HDL Metabolism and the Role of HDL in the Treatment of High-risk Patients with Cardiovascular Disease

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Developing new therapeutic approaches to treating residual cardiovascular risk of recurrent clinical events in statin-treated patients has been a major challenge for the cardiovascular field. Data from epidemiological evidence, animal models, and initial clinical trials indicate that increasing high-density lipoprotein (HDL) may be an effective new target for treating residual cardiovascular risk. Over the past several years, major advances have occurred in our understanding of HDL metabolism and of the important roles of the ABCA1 and ABCG1 transporters as well as the SR-BI receptor in cholesterol transport. Current approaches to HDL therapy include acute HDL infusion therapy in acute coronary syndrome patients and chronic oral HDL therapy in stable patients with cardiovascular disease. Definitive clinical trials will now be required to establish the safety and efficacy of increasing HDL in the treatment of patients with cardiovascular disease.

Introduction

Over the past several decades, epidemiologic studies have identified low-density lipoprotein (LDL) cholesterol (LDL-C) and high-density lipoprotein (HDL) cholesterol (HDL-C) as independent risk factors that modulate cardiovascular disease (CVD) risk [1,2]. Clinical trials of LDL-decreasing drugs have definitively established that reductions in LDL levels are associated with a 25% to 45% reduction in clinical events [3–7]. However, despite lowered LDL levels, many high-risk patients continue to have cardiac events. This residual cardiovascular risk has been the focus of a great deal of interest. Because low HDL is often present in high-risk CVD patients [8], clinical and research programs have focused on using dietary, pharmacologic, or genetic manipulations to increase plasma HDL levels as a potential supplement to current statin therapy to further reduce residual cardiovascular risk.

Several lines of evidence have suggested that increasing HDL would decrease the risk of CVD. In addition to epidemiologic studies, several animal studies including HDL infusions in the form of apolipoprotein (apo)A-I/phospholipids complexes were associated with atherosclerosis regression in cholesterol-fed rabbits [9]. Increased plasma HDL levels associated with overexpression of both human apoA-I in transgenic mice [10,11] and lecithin-cholesterol acyltransferase (LCAT) in transgenic rabbits were also associated with decreased atherosclerosis [12]. Although limited in number, human clinical trials have supported data that increasing HDL may decrease clinical events. In acute coronary syndrome patients, five weekly infusions of apoA-I Milano were associated with reduction of total atheroma volume by 4.2% in 36 patients compared with 11 controls using intravascular ultrasound (IVUS) to quantitate coronary atheroma [13]. In the ARBITER 3 trial of 253 patients, the addition of niacin—which increased HDL by approximately 20%—to LDL therapy in combination with statins (n = 61) resulted in regression of carotid intimal-medial thickness (cIMT) by 0.0225 mm (n = 125) and 0.037 mm (n = 67) at 12 and 24 months, respectively [14]. The combined results of the epidemiologic, animal, and clinical studies provide support for the concept that raising HDL will be an effective additional therapeutic target for CVD prevention.

Overview of ApoA-I and Cholesterol Metabolism

Lipoprotein metabolism consists of two interconnected cascades: apoB-containing lipoproteins and apoA-I–containing lipoproteins (HDL). The apoB cascade includes both the chylomicron–chylomicron remnant pathway, which transports dietary lipids from the intestine to peripheral cells and liver, and the very-low-, intermediate-, low-density lipoprotein (VLDL–IDL–LDL) pathway,

