Atherosclerotic Plaque Imaging for Evaluation of HDL Targeting Therapy

Yu Kataoka, Stephen J. Nicholls

Persistent risk of cardiovascular diseases despite using established medical therapies require additional therapeutic approach. Considerable attentions have focused on novel agents targeting high-density lipoprotein (HDL) because of its atheroprotective functions. HDL has the abilities to promote reverse cholesterol transport and modify inflammatory, oxidative, apoptotic, and endothelial pathways. Numerous basic and preclinical studies have demonstrated the beneficial effects of HDL targeting therapy on atherosclerosis. While recent clinical trials failed to prove favourable effect of HDL targeting therapies on cardiovascular outcomes, further search still continues to identify effective therapeutic approach targeting HDL due to its attractive anti-atherosclerotic properties. Recently, different types of agents modulating HDL have been developed and investigated or currently under clinical trial. Plaque imaging has been increasingly employed to evaluate the efficacy of these therapies because of its association with cardiovascular outcomes. This modality will further play an important role in the assessment of novel drugs modulating HDL in the future. The current article will review characteristics of HDL, its atheroprotective effects, HDL targeting therapies and its efficacy on atherosclerosis evaluated by plaque imaging modalities.

KEY WORDS: high-density lipoprotein, atherosclerosis, intravascular imaging, plaque progression

I. Introduction

Over the course of the last 2 decades, large randomized controlled trials have consistently demonstrated anti-atherosclerotic effect of lowering low-density lipoprotein (LDL) cholesterol with statin.\(^1,2\) As a result, LDL cholesterol lowering has become an integral component of therapeutic strategies in the prevention of cardiovascular diseases.\(^3,4\) However, despite using a statin, substantial amount of cardiovascular events is still observed.\(^5,6\) These observations indicate the need to develop new therapeutic strategies modulating additional therapeutic target associated with residual cardiovascular risks.

In the recent years, considerable attentions have focused on therapeutic approach targeting high-density lipoprotein (HDL) because of its biological properties which include promoting reverse cholesterol transport and modifying inflammatory, oxidative, apoptotic, and endothelial pathways.\(^7-9\) An inverse relationship between HDL cholesterol (HDL-C) level and cardiovascular outcomes in population studies supports this therapeutic concept.\(^10-12\) In addition, infusing HDL or transgenic expression of its major protein has been shown to favourably modify plaque size and composition in animal models.\(^13-17\) While recent clinical trials fail to prove the benefit of HDL-C raising therapy to cardiovascular outcomes,\(^18-21\) further search still continues to identify effective therapeutic approach targeting HDL.

Technological advances in plaque imaging modalities enable to directly visualize atherosclerotic plaques within vascular beds. Recently, plaque imaging has been increasingly employed to evaluate the efficacy of novel therapies on atherosclerotic lesions because several measures evaluated by imaging modalities have been reported to associate with cardiovascular outcomes.\(^22-24\) As a result, it is likely that atherosclerosis imaging will become an integral component of early clinical development of novel therapies including HDL modifying agent. This review will summarize characteristics of HDL, therapeutic approaches modulating HDL and its efficacy on atherosclerosis evaluated by plaque imaging modalities.

II. Structure of HDL

The HDL particles consist of an outer amphipathic layer of free cholesterol, phospholipid, and several apolipoproteins on the surface, with a triglyceride and cholesterol ester-rich hydrophobic core.\(^25,26\) The main protein component of HDL is apolipoprotein A-I (apoA-I). It plays a key role in the biogenesis and function of HDL. HDL particles also carry enzymes, such as paraoxonase (PON), platelet activating factor-acetylhydrolase (PAF-AH), lecithin cholesterol acyltransferase, and cholesteryl ester transfer protein (CETP). As HDL particles are being
constantly remodelled through transportation of cholesterol between cells and other lipoproteins, considerable heterogeneities exists in the relative content of apolipoproteins and lipids within HDL. Various isolation methods such as ultracentrifugation, electrophoresis, or nuclear magnetic resonance enable to characterize subtypes of HDL particles. Major subpopulations of HDL particles differ in shape (discoidal or spherical), density and size (HDL2b, 2a, 3a, 3b or 3c), apolipoprotein composition (only apoA-I or both apoA-I and -II) and electrophoretic mobility [pre-β (very small pre-β1, large pre-β2 and pre-β3), α1, 2, 3 or 4] (Fig. 1). It has become increasingly important to determine the clinical relevance of each individual HDL subfraction associated with atheroprotective property because recent study reported that atheroprotective ability of HDL varies according to its subclasses. It is important to assess the effects of therapeutic intervention in HDL on its structural heterogeneity.

III. Protective Properties of HDL toward Atherosclerosis

HDL has been demonstrated to exhibit a variety of atheroprotective properties (Table 1).

