Exploiting the Vascular Protective Effects of High-Density Lipoprotein and its Apolipoproteins

An Idea Whose Time for Testing Is Coming, Part II

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Despite the benefits of currently available preventative and therapeutic interventions, atherosclerotic vascular disease continues to be a major cause of morbidity and mortality in much of the Western world. This emphasizes the need for additional preventive and therapeutic interventions exploiting new targets to complement and augment the results of LDL lowering and other current strategies. One such potential target is HDL and its apolipoproteins. A large body of experimental evidence suggests that augmenting the levels and/or function of HDL and its apolipoproteins can have major vascular protective effects ranging from prevention to stabilization and regression, independent of total or non-HDL cholesterol levels. Therefore, we think that the time is ripe for the development and clinical testing of this new frontier in antiatherogenic strategy. In the present article, we will review the structure/function of HDL and explore means of enhancing the levels and/or function of HDL and its apolipoproteins for vascular protection.

Structural and Functional Heterogeneity of HDL Particles

HDL cholesterol particles consist of an outer amphipathic layer of free cholesterol, phospholipid, and several apolipoproteins (apolipoprotein A-I [the most abundant] and apolipoproteins AII, C, E, AIV, J [clusterin], and D) on the surface, with a triglyceride and cholesterol ester–rich hydrophobic core. Apolipoprotein A-I constitutes ~70%; and apolipoprotein A-II makes up ~20% of the protein content of HDL. Apolipoprotein A-I is synthesized in the liver and intestines in man and mice (and mostly in the intestines in rabbits), whereas apolipoprotein A-II is synthesized mostly in the liver. The gene for apolipoprotein A-I is located on chromosome 11 adjacent to the gene cluster for apolipoproteins CIII and A-IV. Apolipoprotein A-I is synthesized as a 260-amino acid prepropeptide, which is cleaved to a 249-amino acid prepeptide, which in turn is cleaved to the 243-amino acid, mature apolipoprotein A-I after secretion out of the cell.

Apolipoprotein A-I is the principal protein of HDL; it contains a globular N-terminal domain (residues 1 to 43) and a lipid-binding C-terminal domain (residues 44 to 243). Human apolipoprotein A-I possesses multiple tandem repeating 22-mer amphipathic α-helixes. Computer analysis and studies of model synthetic peptides and recombinant protein-lipid complexes of phospholipids have suggested that apolipoprotein A-I interacts with HDL surface lipids through cooperation among its individual amphipathic helical domains. In mature human apolipoprotein A-I, the amino acid residues 1 to 43 are encoded by exon 3, whereas residues 44 to 243 are encoded by exon 4 of the apolipoprotein A-I gene. The region encoded by exon 4 of the apolipoprotein A-I gene contains 10 tandem amphipathic α-helixes; their location and the class to which they belong are as follows: helix 1 (44 to 65, class A1), helix 2 (66 to 87, class A1), helix 3 (88 to 98, class Y), helix 4 (99 to 120, class Y), helix 5 (121 to 142, class A1), helix 6 (143 to 164, class A1), helix 7 (165 to 186, class A1), helix 8 (187 to 208, class A1), helix 9 (209 to 219, class Y), and helix 10 (220 to 241, class Y). 1

The antiatherogenic effects of HDL are thought to be largely due to apolipoprotein A-I; however, apolipoprotein AIV and apolipoprotein E may also have antiatherogenic effects. 2–9 HDL particles also carry other molecules, such as paraoxonase, platelet activating factor-acetylhydrolase, lecithin cholesterol acyltransferase (LCAT), and cholesterol ester transfer protein (CETP). HDL particles demonstrate α-mobility and have been characterized into several subtypes on the basis of density ultracentrifugation; these subtypes include HDL-2 (density, 1.063 to 1.125 g/mL; particle size, 11 to 12 nm) and HDL-3 (density, 1.125 to 1.21 g/mL; particle size, 9 to 10 nm). The differences in particle size results mainly from the number of apolipoprotein molecules and the volume of the cholesterol ester in the core of the particle. The immunosaffinity technique has shown that some of the HDL particles carry only apolipoprotein A-I (LPA-I), whereas others carry both apolipoprotein A-I and A-II (LPA-I-A-II). On agarose gel electrophoresis, HDL separates as an α-particle, although a small fraction of HDL, pre-β-HDL, has been identified by Fielding and Fielding. 10 This particle may play a key role in the early steps of reverse cholesterol transport.