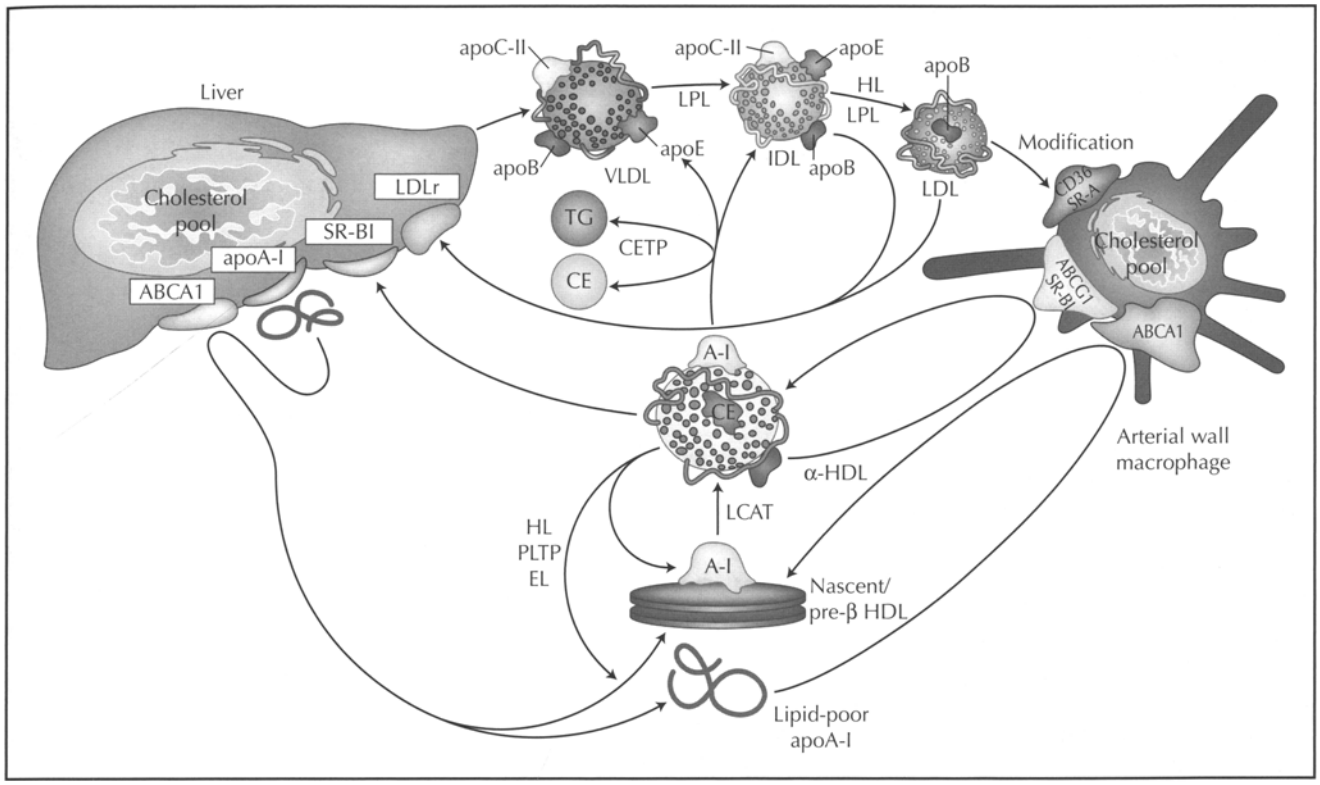

Figure 1. Lipoprotein metabolism consists of two interconnected cascades: apolipoprotein (apo)B-containing lipoproteins and apoAI-containing lipoproteins (high-density lipoprotein [HDL]). The apoB cascade includes the very-low-, intermediate-, low-density lipoprotein (VLDL–IDL–LDL) pathway. ApoA-I is synthesized in both the liver and intestine and is secreted as lipid-poor apoA-I. The HDL cascade includes 1) ATP binding cassette A1 (ABCA1) transporter–mediated lipidation of poorly lipidated apoA-I secreted from the liver to form nascent, pre-β HDL; 2) ABCA1-mediated cholesterol efflux from cholesterol-filled macrophages to lipid-poor apoA-I to form pre-β HDL; 3) conversion of pre-β HDL to mature α-HDL by lecithin-cholesterol acyltransferase (LCAT); 4) scavenger receptor class B type I (SR-BI) and ATP binding cassette G1 (ABCG1) transporter–mediated cholesterol efflux to mature α-HDL; 5) HDL cholesterol return to the liver by transfer of cholesterol esters to the VLDL–IDL–LDL lipoproteins via cholesteryl ester transfer protein (CETP), with uptake by the liver via the LDL receptor; 6) HDL cholesterol return to the liver by selective uptake of cholesteryl esters (CE) by the hepatic SR-BI receptor; 7) HDL remodeling by hepatic lipase (HL), phospholipid transfer protein (PLTP), and endothelial lipase (EL) to generate lipid-poor and pre-β HDL following hepatic uptake of cholesterol via the SR-BI receptor. FC—free cholesterol; LPL—lipoprotein lipase; TG—triglycerides.