1) Reverse cholesterol transport

Promoting reverse cholesterol transport is the main anti-atherosclerotic effect of HDL. This includes cholesterol efflux from macrophages to HDL in the arterial wall with subsequent transfer of cholesterol ester to plasma or hepatic acceptor proteins. Cholesterol efflux can occur by several mechanism; unidirectional ATP-dependent pathway mediated by ATP-binding cassette transporter A1 (ABCA1), a unidirectional ATP-dependent pathway mediated by the ATP binding cassette G1 transporter (ABCG1), an ATP-independent, bidirectional pathway involving scavenger receptor class B type I (SR-B1), and receptor-independent passive diffusion according to cholesterol concentration gradient. The relative efficacy of different HDL subpopulations in promoting cholesterol efflux via these pathways depends on the receptors involved. Lipid-free/lipid-poor apoA-I as well as small pre-β1 HDL contribute to ABCA1-mediated cellular cholesterol efflux. As such, small HDL particles play a key role in cellular cholesterol efflux, consistent with the capacity of ABCA-1 to account for the greater part of cholesterol efflux from macrophages compared to other HDL receptors. Large HDL interacts with SR-B1 and ABCG1 and significantly promotes net cholesterol efflux. Similarly, large HDL2 has a greater capacity to stimulate cholesterol efflux via SR-B1 compared to small HDL3. Thus, these three pathways interacting with different size of HDL particles are involved in cholesterol efflux. In a recent study, cholesterol efflux capacity has been demonstrated to inversely associate with carotid intima-media thickness (β coefficient per 1-SD increase in efflux capacity; −0.03, 95% CI; −0.06 to −0.01, p=0.003) and the likelihood of angiographic coronary artery disease (odds ratio per 1-SD
increase, 0.75; 95% CI, 0.63 to 0.90; P=0.002), independently of the HDL-C level.36) This finding supports the important role of HDL-mediated cholesterol efflux in the development of atherosclerosis.

2) Anti-oxidant effect

Inhibiting oxidative modification of LDL is another important atheroprotective property of HDL.35, 37) ApoA-I and apoA-II are considered as antioxidant apolipoproteins to reduce cholesteryl ester hydroperoxides to cholesteryl ester hydroxides by a process that involves the concomitant oxidation of apoA-I.37, 38) Additionally, free apoA-I removes seeding molecules such as hydroperoxideicosa- and hydroperoxyeicosadienoic acids. Other HDL apolipoproteins such as apolipoprotein-E, -J, apoA-II and apoA-IV may also possess antioxidant activity through protection of LDL from free radical-induced oxidation.38) Another anti-oxidant mechanism of HDL is derived from its ability to accept phospholipid-containing hydroperoxides and other lipid peroxidation products from oxidized LDL.38) This process requires HDL-associated enzymatic components including PON and PAF-AH. PON is an HDL-associated calcium-dependent enzyme that catalyses the hydrolyses of lipid peroxides and prevents their accumulation in LDLs.39, 40) A recent prospective study has demonstrated that participants with the PON1 QQ192 genotype exhibited an increased risk of all-cause mortality (adjusted hazard ratio, 2.05; 95% confidence interval [CI], 1.32–3.18, P=0.001) and of major adverse cardiac events (adjusted hazard ratio, 1.48; 95% CI, 1.09–2.03; P=0.01).40) In addition, the incidence of major adverse cardiac events was significantly lower in participants in the highest PON1 activity quartile compared with those in the lowest activity quartile (7.3 vs 25.1%, p<0.001). PAF-AH, a calcium-independent serine esterase, protects against LDL oxidation by hydrolyzing oxidized phospholipids with short sn-2 acyl chains. Population study has shown the association of PAF-AH activity with the presence of angiographic coronary artery disease independent of established risk factors (odds ratio, 1.39, 95% CI, 1.26–1.54, p<0.001).42) Other HDL-mediated antioxidative effects include reduction of superoxide production, inactivation of neutrophil NADPH oxidase and removal of cytotoxic lipid hydroperoxides from the circulation via CETP.

3) Anti-inflammatory effect

HDL inhibits the expression of several cellular adhesion molecules, including intercellular adhesion molecule-1 and vascular cell adhesion molecule-1.43–46) HDL has been also demonstrated to inhibit the release of monocyte chemoattractant protein 1 and monocyte transmigration into endothelia cells.47, 48) Reconstituted HDL containing apoA-I and phosphatidylcholine decrease the adherence of lipopolysaccharide-stimulated polymorphonuclear neutrophils.49) These effects have been reported in animal studies showing the reduction of adhesion molecule expression, monocyte/macrophage infiltration within established atherosclerotic lesions following reconstituted HDL administration.50–52) Despite these properties, recent studies suggest the potential heterogeneity of HDL mediated anti-inflammatory activities including the ability to inhibit adhesion molecule expression. Small, dense, protein-rich HDL3 has the better capacity to inhibit VCAM-1 expression in endothelial cells compared to large, light, lipid-rich HDL2.53) This difference in anti-inflammatory effect of HDL2 and HDL3 can be due to their phospholipid composition.54) Thus, HDL favourably modulates inflammation associated with atherosclerosis although heterogeneity exists.

4) Vasodilatory Effect

HDL possesses the ability to maintain vascular endothelium function by stimulating nitric oxide (NO) release and production of prostacyclin from endothelial cells.55, 56) The interaction of HDL with SR-BI promotes NO production via activating the phosphatidylinositol-3-kinase/Akt signaling pathway and the phosphorylation of endothelial nitric oxide synthase (eNOS).57) Another pathway associated with eNOS activity and endothelium-dependent vasorelaxation is mediated by ABCG1 and

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Table 1  Atheroprotective Properties of HDL

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<th>Function</th>
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<td>Reverse cholesterol transport</td>
<td>Cholesterol efflux via ABCA1, ABCG1 and SR-BI</td>
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<td>Anti-oxidant effect</td>
<td>Inhibiting oxidative modification of LDL</td>
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<td>Anti-inflammatory effect</td>
<td>Inhibiting the expression of cellular adhesion molecules, monocyte chemoattractant protein 1 and monocyte transmigration into endothelia cells</td>
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<tr>
<td>Vasodilatory effect</td>
<td>• Stimulating nitric oxide release and production of prostacyclin from endothelial cells</td>
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<td></td>
<td>• Stimulating eNOS activity and endothelium-dependent vasorelaxation</td>
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<tr>
<td>Anti-thrombotic effect</td>
<td>• Promoting prostacyclin synthesis, leading to the inhibition of platelet aggregation</td>
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<td>• Inhibiting the initiation of thrombus formation</td>
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<td>• Downregulating endothelial cell tissue factor expression, platelet activating factor and thromboxane A2</td>
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<td>• Inhibiting the activation of coagulation factor Va</td>
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<td>• Upregulating endothelial cell thrombomodulin</td>
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involves cholesterol efflux by mature α-HDL. While both HDL2 and HDL3 stimulate secretion of prostacyclin by endothelial cells, HDL2 exerts more potent vasodilatory effect than HDL3.