It has been shown that the antioxidant enzyme paraoxonase is carried only by apolipoprotein A-I and apolipoprop...
tein J–containing HDL subspecies. The cholesterol efflux–promoting capacity is generally attributed to sub-

Species containing apolipoprotein A-I. Several naturally

occurring mutants of apolipoprotein A-I have been described; these mutants are generally associated with reduced HDL cholesterol and apolipoprotein A-I levels, most often related to accelerated catabolism rather than reduced synthesis of apolipoprotein A-I. Although some of these hypoalphalipoproteinemic states are associated with an increased risk of atherosclerotic vascular disease, others do not seem to predispose to accelerated or premature vascular disease. In fact, there is evidence to suggest that one such mutant, apolipoprotein A-I_{Milano}, which is inherited as an autosomal-dominant trait among a small number of inhabitants of Limone Sul Garda in Northern Italy, may actually be associated with longevity and protection from vascular disease.

Apolipoprotein A-I_{Milano} is the result of a point mutation, with an arginine to cysteine substitution at position 173. The carriers of this mutation are all heterozygotes who exhibit hypertriglyce-

eridemia with markedly reduced HDL and apolipoprotein A-I levels, and mutant apolipoprotein A-I constitutes 80% of the total apolipoprotein A-I content. The presence of a cysteine residue at position 173 results in the formation of homodimers with wild-type apolipoprotein A-I and heterodimers with apoli-

ipoprotein A-II. The absence of vascular disease in the carriers of apolipoprotein A-I_{Milano} has been attributed to the increased efficiency of the mutant in promoting reverse cholesterol transport. This conjecture is supported by in vitro data from our laboratory and by data reported by Franceschini et al. However, opposite results have been reported by Bielicki et al using a different in vitro model system.

**Strategies for Increasing Circulating and/or Arterial Wall HDL/Apolipoprotein A-I Levels**

Circulating HDL and apolipoprotein A-I levels can be increased directly by increasing the synthesis of apolipoprotein A-I and/or by inhibiting the clearance of apolipoprotein A-I. Circulating HDL levels tend to increase with regular dynamic exercise, modest alcohol consumption, weight loss, a high fat diet, and smoking cessation, whereas they tend to decrease with obesity, menopause, high carbohydrate diets, and smoking. Pharmacological agents that increase HDL include hydroxymethylglutaryl coenzyme A reductase inhibitors (statins), niacin, fibric acid derivatives, Dilantin (phenytoin), chromium picolinate, and female hormone replacement therapy. However, the magnitude of HDL elevation is variable, ranging from small increases with statins to larger increases with niacin and fibrates. Among statins, there seem to be differences in the magnitude of the HDL increases observed. Thus, high-dose simvastatin produces a relatively greater increase in HDL than does high-dose atorvastatin, despite significant and comparable reductions in LDL cholesterol. These findings suggest that all statins may not be similar in terms of their pleiotropic effects other than LDL-lowering. Statin-induced inhibition of the Rho-signaling pathway activates peroxisomal proliferator activated receptor-α (PPAR-α), thereby inducing the transcription of the apo-

oprotein A-I gene, which thus provides a molecular basis for the increase in HDL by statins.

Given the structural heterogeneity of HDL with respect to its apolipoprotein composition and consequent functional hetero-

geneity, it is unclear which one of these interventions and to what extent they increase the functionally protective species of HDL particles (apolipoprotein A-I only containing particles [LPA-I] and apolipoprotein E containing particles). Other approaches to increasing HDL include use of new drugs/antibodies to inhibit CETP. The relationship between CETP and atherosclerosis is complex, and both proatherogenic and antiatherogenic effects have been suggested. CETP may be atherogenic because of its ability to transfer cholesterol ester from HDL to LDL/VLDL, thus lowering plasma levels of HDL while increasing the levels of LDL/VLDL. Conversely, CETP may also be antiatherogenic by facilitating the production of lipid-poor pre-β-HDL particles, which are efficient stimulators of reverse cholesterol transport. Both experimental and human epidemiological studies have provided conflicting data. A vaccine made from a peptide containing a region of CETP known to be necessary for neutral lipid transfer has been shown to stimulate antibodies against CETP, leading to an inhibition of CETP, an increase in HDL cholesterol, and a reduction of fatty streaks in cholesterol-fed rabbits, thus providing a novel mechanism for increasing HDL cholesterol levels. In view of the complex relationship between CETP and atherosclerosis, it is uncertain whether this approach will provide a viable strategy for humans.