which transports hepatic lipids to peripheral cells and returns cholesterol to the liver via the LDL receptor (Fig. 1). Major advances in our understanding of HDL and cholesterol metabolism have been made in the past several years. ApoA-I, the major structural apolipoprotein of HDL, is synthesized and secreted by the liver and intestine as a lipid-poor apolipoprotein. The discovery of the ATP binding cassette A1 (ABCA1) transporter provided a major breakthrough in understanding the mechanism by which apoA-I lipidation forms HDL as well as HDL's ability to efflux cellular cholesterol. The ABCA1 transporter was identified as the molecular defect in Tangier disease [15–21], a rare genetic dyslipoproteinemia characterized by orange tonsils and low HDL caused by accelerated lipid-poor HDL catabolism resulting from decreased apoA-I lipidation secondary to ABCA1 transporter mutations [22]. The discovery of the ABCA1 transporter solved the enigma of which key receptor pathway regulates cellular cholesterol efflux to HDL. Regulation of cholesterol efflux by the ABCA1 pathway plays a pivotal role in cholesterol efflux from the liver and the intestine and, thus, is a major determinant of the HDL plasma level. The ABCA1 pathway also provided key insight into the mechanism for cholesterol efflux from the cholesterol-loaded macrophage and reverse cholesterol transport. Over the past four decades, the major mechanism by which HDL was proposed to decrease CVD was reverse cholesterol transport, a process in which HDL carries excess cholesterol from peripheral cells, including foam cells in the coronary artery, back to the liver [23].

Recently, the underlying molecular mechanism by which apoA-I and the ABCA1 transporter facilitate the removal of cellular cholesterol has been addressed. Cell culture studies have established that the ABCA1 transporter and apoA-I recycle from the cell membrane to the late endocytic compartment; this appears to be critical in intracellular cholesterol's movement to the cell surface for cholesterol efflux to lipid-poor apoA-I [24,25]. Following cholesterol efflux, lipid-poor apoA-I is converted to nascent or pre-β HDL, which matures into the spherical α-HDL following the esterification of free cholesterol to cholesteryl esters by LCAT (Fig. 1).

In the initial in vitro studies analyzing cellular efflux, the major apolipoprotein acceptor for ABCA1-mediated cholesterol efflux was poorly lipidated apoA-I [26]; however, detailed analysis of several other plasma apolipoproteins, including apoA-I, apoC-I, apoC-III, and apoE, also demonstrated facilitation of excess cellular cholesterol removal [27]. The common structural feature of these apolipoproteins—which provides the basis for lipid binding—is the amphipathic helical structure (one hydrophilic and one hydrophobic surface [28,29]), which was initially recognized following the determination of the amino acid sequences of apoA-I [30], apoA-II [31], and apoC-III [32].

In addition to the ABCA1 transporter pathway, a second pathway using mature α-HDL as the ligand and either of two receptors—scavenger-receptor class B type I (SR-BI) or the ATP binding cassette G1 (ABCG1) transporter—has also been shown to modulate efflux of excess cellular cholesterol. SR-BI has the capacity to transport cholesterol both into and out of the cell depending on the decreased or increased intracellular cholesterol level, respectively [33,34]. The increased atherosclerosis observed following the exchange of bone marrow cells derived from SR-BI knockout mice to control mice by bone marrow transplantation supported a physiological role of SR-BI in the efflux of cellular cholesterol from vascular macrophages, thereby preventing the development of diet-induced atherosclerosis in the mice model [35].