5) Antithrombotic effects
HDL has been also reported to exert antithrombotic effect via a variety of mechanisms. The incubation of cultured endothelial cells with native HDL or delipidated HDL has been reported to induce an increase in prostacyclin production, leading to the inhibition of platelet aggregation. With regard to the effect of HDL on thrombus initiation, the phospholipid components of HDL inhibits the initiation of thrombus formation via downregulating E-selectin expression on endothelial cell surfaces. In addition, reconstituted HDL directly downregulates thrombin-induced endothelial cell tissue factor expression in vitro. HDL, particularly HDL2 or large HDL, plays an important role in inhibiting the activation of coagulation factor Va in vitro, which blunts thrombin generation and fibrin clot formation. Moreover, HDL infusions upregulates endothelial cell thrombomodulin, which is an additional anticoagulant factor. The administration of apoA-I Milano into rats inhibits platelet aggregation. In addition, downregulation of platelet activating factor and thromboxane A2 decrease platelet aggregation and attenuate leukocyte-endothelial cell interactions. Of note, HDL2 is more effective than HDL3 in promoting the antithrombotic effects of HDL. As such, the mechanisms responsible for the antithrombotic effects of HDL are likely to be multiple.

IV. Dysfunctional HDL and Atherosclerosis
While there are numerous studies showing atheroprotective effects of HDL, recent investigations suggest that high levels of HDL are not always protective toward atherosclerosis. Patients with cardiovascular disease have been reported to likely have ‘dysfunctional’ HDL, which lacks typical atheroprotective properties and promotes proinflammatory effects. This observation was also identified in patients with acute coronary syndrome (ACS), type 2 diabetes or inflammatory diseases. Recent evidences suggest the role of myeloperoxidase (MPO) to cause functional impairment of HDL. This concept has been supported by several studies, which elucidated HDL isolated from atherosclerotic lesions contain numerous MPO-derived peptides, including site-specific oxidative modifications by reactive chlorinating and nitrating species. Moreover, MPO selectively binds to apolipoprotein A-I within plasma via a specific region on helix 8 of the lipoprotein. Analysis of apolipoprotein A-I isolated from plasma of subjects with cardiovascular diseases versus that from healthy controls reveals that a greater content of nitrotyrosine and chlorotyrosine per apoA-I particle are observed within subjects with cardiovascular diseases. The degree of this modification was found to correlate with the frequency of coronary artery disease and cardiovascular diseases. Also, increasing levels of apoA-I oxidation has been reported to associate with a specific functional impairment in ABCA1-dependent cholesterol efflux from cholesterol-laden macrophages. These results suggest that MPO-catalyzed oxidation of apoA-I preferentially occurs in the arterial wall, potentially promoting atherosclerosis. Clinical studies also support the contribution of MPO to atherosclerosis and cardiovascular events. Population studies demonstrated the direct association of serum MPO level with mortality or cardiovascular events in patients with or without coronary artery disease. Imaging studies have reported that MPO level was associated with progression of carotid stenosis in patients with low HDL-C level and progression of coronary atherosclerosis in diabetic patients with coronary artery disease. These observations might suggest MPO and its mediated oxidation as an important therapeutic target for the prevention of cardiovascular events.

V. HDL targeting therapy and plaque progression
There are already several available agents to raise HDL-C level (Table 2). Also, novel therapies targeting HDL have been developed and tested or under investigation in clinical trials (Table 2).

1) Current therapeutic approach
a. Statin
Statins, a 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor, not only lowers LDL cholesterol level but also slightly increases HDL-C level by 5–10%. A pooled analysis of 1455 statin-treated patients enrolled in four clinical trials with intravascular ultrasound (IVUS) elucidated that increase in HDL-C level with statin therapy was significantly associated with slowing plaque progression (p=0.002). In particular, plaque regression was observed in patients who achieved both LDL cholesterol level <87.5 mg/dl and percentage increase in HDL-C >7.5% (change in percent atheroma volume, –0.4±3.4%). The SATURN (the Study of Coronary Atheroma by Intravascular Ultrasound: Effect of Rosuvastatin Versus Atorvastatin) study compared the efficacy of 80 mg rosuvastatin and 20 mg rosuvastatin on plaque progression in 1039 patients with coronary artery disease. Patients treated with rosuvastatin achieved a higher level of HDL-C (50.4±0.5 vs 48.6±0.5 mg/dl, p=0.01). On serial IVUS imaging, both therapeutic regimens induced plaque regression (median change in percent atheroma volume, –0.99% vs –1.22%, p=0.17). Of note, further analysis using virtual histology IVUS demonstrated that an increase in HDL-C under maximally intensive statin use induced plaque regression (p=0.001) and reduced necrotic core volume (p=0.03). As such, statin-mediated increase in HDL-C level has beneficial impacts on plaque
### Statin