A more direct approach for exploiting the vascular protective effects of HDL and apolipoprotein A-I would be the actual administration of plasma-derived or recombinant HDL/apolipoprotein A-I as a drug. The feasibility and efficacy of this approach has been demonstrated in our laboratory in the animal model. Studies in our laboratory have shown that repeated administration of the recombinant apolipo-

protein A-I_{Milano}–phospholipid complex substantially reduces il-

eofemoral atherosclerosis in cholesterol-fed rabbits subjected to balloon injury and prevents the progression of athero-

sclerosis. At high doses, it promotes the regression of aortic atheromatosis in cholesterol-fed apolipoprotein E–null mice, despite severe hypercholesterolemia. Furthermore, the recombinant apolipoprotein A-I_{Milano}–phospholipid complex therapy was also shown to decrease lipid and macrophage content, thus promoting a more stable plaque-phenotype in apolipoprotein E–null mice, both during repeated administration and after single high-dose administration. Furthermore, in vitro studies have demonstrated that both wild-type apolipoprotein A-I and apolipoprotein A-I_{Milano} reduce the proapoptotic effect of oxysterols (produced during LDL oxidation) on vascular smooth muscle cells, which may further help preserve the integrity of the smooth muscle cell–rich cap of the atherosclerotic plaque. Wild-type plasma-derived HDL has also been shown to reduce neointimal thickening in a perivascular cuff–induced carotid arterial injury model in apolipoprotein E–null mice. Because inflam-

mation is thought to play an important role in arterial response to injury, the anti-inflammatory effects of HDL may be the underlying mechanism for the reduction in neointimal thickening.
Human Studies With Reconstituted Wild-Type HDL and Pro-Apolipoprotein A-I

Preliminary studies by Nanjee et al. have shown that the intravenous injection of discs containing apolipoprotein A-I and phosphatidylcholine increase the intravascular production of small pre-β-HDL particles in vivo and are associated with an increase in the efflux and esterification of tissue-derived unesterified cholesterol in normal healthy male volunteers. Similarly, Eriksson et al. demonstrated increased fecal excretion of cholesterol after the intravenous injection of liposomes containing pro-apolipoprotein A-I (a precursor of apolipoprotein A-I).

Apolipoprotein A-I or HDL Mimetic Synthetic Peptides

Dimeric lipid-free synthetic peptides containing 18-mer amphiphatic helices of the class found in HDL apolipoproteins (class A) have been shown to promote reverse cholesterol transport in cultured cholesterol-laden fibroblasts and macrophages and to interact with cell-surface HDL binding sites, thus simulating many of the biological effects of HDL. Thus, synthetic peptides comprising dimers of a structural motif common to exchangeable apolipoproteins can mimic apolipoprotein A-I in both binding to putative cell-surface receptors and in clearing cholesterol from cells. Several such peptide mimetics are being developed for further preclinical and clinical testing by different pharmaceutical companies.

Unilamellar Phospholipid Vesicles

Several experimental studies have also suggested that large unilamellar phospholipid vesicles or liposomes, when administered intravenously in large amounts, may stimulate reverse cholesterol transport and, thereby, have potential antiatherogenic effects analogous to those of HDL. Whether these beneficial effects are similar to or different from those of the apolipoprotein A-I–phospholipid complex are presently unknown, because no comparative data are available. However, further evaluation of this approach is warranted.

Apolipoprotein A-I Gene Transfer

Several preclinical studies have shown the feasibility and efficacy of apolipoprotein A-I gene transfer in inhibiting the progression and promoting the regression of atherosclerosis in murine models.

Other HDL-Related Gene Targets

Others that may influence HDL structure or function or be involved in reverse cholesterol transport may also have antiatherogenic effects. These potential HDL-associated genes include lecithin cholesterol acyltransferase (LCAT; especially in species with CETP), apolipoprotein E, apolipoprotein A-IV, paraoxonase, platelet activating factor acetylhydrolase (PAF-AH), scavenger receptor (SR)-B1, and ABC-AI (co-transporter). Although gene therapy continues to evolve as a therapeutic strategy, several obstacles need to be overcome before the promise of gene therapy as a clinically effective modality becomes a reality. Current gene therapy approaches are limited by the inefficiency of the vector, the transient and inefficient nature of gene expression, and the potential for an immunological reaction to the vector and to the transgene. To make gene therapy a viable option, several issues need to be addressed. These include the following: selecting a highly efficient, nonpathogenic vector; selecting the most appropriate method and site of gene delivery for optimum effectiveness; ensuring high levels of transgene expression; ensuring prolonged and stable expression of therapeutic genes, without an adverse immune response; and ensuring that random integration and germline transmission of viral vectors with potentially adverse effects does not occur. At the present time, none of these issues have been resolved, although steady progress is being made. It is likely that over the next several years, improvements in vectors and delivery techniques, which will allow for prolonged or possibly stable perpetual expression of therapeutic genes, will make gene therapy a viable option for atherothrombotic vascular disease.

