic Peptide (FAMP)

The most severe form of HDL deficiency is Tangier disease, which was first described by Fredrickson et al⁴⁹ and is caused by a mutation in *ABCA1*.^{50–52} *Abca1* knockout mice suffer from HDL deficiency and impaired RCT.^{53,54} In contrast, ABCA1 overexpression increases ApoA-I-mediated cholesterol efflux in *Abca1* transgenic mice.^{55,56} These findings indicate that *Abca1* is pivotal for regulating plasma HDL-cholesterol levels as well as cellular cholesterol homeostasis.

Although studies of the use of ApoA-I mimetic peptides, such as D-4F, L-4F (APL180), and L37pA, are underway,^{57–60} none of these agents are currently available for clinical use. Moreover, these peptides have a high affinity for lipids, so they appear to increase cholesterol efflux through a nonspecific, passive pathway. The recently described novel human ApoA-I-mimetic 24-mer peptide [FAMP, ~one-tenth of human ApoA-I in molecular weight], which does not form complexes with phospholipids,⁶¹ significantly enhances the biochemical function of HDL and reduces the formation of aortic plaques by 48.2% in *ApoE* knockout mice fed a high-fat diet.⁶² FAMP differs from other ApoA-I mimetics because it is designed to specifically interact with human ABCA1 without engaging the nonspecific, passive efflux pathway. Therefore, it functions similarly to human ApoA-I. Furthermore, FAMP markedly increases pre- β HDL as well as overall cholesterol efflux from peripheral tissues.

FAMP plays at least 2 possibly distinct roles in HDL metabolism as follows. First, FAMP enhances cellular cholesterol efflux that is mediated through ABCA1-dependent and partly ABCA1-independent mechanisms, generating nascent pre-β HDL particles. Second, FAMP incubation with human HDL or plasma generates pre-β-HDL-like small HDL particles and charged ApoA-I-rich particles that accelerate the generation of pre-β HDL, which is derived from mature HDL (Figure 3). Although 16-week treatment of *Apoe* knockout mice with FAMP did not significantly increase the level of HDL-cholesterol, the free cholesterol levels of the small-size HDL subfractions, reflecting pre-β₁ HDL particles, were significantly increased, and this should reflect enhanced *de novo* generation of HDL through cholesterol efflux from peripheral tissue in the treated mice. On the one hand, CETP inhibition greatly increasing HDL-cholesterol levels is attended to grow larger HDL particle size; on the other hand, the apoA-I mimetic peptide FAMP reduces the particle size without changing the HDL-cholesterol level; thus these phenomena seem to be precisely opposing.

Moreover, the biological function of HDL in FAMP-treated *Apoe* knockout mice is significantly increased, according to the results of an analysis of *ex vivo* HDL efflux capacity that is strongly predictive of coronary heart disease.⁶³ Furthermore, FAMP promotes ABCA1-dependent HDL efflux *ex vivo*, HDL

turnover *in vivo*, and RCT in the macrophages of transgenic mice that express human CETP, despite decreased plasma HDL-cholesterol levels.⁶⁴ These findings support the conclusion that the prospects are promising for successful treatment of atherosclerotic disease with FAMP and other mimetics.

HDL may play a significant role in the pathogenesis of diseases other than those of the cardiovascular system. For example, mice with complete HDL deficiency caused by targeted deletions of *Apoa1* and *Apoe* exhibit a phenotype characterized by deep alterations in skin structure, with a massive dermal accumulation of cholesterol clefts, foam cells, and T lymphocytes.⁶⁵ This phenotype resembles that of humans with inherited or secondary hyperlipidemic conditions referred to as xanthomas or xanthelasmas. Therefore, HDL-targeted therapy may be applicable to patients with xanthomas, as well as to those with dermatitis.

Besides its therapeutic potential, FAMP exhibits interesting properties that can be applied to the analysis of atherosclerotic plaque. ApoA-I or its mimetics must penetrate atherosclerotic plaques to remove cholesterol, and this characteristic may be exploited to detect activated arterial plaques. FAMP serves as a unique tracer for positron electron emission tomography (PET). When FAMP is functionalized using the chelator, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid and labeled with ⁶⁸Ga (⁶⁸Ga-DOTA-FAMP), it can be used to

specifically image atherosclerotic plaques. Thus, atherosclerotic plaques incorporate ^{68}Ga -DOTA-FAMP at a high rate, generating impressive PET images of an aortic plaque in vivo.⁶⁶ Uchida et al have also investigated plaque characteristics using color fluorescent microscopy and shown the fluorescent characteristics of HDL deposits with plaque formation, but not in the advanced stage of plaque in the human coronary arterial wall.⁶⁷

We believe that HDL-targeted therapy, including the use of FAMP, has tremendous atheroprotective potential and likely represents a new therapeutic tool for treating atherosclerotic cardiovascular disease. Although most research is focused on the therapeutic use of HDL, an ApoA-I mimetic peptide may contribute to the development of a technique to diagnose lipid-rich, unstable plaques.

Conclusions

LDL-lowering statin therapy is the standard treatment for cardiovascular diseases; however, it lacks benefit for preventing or mitigating adverse vascular events experienced by numerous patients. Therefore, the studies described here indicate that targeting HDL has very bright prospects for treating patients with atherosclerotic diseases. In particular, novel ApoA-I mimetics, such as FAMP, will likely enhance the pharmacological armamentarium available for treating these diseases.

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