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<td>Nicholls, et al. (81)</td>
<td>JAMA 2007; 297: 499–508</td>
<td>Statin use in four IVUS clinical trials (REVERSAL, CAMELOT, ACTIVATE, ASTEROID)</td>
<td>Serial IVUS imaging</td>
<td>Increase in HDL-C level was significantly associated with slowing plaque progression (beta coefficient, –0.04; 95% CI, –0.06 to –0.01; p=0.002). Plaque regression was observed in patients who achieved both LDL cholesterol level &lt;87.5 mg/dl and percentage increase in HDL-C &gt;7.5%.</td>
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<td>Nicholls, et al. (82)</td>
<td>N Engl J Med 2011; 365: 2078–2087</td>
<td>80 mg atorvastatin vs 20 mg rosuvastatin (SATURN study)</td>
<td>Serial IVUS imaging</td>
<td>Patients treated with rosuvastatin achieved a higher level of HDL-C (50.4±0.5 vs 48.6±0.5 mg/dl, p=0.01). Both therapeutic regimens induced plaque regression (median change in percent atheroma volume in atorvastatin and rosuvastatin, –0.99 vs –1.22%, p=0.17).</td>
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<tr>
<td>Puri, et al. (83)</td>
<td>Eur Heart J Imaging 2014; 15: 380–388</td>
<td>80 mg atorvastatin vs 20 mg rosuvastatin (sub-analysis of the SATURN study)</td>
<td>Serial VH-IVUS imaging</td>
<td>Increase in HDL-C under rosuvastatin/atorvastatin induced plaque regression (p=0.001) and reduced necrotic core volume (r=−0.27, p=0.03).</td>
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### Fibrates

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<td>Elkeles, et al. (84)</td>
<td>Diabetes Care 1998; 21: 641–648</td>
<td>Bezafibrate vs placebo (SENDCAP study)</td>
<td>Serial B-mode ultrasound of carotid and femoral arteries</td>
<td>Bezafibrate significantly increased HDL-C level (+6 vs –2%, p=0.02). There was no beneficial effect of bezafibrate on aorta and femoral atherosclerosis progression.</td>
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<td>Hiukka, et al. (68)</td>
<td>J Am Coll Cardiol 2008; 52: 2190–2197</td>
<td>Fenofibrate 200 mg vs placebo (from the FIELD trial)</td>
<td>Serial B-mode ultrasound of carotid intima-media thickness</td>
<td>Carotid intima-media thickness did not significantly change in diabetic patients treated with fenofibrate compared to placebo (+0.054 vs +0.069 mm/year, p=0.98).</td>
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<td>Davidson, et al. (69)</td>
<td>Arterioscler Thromb Vasc Biol 2014; 34: 1298–1306</td>
<td>Fenofibr acid vs placebo (FIRST trial)</td>
<td>Serial B-mode ultrasound of carotid intima-media thickness</td>
<td>No significant difference in carotid-intima media thickness between fibrate vs placebo (−0.006 vs ±0.00 mm/year, p=0.22).</td>
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### Niacin

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<td>Cashin-Hemphill, et al. (71)</td>
<td>JAMA 1990; 264: 3013–3017</td>
<td>Colestipol-niacin vs placebo (CLAS II study)</td>
<td>Coronary angiography</td>
<td>Colestipol-niacin therapy was associated with angiographic regression (18 vs 6%, p=0.04), fewer new lesions in native arteries (14 vs 40%, p=0.001) and bypass grafts (16 vs 38%, p=0.006).</td>
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<td>Taylor, et al. (72)</td>
<td>Circulation 2004; 110: 3512–3517</td>
<td>Extended-release niacin vs placebo adding to a statin (ARBITER2 study)</td>
<td>Serial B-mode ultrasound of carotid intima-media thickness</td>
<td>Mean CIMT increased significantly in the placebo group (0.04±0.100 mm; P&lt;0.001) and was unchanged in the niacin group (0.01±0.104 mm; p=0.23). Niacin significantly reduced CIMT progression in subjects without insulin resistance (p=0.02).</td>
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### Thiazolidinediones

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<td>Nissen, et al. (75)</td>
<td>JAMA 2008; 299: 1561–1573</td>
<td>Glimperide vs pioglitazone (PERISCOPE study)</td>
<td>Serial IVUS imaging</td>
<td>Pioglitazone administration was associated with an increase in HDL-C (+5.7 vs +0.9 mg/dl, p&lt;0.001). Patients treated with glimepiride exhibited plaque progression (change in percent atheroma volume = +0.73%), whereas pioglitazone was associated with decrease in percent atheroma volume (−0.16%, p=0.002 between groups).</td>
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<td>Mazzone, et al. (77)</td>
<td>JAMA 2006; 296: 2572–2581</td>
<td>Glimperide vs pioglitazone (CHICAGO study)</td>
<td>Serial B-mode ultrasound of carotid intima-media thickness</td>
<td>Progression of mean CIMT was less with pioglitazone vs glimepiride (−0.001 mm vs +0.012 mm, p=0.02). Pioglitazone also slowed progression of maximum CIMT compared with glimepiride (p=0.008).</td>
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### Cholesteryl ester transfer protein inhibitor

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<td>Bots, et al. (89)</td>
<td>Lancet 2007; 370: 153–160</td>
<td>Atorvastatin alone vs atorvastatin + torcetrapib (RADIANCE 2 study)</td>
<td>Serial B-mode ultrasound of carotid intima-media thickness</td>
<td>Torcetrapib was associated with 63.4% increase in HDL-C level. However, there was no significant difference in change in carotid-intima media thickness (+0.025±0.005 vs 0.030±0.005 mm, p=0.46). A significant increase in systolic blood pressure was observed in torcetrapib group (+6.6 vs +1.5 mmHg, p&lt;0.0001).</td>
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Fibrate mainly lowers triglyceride by 35 to 50%, but this does not slow disease progression and its vulnerability.