We recently demonstrated successful transfer of the apolipoprotein A-Imilano gene into vascular smooth muscle cells, endothelial cells, and bone marrow cells using recombinant adenovirus (AAV) driven by Rous sarcoma virus and cymotegamovirus promoters. Vessel wall–specific targeting of the apolipoprotein A-I gene may also be achieved through local delivery of the gene at the time of endovascular intervention, intrapericardial delivery for coronary artery targeting, or through bone marrow or stem cells/macrophage–mediated gene transfer.

Other Approaches

Recent unraveling of critical steps involved in reverse cholesterol transport, particularly the involvement of ABC-AI and SRB-1, have created additional novel targets for therapeutic exploitation against atherosclerosis. Thus, pharmacologically increasing the expression or functional activity of ABC-AI to augment cholesterol efflux to apolipoprotein A-I/HDL or increasing the hepatic uptake of HDL cholesterol through the enhancement of the expression or activity of SRB-1 are strategies worth exploring. The ABC-AI gene contains elements in its promoter that are responsive to several nuclear hormone receptors, such as PPARα, PPARδ, liver X receptor (LXR), and retinoid X receptor (RXR). Recent studies have shown that a selective orally effective synthetic PPARδ agonist increases HDL cholesterol, reduces triglycerides and small dense LDL, enhances cholesterol efflux, and reduces insulin levels in a primate model of insulin resistance–type dysmetabolic syndrome. Similarly, a high-affinity agonist of RXR (tianoids) was shown to inhibit atherosclerosis markedly in apolipoprotein E–null mice; this was most likely due to the stimulation of ABC-AI mRNA levels and cholesterol efflux from macrophages in an LXR-dependent fashion. Ligands of LXR also seem to (1) stimulate the expression of cholesterol 7α-hydroxylase, leading to increased biliary excretion of bile acids, (2) increase cholesterol efflux from peripheral tissues by activating ABC-AI and possibly other ABC transporters, such as ABC-G1, and (3) inhibit cholesterol absorption from the gut, possibly by activating other ATP binding transporters (ie, ABC-G5 and ABC-G8). These observations suggest that RXR and some of its heterodimer partners may also play an important role in reverse cholesterol transport, providing attractive targets for therapeutic exploitation. Thus, the activation of specific subtypes of nuclear hormone receptors may provide a novel approach toward enhancing HDL and ABC-AI–mediated reverse cholesterol transport, with significant implications for atherosclerosis prevention and regression.
Potential Clinical Benefits of HDL/Apolipoprotein A-I Therapy

The multiple biological actions of HDL/apolipoprotein A-I coupled with promising experimental studies suggest that an HDL/apolipoprotein A-I–based therapeutic strategy could be useful against a wide variety of vaso-occlusive disorders, such as coronary artery disease (ie native coronary artery disease, vein-graft atherosclerosis, and transplant vasculopathy) and the restenosis that occurs after percutaneous transluminal coronary angioplasty (PTCA)/stenting. Therefore, we believe that the time for the evaluation of the vascular protective effects of HDL/apolipoprotein A-I is here. Therapeutic interventions targeted at enhancing HDL/apolipoprotein A-I levels or function and/or the direct administration of HDL/apolipoprotein A-I may have a major potential role against vaso-occlusive disease.

On the basis of the body of experimental and clinical data reviewed, this novel therapeutic strategy offers the possibility of promoting stabilization of atherosclerotic lesions by stimulating plaque-lipid and macrophage depletion and by improving endothelial dysfunction. Furthermore, preliminary experimental studies also raise the tantalizing possibility that adverse vascular remodeling and intimal hyperplasia responsible for post-PTCA and post-stent restenosis may also be inhibited. The antioxidant and anti-inflammatory effects of HDL/apolipoprotein A-I may contribute to the prevention of the progression of atherosclerotic lesions and possibly promote the regression of atherosclerotic disease involving the coronary, carotid, or peripheral circulation.

Conclusions

Statins and other LDL-lowering strategies have clearly contributed significantly to improved clinical outcomes in atherosclerotic vascular disease; however, heart attacks and other adverse vascular events have not disappeared with the passage of the 20th century. The time for the development and testing of novel antiatherogenic strategies, including but not limited to those exploiting the vascular protective effects of HDL/apolipoprotein A-I and, possibly, other HDL-related atheroprotective molecules is coming.

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