Regulation of Plasma High-Density Lipoprotein Levels by the ABCA1 Transporter and the Emerging Role of High-Density Lipoprotein in the Treatment of Cardiovascular Disease

H. Bryan Brewer, Jr, Alan T. Remaley, Edward B. Neufeld, Federica Basso, Charles Joyce

Abstract—High-density lipoproteins (HDL) protect against cardiovascular disease. HDL removes and transports excess cholesterol from peripheral cells to the liver for removal from the body. HDL also protects low-density lipoproteins (LDL) from oxidation and inhibits expression of adhesion molecules in endothelial cells, preventing monocyte movement into the vessel wall. The ABCA1 transporter regulates intracellular cholesterol levels in the liver and in peripheral cells by effluxing excess cholesterol to lipid-poor apoA-I to form nascent HDL, which is converted to mature \( \alpha \)-HDL by esterification of cholesterol to cholesteryl esters (CE) by lecithin cholesterol acyltransferase. The hepatic ABCA1 transporter and apoA-I are major determinants of levels of plasma \( \alpha \)-HDL cholesterol as well as poorly lipidated apoA-I, which interact with ABCA1 transporters on peripheral cells in the process of reverse cholesterol transport. Cholesterol in HDL is transported directly back to the liver by HDL or after transfer of CE by the cholesteryl ester transfer protein (CETP) by the apoB lipoproteins. Current approaches to increasing HDL to determine the efficacy of HDL in reducing atherosclerosis involve acute HDL therapy with infusions of apoA-I or apoA-I mimetic peptides and chronic long-term therapy with selective agents to increase HDL, including CETP inhibitors. (Arterioscler Thromb Vasc Biol. 2004;24:1755-1760.)

Key Words: ABCA1 transporter ▪ cholesterol ▪ cholesteryl ester transfer protein ▪ cholesteryl ester transfer protein inhibitor ▪ apoA-I

Low levels of high-density lipoprotein (HDL) cholesterol constitute an independent risk factor for cardiovascular disease (CVD).1-3 Recent studies have elucidated molecular mechanisms by which HDL acts to reduce cardiovascular risk. These findings bolster the rationale for increasing HDL as a new therapeutic target for treatment of the patient with high-risk CVD.

Over the past 4 decades, HDL has been proposed to decrease CVD by reverse cholesterol transport, a process by which HDL carries excess cholesterol from peripheral cells, including foam cells in the coronary artery, back to the liver for removal from the body.4 A major breakthrough in our understanding of the mechanism of reverse cholesterol transport came with the discovery of the ABCA1 transporter as the molecular defect in Tangier disease, a rare genetic disease characterized by orange tonsils, low-plasma HDL, and increased CVD.5 The low HDL characteristic of Tangier patients, including the original kindred, was caused by decreased cellular cholesterol efflux resulting from ABCA1 transporter mutations6-11 and increased catabolism of the poorly lipidated apoA-I.12 These findings identified ABCA1 as a transporter that effluxes excess cellular cholesterol to poorly lipidated apoA-I, thus playing a pivotal role in the reverse cholesterol transport process.

Regulation of ABCA1 transporter gene expression plays a key role in determining intracellular cholesterol levels. After the discovery of the ABCA1 transporter, we determined the complete genomic structure of the ABCA1 transporter gene, including the promoter region.9,13 The ABCA1 transporter spans 149 kb and contains 50 exons and 2261 amino acids. Analysis of the ABCA1 promoter revealed several promoter elements, including an LXRE14-17 and an E-box, as well as binding motifs for Sp1, Sp3, and AP1.18 Both USF1 and USF2 have been shown to bind to the E-box in the promoter of the ABCA1 transporter gene.18 ABCA1 transporter gene expression is also enhanced by intracellular cholesterol via the LXR pathway. Intracellular cholesterol is converted to oxysterols that stimulate the LXR pathway; LXR binds the LXRE in the ABCA1 promoter, resulting in increased ABCA1 promoter activity.14-17 Enhanced expression of the ABCA1 transporter increases efflux of intracellular cholesterol to lipid-poor apoA-I to form preβ HDL or nascent...
HDL. The cholesterol in this nascent HDL is esterified by lecithin-cholesterol acyltransferase (LCAT) with conversion of the nascent HDL to mature spherical α-HDL in plasma. Thus, the cholesterol content of cholesterol-loaded cells is decreased by stimulation of the LXR pathway and upregulation of the ABCA1 transporter.