### b. Fibrate

Fibrate mainly lowers triglyceride by 35 to 50%, but this agent also increases HDL-C level by about 10%. The anti-atherosclerotic efficacies of fibrates have been investigated in several clinical trials. In the SENDCAP (St. Mary’s Ealing, Northwick Park Diabetes Cardiovascular Disease Prevention) study, there was no beneficial effect of bezafibrate on aorta and femoral thickness in mixed dyslipidaemia, REVERSAL: Reversal of Atherosclerosis with Aggressive Lipid Lowering, SATURN: Study of Coronary Regression of Intravascular Sonographic Coronary Obstruction Prospective Evaluation, RADIANCE: Torcetrapib and carotid intima-media Inhibition and HDL Elevation, IVUS: intravascular ultrasound, HDL-C: high-density lipoprotein cholesterol, PERISCOPE: Pioglitazone Effect on Therapy, ILLUSTRATE: Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis by CETP Choline Fenofibrate on Carotid Intima-Media Thickness in Subjects with Type IIb Dyslipidemia with Residual Risk in Addition to Atorvastatin Cholesterol-Lowering Atherosclerosis Studies, FIELD: Fenofibrate Intervention and Event Lowering in Diabetes, FIRST: the Evaluation of Thrombosis, CIMT: carotid intima-media thickness, CHICAGO: Carotid Intima-Media Thickness in Atherosclerosis Using Pioglitazone, CLAS: Intravascular Ultrasound-Derived Coronary Atheroma Burden, CAMELOT: Comparison of Amlodipine Versus Enalapril to Limit Occurrences of Intravascular Ultrasound for Coronary Atheroma Regression Evaluation, ASTEROID: A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound-Derived Coronary Atheroma Burden, Nissen, et al. 2007; 356: 1304–1316. Atorvastatin alone vs atorvastatin + torcetrapib (ILLUSTRATE study). Serial IVUS imaging Torcetrapib increased HDL-C by 61% with LDL-C reduction by 20%. However, this therapy did not slow disease progression (change in percent atheroma volume, +0.19 vs +0.12%, p=0.72). Torcetrapib was also associated with an increase in systolic blood pressure of 4.6 mmHg.

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<td>Nissen, et al. (90)</td>
<td>2007</td>
<td>Atorvastatin alone vs atorvastatin + torcetrapib (ILLUSTRATE study)</td>
<td>Serial IVUS imaging</td>
<td>Torcetrapib increased HDL-C by 61% with LDL-C reduction by 20%. However, this therapy did not slow disease progression (change in percent atheroma volume, +0.19 vs +0.12%, p=0.72). Torcetrapib was also associated with an increase in systolic blood pressure of 4.6 mmHg.</td>
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<td>Nicholls, et al. (91)</td>
<td>2008</td>
<td>Sub-analysis of ILLUSTRATE study</td>
<td>Serial IVUS imaging</td>
<td>The greatest increase in HDL-C with torcetrapib showed significant plaque regression (change in percent atheroma volume, –0.69% p=0.01 compared to baseline).</td>
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<td>Fayad, et al. (93)</td>
<td>2011</td>
<td>Dalceptrapib vs placebo (dal-PLAQUE study)</td>
<td>Serial MRI imaging of aorta and PET/CT assessment</td>
<td>Dalceptrapib increased HDL-C by 31%. However, there was no significant change in vessel wall area of aorta between dalceptrapib and placebo (least square mean; 0.49 vs 2.69 mm², p=0.12). Carotid artery analysis showed a 7% reduction in most-diseased-segment TBR in the dalceptrapib group compared with the placebo group (–7.3 [90% CI –15.5 to –0.8]; nominal p=0.07).</td>
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<td>Nissen, et al. (101)</td>
<td>2003</td>
<td>Placebo vs ETC-216 15 mg/kg vs 45 mg/kg</td>
<td>Serial IVUS imaging</td>
<td>The percent atheroma volume decreased by –1.06% in the combined ETC-216 group (p=0.02 compared with baseline). In the placebo group, percent atheroma volume increased by 0.14% (p=0.97 compared with baseline).</td>
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<td>Tardif, et al. (102)</td>
<td>2007</td>
<td>Placebo vs CSL-111 40 mg/kg vs 80mg/kg (ERASE trial)</td>
<td>Serial IVUS imaging</td>
<td>There was no significant difference between combined group of HDL infusion vs placebo (change in percent atheroma volume, –3.4 vs –1.6%, p=0.48, change in total atheroma volume, –5.3 vs –2.3 mm³, p=0.39). Improvements in the plaque characterization index (–0.0067 vs +0.0128 mm³, p=0.01) and coronary score (–0.039 vs –0.071 mm, p=0.03) were observed.</td>
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<td>Waksman, et al. (103)</td>
<td>2010</td>
<td>Control vs HDL selective delipidated plasma apheresis</td>
<td>Serial IVUS imaging</td>
<td>There was a favourable trend toward regression of total atheroma volume (–12.18±36.75 mm³) in infusion of delipidated HDL group compared to placebo (+2.8±21.25 mm³, p=0.26 between the groups) in a total of 28 patients.</td>
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<td>Tardif, et al. (104)</td>
<td>2014</td>
<td>Placebo vs 3 mg/kg vs 6 mg/kg vs 12 mg/kg CER-001 (CHI-SQUARE trial)</td>
<td>Serial IVUS imaging</td>
<td>This study did not find any significant differences with regard to plaque progression in placebo or 3, 6 or 12 mg/kg CER-001 group.</td>
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<tr>
<td>Nicholls, et al. (107)</td>
<td>2013</td>
<td>Placebo vs RVX-208 (ASSURE trial)</td>
<td>Serial IVUS imaging</td>
<td>Both RVX-208 and placebo increased apoA-I (12.8 vs 10.6%) and HDL-C levels (10.9 vs 7.7%), and there were no significant differences between the groups (p=0.18 and 0.32, respectively). Change in percent atheroma volume (–0.40 vs –0.30%, p=0.81) and total atheroma volume (–4.2 vs –3.8 mm³, p=0.86) was comparable between the groups.</td>
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atherosclerosis progression in 164 type 2 diabetic patients despite a significant increase in HDL-C level (+6 vs −2%, p=0.02). 6) In another study analyzing a subset from the FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) trial, carotid intima-media thickness did not significantly change in 170 diabetic patients treated with fenofibrate compared to placebo (+0.054 vs +0.069 mm/year, p=0.98). 8) Also, a recent clinical trial (FIRST: the Evaluation of Choline Fenofibrate on Carotid Intima-Media Thickness in Subjects with Type Iib Dyslipidemia with Residual Risk in Addition to Atorvastatin Therapy) did not demonstrate the benefit of adding a fibrate to a statin on carotid intima-media thickness in 682 patients with mixed dyslipidemia (fibrate-atorvastatin vs atorvastatin alone; −0.006 vs ±0.00 mm/year, p=0.22). 6