Recently, α-HDL has been also shown to bind to a second transporter, ABCG1, which modulates cholesterol efflux from cholesterol-loaded macrophages [36,37]. In addition to normal α-HDL, the large HDL isolated from cholesteryl ester transfer protein (CETP)–deficient patients binds to the ABCG1 transporter and mediates cholesterol efflux [38•]. An increased level of LCAT as well as apoE present on the large HDL particles that are isolated from CETP-deficient patients have been reported to markedly increase the cholesterol efflux from cholesterol-loaded macrophages. The effect on experimental atherosclerosis in control animals following bone marrow transplantation with cells derived from ABCG1 transporter knockout mice is still controversial. In one report, the atherosclerosis was decreased [39], whereas it was increased in a second report [40].

The cellular levels of the ABCA1 and ABCG1 transporters are major determinants regulating cholesterol efflux. The expression level of the ABCA1 and ABCG1 transporter genes plays a key role in determining intracellular cholesterol levels. The expression of both the ABCA1 and ABCG1 transporter genes is enhanced by increased intracellular oxysterols concentrations via the liver X receptor (LXR) pathway. Excess intracellular cholesterol is converted to oxysterols that stimulate the LXR pathway; LXR binds to the LXR response elements (LXREs) in the ABCA1 and ABCG1 promoters, resulting in increased ABCA1 and ABCG1 gene expression [41–45]. Enhanced expression of the ABCA1 and ABCG1 transporters increases intracellular cholesterol efflux to lipid-poor apoA-I to form pre-β HDL and α-HDL, respectively. Thus, the overall effect is to decrease the cholesterol content of cholesterol-loaded cells by stimulation of the LXR pathway and upregulation of the ABCA1 and ABCG1 transporters.

The cholesterol in plasma HDL derived from the arterial wall is transported to the liver by two pathways (Fig. 1) [46,47]. The first involves the exchange of cholesteryl esters within HDL with the apoB-containing lipoproteins VLDL–IDL–LDL by CETP. This cholesterol ester–triglyceride exchange will increase the cholesterol and triglyceride content of the apoB-containing lipoproteins and HDL, respectively. The cholesterol exchanged into the apoB-containing lipoproteins will be transferred to the liver following interaction of the apoB-containing lipoproteins with the LDL receptor.

The second pathway involves the direct delivery of the HDL-C to the liver following interaction with the SR-BI receptor. The hepatic transfer of HDL-C via the SR-BI receptor involves selective uptake of the lipid component with virtually no HDL particle degradation. Associated with the delivery of cholesterol to the liver, HDL undergoes remodeling by hepatic lipase, phospholipid transfer protein, and endothelial lipase with regeneration of lipid-poor HDL, pre-β HDL, and poorly lipidated α-HDL [48–50]. The lipid-poor HDL can once again bind to the ABCA1 transporter, allowing the cholesterol cycle from the periphery to the liver to continue (Fig. 1).

Overview of the Role of HDL in Protection Against CVD

Several major mechanisms by which HDL protects against the development of CVD have now been identified. As outlined above, HDL-mediated efflux of cholesterol from cholesterol-loaded macrophages is a well-established antiatherogenic function of HDL.

A second important mechanism by which HDL may protect against CVD is the reduction of inflammation through the selective decrease of endothelial cell adhesion molecules that facilitate the binding of mononuclear cells to the vessel wall and promote lesion development. In vitro studies using cultured endothelial cells demonstrated a marked reduction in vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) following incubation with HDL or reconstituted HDL [51]. An in vivo rabbit model using a carotid artery cuff substantiated the reduction in inflammatory cells in the vessel wall following an infusion of reconstituted HDL or apoA-I before, at the time of cuff application, or for a few hours following cuff placement [52,53]. HDL may also decrease atherosclerosis by protecting LDL from oxidation. Oxidized or modified LDL, unlike normal LDL, is readily taken up by the scavenger receptor SR-A or CD36

on macrophages, resulting in cholesteryl ester accumulation with foam cell formation. The cholesterol-loaded macrophage secretes several inflammatory cytokines that stimulate monocyte chemotactic protein-1 (MCP-1) as well as endothelial cell adhesion molecules. Oxidized lipids are transferred to HDL from LDL and may be hydrolyzed by HDL paraoxonase and PAF acetylhydrolase [54,55].