Whereas the major apolipoprotein acceptor for ABCA1-mediated cholesterol efflux is poorly lipidated apoA-I, detailed analysis of several other plasma apolipoproteins, including apolipoproteins A-I, C-I, C-III, and E, established that these apolipoproteins also facilitate the removal of excess cellular cholesterol in vitro. The common structural feature of these apolipoproteins is an amphipathic helical structure with one surface hydrophilic and the other hydrophobic, initially recognized when we determined the amino acid sequence of apoA-I, apoA-II, and apoC-III. This structural motif increases the ability of the apolipoprotein to interact with lipid surfaces and remove cholesterol from cell membranes. The recognition of the importance of the amphipathic helix to lipid binding resulted in the initiation of studies to develop short amphipathic apoA-I mimic peptides of 18 to 36 amino acids that could also efflux cholesterol from cells. In initial in vitro studies, we established that synthetic D and L amino acid amphipathic peptides were able to efflux cholesterol from the ABCA1 transporter pathway.

Of particular interest has been the underlying mechanism by which apoA-I and the ABCA1 transporter facilitate the removal of cellular cholesterol. In a series of studies, we have shown that the ABCA1 transporter and apoA-I recycles from the cell membrane to the late endocytic compartment, which appears to be critical in the movement of intracellular cholesterol to the cell surface for cholesterol efflux. Recent studies have also established that apoA-I and synthetic apoA-I mimic peptides are able to stabilize the ABCA1 transporter and increase cholesterol efflux presumably by preventing degradation by calpain.

In addition to the ABCA1 transporter pathway, HDL facilitates the efflux of excess cellular cholesterol by the SR-BI pathway and by passive diffusion (Figure 1). Additional mechanisms by which HDL protects against CVD include reduction of the oxidative modification of low-density lipoproteins (LDL) and inhibition of cytokine-induced expression of endothelial adhesion molecules, which increases monocyte movement into the vessel wall (Figure 2).

**Overexpression of ABCA1 Transporter**

To gain additional information on the role of the ABCA1 transporter in cholesterol metabolism, we developed transgenic mice overexpressing the ABCA1 transporter. ABCA1 overexpression was associated with a marked increase in total cholesterol, HDL cholesterol, and apoA-I, as well as a modest increase in non-HDL and LDL cholesterol and apolipoprotein B, when compared with control mice. ABCA1 transgenic mice fed a regular chow diet had a 2-fold increase in both apoA-I-mediated cholesterol efflux from macrophages and plasma HDL cholesterol levels, as well as a 1.5-fold increase in net hepatic delivery of exogenous radiolabeled cholesteryl ether HDL compared with control mice. The elevation in HDL cholesterol levels was caused by increased HDL synthesis and decreased HDL catabolism. These combined results indicated that activation of the ABCA1 transporter results in increased levels of plasma HDL, increased reverse cholesterol transport, and increased delivery of cholesterol back to the liver in this transgenic animal model. Overexpression of the ABCA1 transporter in transgenic mice was associated with decreased cholate diet-induced atherosclerosis.