c. Niacin

Niacin is presently the most effective commercially available agent for raising HDL-C by as much as 35%. This drug also changes the quality of HDL particles as it leads to retention of cholesterol ester in HDL, an increase in HDL particle size, and a rise in apoA-I levels. 7) The beneficial effect of niacin on cardiovascular risk was reported in previous clinical studies. The CLAS (Cholesterol-Lowering Atherosclerosis Studies I and II) showed angiographic regression (18 vs 6%, p=0.04) of coronary artery stenosis on high-dose niacin with colestipol. 8 Similar observations were observed in ARBITER2 (Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol 2) study, which demonstrated that placebo group showed progression of carotid-intima media thickness (+0.044±0.100 mm, p=0.001 vs baseline), whereas this measure did not change in the niacin group (+0.014±0.104 mm, p=0.23 vs baseline) in individuals with known coronary heart disease and moderately low HDL-C level. 9 However, recent large-scale clinical trials did not show any benefit of niacin to cardiovascular outcomes. The AIM-HIGH (Atherothrombosis Intervention in Metabolic Syndrome with low HDL cholesterol/High triglyceride and Impact on Global Health Outcomes) study was stopped early after a mean follow-up of 3 years when niacin showed no signs of additional effects over placebo in patients already at target levels of LDL-C (cardiovascular events; 16.4 vs 16.2%, p=0.79). 10 The HPS2-THRIVE (Heart Protection Study 2-Treatment of HDL to Reduce the Incidence of Vascular Events) also failed to prove the benefit of extended-release niacin to the risk of major cardiovascular events in 25673 patients with atherosclerotic vascular disease (13.2 vs 13.7%, p=0.29). 11

d. Thiazolidinediones

Thiazolidinediones are agonists for PPARα and PPARγ regulating both glucose and lipid metabolism. 12 Pioglitazone is PPARγ agonists, which were found to increase both HDL-C and the average size of HDL particles in patients with type 2 diabetes mellitus and dyslipidemia. 13 The PERISCOPE (Pioglitazone Effect on Regression of Intravascular Sonographic Coronary Obstruction Prospective Evaluation) study compared progression of coronary atherosclerosis in patients with type 2 diabetes mellitus and coronary artery disease receiving either glimepiride or pioglitazone. 14 Pioglitazone significantly increased HDL-C (+5.7 vs +0.9 mg/dl, p<0.001) and decreased both triglyceride (–16.3 vs +3.3 mg/dl, p<0.001) and c-reactive protein (–1.0 vs –0.4 mg/dl, p<0.001) levels with favourable glycated hemoglobin control (–0.55 vs –0.36%, p=0.03). On serial evaluation of atheroma burden, pioglitazone was associated with decrease in percent atheroma volume compared to glimepiride (change in percent atheroma volume, +0.73% vs −0.16%, p=0.002). Subsequent analysis revealed that the strongest independent predictor of the benefit of pioglitazone in slowing disease progression was the ratio of triglyceride to HDL-C (p=0.03). 15 The CHICAGO (Carotid Intima-Media Thickness in Atherosclerosis Using Pioglitazone) trial also demonstrated that pioglitazone was associated with less progression of mean (–0.001 vs +0.012 mm, p=0.02) and maximum carotid intima-media thickness (+0.002 vs +0.026 mm, p=0.008) compared to glimepiride. 16 These findings highlight the importance of the atherogenic dyslipidemia phenotype in patients with diabetes and the potential benefit from therapies raising HDL-C levels.

2) Novel therapeutic approach

a. Cholesterol ester transfer protein inhibitor

CETP is a hydrophobic glycoprotein that is synthesized in several tissues but mainly in the liver. It promotes the transfer of esterified cholesterol from HDL to very low-density lipoprotein and LDL particles, in exchange for triglycerides. 17 Previous observational study reported that individuals in fishing villages in Japan with CETP mutations have elevated HDL-C levels and decreased coronary heart disease. 18, 19 Also, animal studies have shown an elevated HDL-C levels and less atherosclorotic development in rodents without plasma CETP activity. 20 Subsequent population studies demonstrated that low CETP levels were associated with relative cardioprotection in some cohorts. 21, 22 These observations stimulated considerable attentions toward development of CETP inhibitors which would represent an attractive therapeutic strategy potentially to reduce cardiovascular events. To date, a couple of CETP inhibitors has been developed and investigated in clinical trials already. Another two novel CETP inhibitors are under the investigation on clinical trials.