HDL has been reported to have a number of other important effects on endothelial cells. In addition to reducing adhesion molecules, HDL and reconstituted HDL increase nitric oxide production by SR-BI–mediated stimulation of the endothelial nitric oxide synthase (eNOS) pathway [56], increase endothelial precursor cell levels in the plasma and at the site of injury [57], and decrease endothelial cell apoptosis [58].

Reports from experimental animal models that HDL as well as apoA-I Milano can reduce infarct size suggest other potential important clinical effects of HDL [59,60•]. α-HDL can also function as a transport vesicle for a number of different proteins identified by proteomic analyses that may ultimately be shown to have several important physiological functions [61].

Future Approaches for Increasing HDL and Protection Against Atherosclerosis

Based on current information, two separate conceptual approaches—acute and chronic HDL therapy—are under development to raise HDL and potentially decrease clinical events [62]. Acute HDL therapy is directed toward acutely increasing lipid-poor, nascent, pre-β HDL by infusions in acute coronary syndrome patients at high risk for repeated clinical events. The underlying hope is that HDL infusions will reduce the number of vulnerable plaques that rupture and result in acute cardiac events. The first clinical data to suggest that infusions of lipid poor apoA-I would be effective in decreasing atherosclerosis were provided by the apoA-I Milano infusion study, in which patients with the acute coronary syndrome were given five weekly infusions of either saline or apoA-I Milano/phospholipid complexes; the coronary atherosclerosis was quantitated by IVUS [13] as outlined above. The potential to significantly reduce atherosclerosis within weeks rather than months led to the concept of "acute HDL infusion therapy," in which high-risk acute coronary syndrome patients would receive weekly HDL infusion for a short period of time (6–8 weeks) to acutely decrease atherosclerosis and potentially decrease recurrent cardiac events. Additional approaches for acute HDL therapy under development include infusion of synthetic apoA-I mimetic peptides based on the amphipathic structure of apoA-I and reinfusion of autologous delipidated HDL. Further detailed clinical trial data will now be required to definitively establish whether acute HDL infusion therapy will protect against cardiovascular events.

A second approach to HDL therapy is the development of oral agents that significantly increase plasma HDL and can be used for a long or indefinite period of time. Currently, available oral drugs to increase HDL include statins, fibrates, and niacin. Until recently, the most promising new approach to significantly raise HDL was CETP inhibition. CETP inhibitors both increase HDL and reduce LDL. As previously outlined, CETP mediates exchange of cholesteryl esters for triglycerides between HDL and the apoB-containing lipoproteins, VLDL–IDL–LDL. The efficacy of CETP inhibition as an approach to increase HDL has been controversial because patients with complete CETP deficiency have been reported to be both protected against or at an increased risk for atherosclerosis development [63,64]. Because of the small number of patients with complete CETP deficiency, it has been difficult to make a definitive conclusion regarding the risk or benefit of CETP inhibition on CVD.

Studies with the chemical oral CETP inhibitor, JTT-705, administered to cholesterol-fed rabbits led to approximately a two-fold increase in HDL-C, a 50% decrease in non–HDL-C, and a 70% decrease in atherosclerosis [65]. A second study in which markedly hypercholesterolemic rabbits were fed the JTT-705 inhibitor was not associated with decreased atherosclerosis, presumably due to the marked hyperlipidemia and atherosclerosis present in these animals [66]. A second oral CETP inhibitor, torcetrapib, was associated with a three-fold increase in HDL-C levels, with no change in non–HDL-C and a 60% reduction in aortic atherosclerosis in cholesterol-fed rabbits [67]. Combined, these results suggest that increasing HDL by CETP inhibition would be anticipated to reduce coronary atherosclerosis and decrease cardiac events in high-risk patients with CVD.