The site of synthesis of the increased plasma HDL cholesterol in transgenic mice overexpressing ABCA1 in the liver and macrophages was then evaluated. Our initial studies established that the ABCA1 transporter was localized to the basal-lateral side of the hepatocyte, consistent with the ABCA1-mediated cholesterol efflux directed toward plasma rather than the bile. The macrophage was eliminated as a major source of the plasma HDL because studies with bone marrow transplantation of macrophages in control and ABCA1 knockout mice established that the HDL cholesterol
pool generated by macrophages did not make a significant contribution to plasma HDL levels. In additional studies, selective hepatic expression of the ABCA1 transporter using an ABCA1 adenoviral vector resulted in a marked increase in plasma HDL cholesterol levels. These combined studies established the liver as the major site of HDL cholesterol synthesis in the ABCA1 transgenic mice. The analysis of transgenic mice overexpressing ABCA1 in the liver provided the unique opportunity to discover that the hepatic ABCA1 transporter plays a major role in the determination of plasma HDL levels, as well as a pivotal mechanism for the regulation of the hepatic intracellular levels of cholesterol.

These combined results provide a new concept in HDL metabolism in which the liver is a site of synthesis of apoA-I as well as a major source of poorly lipidated apoA-I/pre-β HDL and mature α-HDL. The poorly lipidated apoA-I may interact with the hepatic ABCA1 transporter, removing excess cholesterol and ultimately being converted to mature α-HDL after esterification of the hepatic-derived FC to CE by LCAT. Alternatively, the poorly lipidated apoA-I may be transported to the periphery and interact with the ABCA1 transporter on peripheral cells. Thus, both the hepatic-derived poorly lipidated apoA-I and mature α-HDL may traffic to periphery cells (eg, cholesterol-filled vascular macrophages) and remove the excess cellular cholesterol by interaction with the ABCA1 transporter and SR-BI receptor, respectively; Figure 1), and return the cholesterol within HDL to the liver for removal from the body by biliary secretion of cholesterol or bile acids.

Plasma non-HDL cholesterol and apoB were modestly increased in ABCA1 transgenic mice on the normal chow diet and decreased on the high-cholesterol/cholate diet. When ABCA1-Tg mice were put on a Western diet, there was a marked increase in LDL levels and the apoB-lipoproteins when compared with control mice or transgenic mice on a regular chow diet. Based on these results, we propose that the increased cholesterol absorption on the high-cholesterol diet resulted in an increase in flux of cholesterol to the liver and upregulation of the hepatic ABCA1 transporter with increased synthesis of HDL.

The increase in cholesterol levels in the apoB-lipoproteins in the mouse, which is deficient in cholesteryl ester transfer protein (CETP), is caused by the rapid transfer of free cholesterol from HDL to the apoB-lipoproteins. In humans, the interchange of cholesterol between the HDL and apoB-lipoprotein pathways occurs as either free cholesterol or cholesteryl ester (CE) facilitated by CETP. The free cholesterol transferred to the apoB-lipoproteins can be esterified by the LCAT activity present on the apoB lipoproteins.

These combined results raise the interesting possibility that overexpression of the hepatic ABCA1 transporter as would be achieved with LXR agonist may be associated with increased rather than decreased atherosclerosis, depending on the genetic makeup of the animal model or patient. Hepatic ABCA1 overexpression with increased flux of cholesterol into apoB lipoproteins in LDL receptor deficiency may be associated with potentially increased atherosclerosis. In contrast, selective overexpression of the ABCA1 transporter in macrophages using LXR agonist or other small molecules to increase the ABCA1 transporter would be anticipated to result in decreased atherosclerosis.

Schematic Model of Lipoprotein Metabolism

An overview of our current working model of lipoprotein metabolism in humans is illustrated in Figure 3. This new updated model illustrates the important roles of the hepatic ABCA1 transporter in determining the plasma level of HDL cholesterol and the pathways for the metabolism of the apoB-lipoproteins. ApoB-lipoprotein metabolism includes the chylomicron–chylomicrons remnant pathway, which transports dietary lipids from the intestine to peripheral cells and returns cholesterol back to the liver via the LDL receptor (LDLr).

The metabolism of HDL (apoA-I lipoproteins) and the apoB lipoproteins involves 2 cascades interconnected by CETP, which mediates exchange of CE and triglycerides between the 2 lipoprotein pathways.