Torcetrapib was the first CETP inhibitor to increases in HDL-C, by 50% to 100%, in addition to lowering of LDL cholesterol, by up to 20%. 23 The clinical efficacy of this agent was evaluated in the ILLUMINATE (Investigation of Lipid Level Management To Understand its Impact in Atherosclerotic Events) study, which compared cardiovascular events in 15,067 patients at high
cardiovascular risk treated with either placebo or torcetrapib.\(^{18}\) This study was stopped early due to a 58% increase in mortality and 25% increase in cardiovascular events in torcetrapib-treated patients despite an increase in HDL-C by 72.1% and a decrease in LDL cholesterol by 24.9% with torcetrapib. The RADIANCE 2 study investigated the effect of torcetrapib on carotid-intima-media thickness in mixed dyslipidaemia.\(^{89}\) While torcetrapib increased HDL-C levels by 63.4%, there was no significant difference in carotid-intima media thickness (+0.025±0.005 vs 0.030±0.005 mm, p=0.46). In addition, a significant increase in systolic blood pressure was observed in torcetrapib group (+6.6 vs +1.5 mmHg, p=0.0001). The ILLUS-TRATE study investigated the effect of torcetrapib on progression of coronary atherosclerosis using serial IVUS imaging in 1188 patients with CAD who already treated with atorvastatin.\(^{90}\) Despite a 61% increase in HDL-C and 20% decrease in LDL cholesterol, torcetrapib did not slow disease progression (change in percent atheroma volume, +0.19 vs +0.12%, p=0.72). Torcetrapib was also associated with an increase in systolic blood pressure of 4.6 mmHg.

Considerable debate subsequently focused on a deleterious effect of torcetrapib on HDL function and reverse cholesterol transport. However, further investigation revealed that the greatest increase in HDL-C with torcetrapib showed significant plaque regression (change in percent atheroma volume, −0.69% vs 0.01 compared to baseline).\(^{91}\) This finding further supported the concept of intact HDL functionality in patients treated with torcetrapib. Because of off-target effects on blood pressure and renin-angiotensin-aldosterone system, this drug has been stopped to develop.

Dalcetrapib is a less potent CETP inhibitor, which raises HDL-C levels by 25% to 35% but does not affect LDL cholesterol levels.\(^{92}\) The dal-PLAQUE study investigated the efficacy of dalcetrapib on atherosclerotic plaques and arterial inflammation in 130 patients.\(^{93}\) Despite 31% increase in HDL-C level following dalcetrapib use, there was no significant change in vessel wall area of aorta between dalcetrapib and placebo (least square mean; 0.49 vs 2.69 mm\(^2\), p=0.12). Dal-OUTCOMES study evaluated cardiovascular outcomes in 15871 patients who had had a recent acute coronary syndrome receiving dalcetrapib or placebo.\(^{94}\) Although HDL-C levels increased by 31 to 40% in the dalcetrapib group, this study was stopped early because dalcetrapib did not reduce the primary end point compared to placebo (cumulative event rate; 8.3% vs 8.0%, p=0.52). In both trials, dalcetrapib therapy for 24 months did not have any adverse effect on blood pressure.

Anacetrapib is a more potent CETP inhibitor, with the ability to raise HDL-C by 138.1% and lower LDL cholesterol by 39.8%.\(^{95}\) A large phase 2 safety study demonstrated that anacetrapib increased HDL-C levels by 138.1% and reduced LDL cholesterol levels by 39.8% without any adverse effects on blood pressure and mineralocorticoid activity in 1,623 patients with coronary heart disease or at high risk for coronary heart disease.\(^{95}\) The impact of anacetrapib on cardiovascular outcomes is currently being evaluated by REVEAL (Randomized Evaluation of the Effects of Anacetrapib through Lipid-modification) study.

Evacetrapib is the most recent CETP inhibitor which is novel benzazepine compound with selective and potent CETP inhibitory activity.\(^{96}\) In a phase 2 study, the lipid efficacy, safety, and tolerability of evacetrapib were investigated over a 12-week treatment period in 398 patients with elevated LDL cholesterol or low HDL-C levels.\(^{97}\) A dose-dependent increase in HDL-C (from 53.6% to 128.8%) and decrease in LDL cholesterol (13.6% to 35.9%) were observed in patients receiving evacetrapib. A significant reduction in triglyceride levels was also observed with administration of evacetrapib 500 mg daily. In addition, there was no adverse effect of evacetrapib on blood pressure or mineralocorticoid activity. Currently, ACCELERATE (Assessment of Clinical Effects of Cholesteryl Ester Transfer Protein Inhibition With Evacetrapib in Patients at a High-Risk for Vascular Outcomes) study is conducted to evaluate the efficacy of evacetrapib on cardiovascular outcomes in 12000 patients with atherosclerotic vascular disease.