Extensive clinical data on the changes in the plasma lipoproteins with CETP inhibitors are now available. Initial human studies with JTT-705 were performed in 198 healthy, mildly hyperlipidemic subjects in a 4-week phase II trial at doses of 300, 600, and 900 mg/d. At the 900-mg/d dose, CETP activity decreased 37%, HDL-C increased 34%, and LDL-C decreased 7% [68]. Extensive clinical data are now available with the torcetrapib CETP inhibitor. Torcetrapib, over the dose range of 10 to 240 mg/d, increased HDL and decreased LDL by 10% to 90% and 10% to 40%, respectively [69•]. In the initial phase II studies, there was a 2.2 mm Hg increase in systolic blood pressure with the torcetrapib 60-mg dose, and, as the clinical experience with torcetrapib continued, the systolic blood pressure was shown to increase by 4 to 5 mm Hg [70]. Analysis of 10 phase II studies revealed that approximately 4% of the patients who were treated with the torcetrapib plus atorvastatin combination had a greater than 15 mm Hg increase in blood pressure [70]. These results suggest that there was a subgroup of people who appeared to be more sensitive to the increased blood pressure effect of torcetrapib.

The phase III torcetrapib program included both imaging and morbidity and mortality trials to evaluate the effect of atorvastatin alone compared with atorvastatin plus torcetrapib (60 mg) on safety and vascular disease events. The imaging trials included a IVUS (ILLUSTRATE) trial (in acute coronary syndrome patients), and two cIMT trials (RADIANCE I in familial hypercholesterolemic patients, and RADIANCE II in mixed hyperlipidemic patients). As reported in the IVUS - ILLUSTRATE trial [71•], as well as the cIMT RADIANCE I and II trials [72•,73•], atherosclerosis was not decreased by either IVUS or cIMT methodology using the combination of torcetrapib plus atorvastatin compared with atorvastatin alone; however, there was a significant increase in HDL and reduction in LDL. In the ILLUMINATE morbidity and mortality study, 15,000 patients were randomized to atorvastatin or torcetrapib plus atorvastatin. However, in December 2006, the ILLUMINATE trial was terminated due to increased deaths and vascular events in the torcetrapib plus atorvastatin arm of the trial. The increased toxicity associated with torcetrapib has raised the critical question as to whether the toxicity is due to CETP inhibition or an off-target torcetrapib toxicity. It is important to note that torcetrapib is able to raise blood pressure in both the mouse and rat, neither of which expresses CETP; therefore, the increase in blood pressure is independent of CETP inhibition. Furthermore, the increase in blood pressure may not be the potential major factor in the toxicity of torcetrapib; rather it may represent a biomarker for a more systemic vascular toxicity leading to endothelial dysfunction, thrombosis, or some other form of vascular injury. A more definitive conclusion regarding the reason for the torcetrapib toxicity awaits a detailed and definitive analysis of the patients who participated in the ILLUMINATE clinical trial. This information is necessary before further studies can be conducted with this interesting and important new class of HDL-raising drugs.

Conclusions

The residual cardiac events present in statin-treated patients provide a challenge to the cardiovascular field to develop supplementary therapy to statin administration to reduce these recurrent clinical events. The combined data from epidemiology, animal models, and initial clinical trials support the concept that raising HDL may be an effective new target to decrease clinical events. However, the question remains: what is the best method to increase HDL? Definitive clinical trials focusing on both safety and efficacy will be required to establish that increasing HDL—both in terms of acute infusion HDL therapy and long-term chronic oral therapy—will reduce clinical events and provide the additional therapy necessary to further reduce vascular disease in high-risk patients.

Clinical Trials Acronyms

ARBITER—Arterial Biology for Investigation of the Treatment Effects of Reducing Cholesterol; ILLUSTRATION—Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis by CETP Inhibition and HDL Elevation; RADIANCE—Rating Atherosclerotic Disease Change by Imaging With a New CETP Inhibitor.

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