b. HDL infusion

HDL infusion agents encompass isolated, partially delipidated HDL proteins and native apoA-I or genetic variants such as apolipoprotein-A-I Milano, complexed with phospholipids.\(^{98}\) This therapeutic approach enables to induce a rapid, dose-proportional, and time-dependent elevation in apoAI and preβ-HDL particles. The favourable benefits of HDL infusion to atherosclerosis have been demonstrated in animal studies. In rabbit atherosclerosis models, administration of HDL infusion has been shown to reduce atheroma size by 36% (p<0.05) and modify its composition including smooth muscle cell and macrophages.\(^{15}\) Previous studies demonstrated that lipid-depleted forms of HDL effectively promote cholesterol efflux.\(^{99}\) In human, infusion of either HDL apolipoprotein or apolipoprotein-phospholipid complexes enhance fecal sterol excretion and improve endothelial function in patients with low HDL-C level or hypercholesterolemia.\(^{100}\) These attractive effects on atherosclerosis have been investigated in a couple of small study by IVUS imaging. Nissen, et al. demonstrated significant reduction in atheroma burden in 57 ACS patients receiving intravenous infusions of reconstituted HDL containing recombinant human apoA-I Milano for 5 weeks (change in percent atheroma volume, −1.29 and −0.73% in 15 and 45 mg/kg infusional HDL, respectively).\(^{101}\) The ERASE (Evaluation the
Effects of Reconstituted High-density Lipoprotein) trial similarly evaluated the impact of infusing HDL containing wild-type apoA-I for 4 weeks in ACS patients. Although disease regression was observed in patients receiving reconstituted HDL infusion, there was no significant difference in this IVUS measure between reconstituted HDL infusion and placebo (change in percent atheroma volume, -3.4 vs -1.6%, p=0.48, change in total atheroma volume, -5.3 vs -2.3 mm³, p=0.39). However, improvements in the plaque characterization index (-0.0067 vs +0.0128 mm, p=0.01) and coronary score (-0.039 vs -0.071 mm, p=0.03) on quantitative coronary angiography were observed, suggesting potential plaque stabilization effect of HDL infusion. Waksman, et al. evaluated the effect of autologous infusion of delipidated HDL on coronary atherosclerosis in 28 ACS patients. 2-D gel electrophoresis delipidated plasmas successfully converted from αHDL to preβ-like HDL. In addition, serial IVUS imaging demonstrated a favourable trend toward regression of total atheroma volume (~12.18±36.75 mm³) in patients receiving infusion of delipidated HDL compared to placebo (+2.8±21.25 mm³, p=0.26 between the groups). The CHISQUARE (Can Hdl Infusions Significantly Quicken Atherosclerosis Regression) study was designed to evaluate the efficacy of infusion of preβ HDL mimetic agent, CER-001 on atherosclerotic plaques in patients with ACS. Although this study did not find any significant differences with regard to plaque progression in placebo or 3, 6 or 12 mg/kg CER-001 group, a trend toward disease regression at the lowest dose of CER-001 (3 mg/kg) might indicate the potential benefit of this therapy to atherosclerosis.

c. ApoA-I induction therapy

Enhancing apoA-I synthesis is another attractive strategy because it has the ability to generate new HDL particles, which act as the vehicle for promotion of reverse cholesterol transport (RCT). RVX-208 is the first oral agent to reach clinical development that selectively induces hepatic synthesis of apoA-I. Mechanistic studies demonstrated that RVX-208 enhances apoA-I transcription in hepatic cell lines and does not appear to modulate PPAR, liver X receptor (LXR), or CETP mediated pathways (Johansson J, unpublished data, September 2010). The safety and efficacy of RVX-208 (50–150 mg bid) was investigated in the ASSERT (ApoA-I Synthesis Stimulation Evaluation in Patients Requiring Treatment for Coronary Artery Disease) study in 299 statin-treated patients with established stable coronary artery disease. RVX-208 was associated with a modest increase in apoA-I levels by up to 5.6%. This agent also increased HDL-C by up to 8.3% and large HDL particles by up to 21.1%. With regard to its safety, a dose-dependent increase in hepatic transaminase levels, peaking at 8 weeks, with no associated elevation in bilirubin, was observed. Following the observation about the enhanced synthesis of apoA-I in ASSERT study, the ASSURE (ApoA-I Synthesis Stimulation and Intravascular ultrasound for Coronary Atheroma Regression Evaluation) study was conducted to investigate the impact of RVX-208 on plaque progression in 310 patients with ACS. Unexpectedly, both RVX-208 and placebo increased apoA-I (12.8 vs 10.6%) and HDL-C levels (10.9 vs 7.7%), and there were no significant differences between the groups (p=0.18 and 0.32, respectively). In addition, change in percent atheroma volume (-0.40 vs -0.30%, p=0.81) and total atheroma volume (-4.2 vs -3.8 mm³, p=0.86) was comparable between the groups.

d. Other on-going development of agents targeting HDL

Several strategies to therapeutically target the metabolism, particle structure and function of HDL are emerging. ApoA-I mimetic peptides are similar to apoA-I with regard to structure and biological activities. It enhances nascent HDL formation, improves cholesterol efflux, antioxidant and anti-inflammatory activities, and favourably modify atherosclerosis in animal models. LXR agonists have been shown to upregulate ABCA1 and ABCG expression, and attenuate atherosclerosis in mice. FXR agonists have the ability to accelerate cholesterol excretion via HDL although it has adverse effects on bile acid metabolism. Endothelial lipase inhibitors are derivatives of sulphonylurea and boron acid to inhibit HDL lipid hydrolysis. MicroRNA antagonists can raise HDL-C level, increase cholesterol efflux from macrophages and exert anti-atherosclerotic actions. Antisense oligonucleotide targeting CETP has been reported to promote macrophage reverse cholesterol transport, increase HDL-C level and reduce cholesterol accumulation within arterial wall of CETP-transgenic mice. Antisense oligonucleotide targeting apolipoproteinC-III has the ability to raise HDL-C level and reduce triglyceride level via stimulating lipolysis of triglyceride-rich lipoproteins. While the potential benefits of these agents are expected, its availability, efficacy and safety require further investigation.

VI. Conclusion

Targeting the biological properties of HDL has emerged as a potential adjunctive therapeutic approach to further reduce cardiovascular risks. With recent failure of HDL targeting therapy and increasing insights into the importance of HDL functionality, novel therapeutic approaches targeting HDL are currently under investigation for their potential impact on cardiovascular risk. Arterial wall imaging has contributed to the better understanding of HDL targeting therapy with regard to its effect on atheroma progression. As a result, it is likely that atherosclerosis imaging will play an important role in the assessment of early clinical development of novel therapies that enhance the functionality of HDL.
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