HDL metabolism involves the synthesis of apoA-I and the ABCA1-mediated lipoprotein (apoA-I) in liver and intestine to form poorly lipidated apoA-I, nascent/pre-β HDL, and mature α-HDL. Pre-β HDL is also formed during the metabolism of triglyceride-rich lipoproteins. Pre-β HDL is converted to mature α-HDL by LCAT. As outlined, in peripheral cells, excess cellular cholesterol is removed by the apoA-I-mediated ABCA1 efflux pathway to form pre-β HDL, and by SR-BI–mediated cholesterol efflux to mature α-HDL. Mature α-HDL may be remodeled by hepatic lipase, phospholipid transfer protein, and SR-B1 with the generation of lipid-poor apoA-I/HDL, which can recycle and stimulate ABCA1-mediated efflux from peripheral cells, including arterial wall macrophages. Cholesterol in HDL is returned directly to the liver by interaction with the hepatic SR-B1 receptor and by transfer of CE by CETP to the VLDL–IDLLDL lipoproteins with ultimate uptake by the liver via the LDLr. The plasma level of pre-β HDL and mature α-HDL reflects the integration of the various lipoprotein metabolic pathways.
Emerging Role of HDL as a New Therapeutic Approach in the Treatment of Cardiovascular Disease

Several lines of evidence support the concept that raising HDL may provide substantial atheroprotective benefit. Epidemiological studies have shown an inverse correlation between HDL and CVD risk. Raising HDL 1 mg has been proposed to reduce the risk of CVD by 2% to 3%. There are several therapeutic approaches that have the potential to increase HDL and decrease cardiovascular disease (Figure 3). In animal models, increasing apoA-I synthesis in apoA-I transgenic mice and transgenic rabbits increased HDL and decreased atherosclerosis. Overexpression of LCAT in hypercholesterolemic transgenic rabbits has also been shown to increase plasma HDL, reduce LDL, and decrease atherosclerosis, consistent with LCAT being an effective potential target for development to increase HDL and decrease CVD.

HDL may also be acutely increased by HDL or apoA-I/phospholipid infusions. Infusion of HDL–VHDL into hyperlipidemic rabbits increased HDL cholesterol levels and reduced atherosclerosis. In addition, apoA-I mimetic peptides have been designed that incorporate the amphiphilic helical structure of apoA-I for potential use to raise HDL and decrease atherosclerosis. Interestingly, an orally administered 18 D-amino acid peptide has been reported that improves the anti-inflammatory properties of HDL, reduces LDL induction of monocyte–chemotactic activity, decreases macrophage penetration into the vessel wall, and reduces atherosclerosis in the apoE knockout and LDL knockout mice with virtually no change in the plasma lipoproteins.

In human studies, a kindred with familial hyperalphalipoproteinemia was identified who had a 2-fold selective increase in apoA-I synthesis and elevated plasma HDL-C levels. Human infusion studies with pro-apoA-I or apoA-I/phospholipid complexes acutely increased HDL cholesterol. The pro-apoA-I infusion was also associated with increased biliary cholesterol and fecal sterol loss. Recently, weekly infusions of apoA-I Milano/phospholipid complexes were shown to regress total atheroma volume by 4.2% in 36 patients compared with 11 controls after an acute coronary event using intravascular ultrasound (IVUS) to quantify coronary atheroma. These results provide support for the concept that elevating HDL will decrease CVD, and that regression of atherosclerotic lesions may occur faster than previously expected. Acute HDL therapy using the infusion of apoA-I, apoA-I Milano, or delipidated HDL as well as amphiphatic apoA-I mimetic peptides all have the potential to increase HDL and decrease CVD.

Of major importance is the development of a long-term, low-risk approach to increasing HDL. To date the most effective potential agent for long-term increase in plasma HDL is a CETP inhibitor. As reviewed, CETP mediates exchange of CE for triglycerides between HDL and VLDL–LDL and may be either proatherogenic, if the CETP-mediated CE in VLDL–LDL is taken up by arterial wall macrophages, or antiatherogenic, if the CETP-mediated VLDL–LDL–CE is returned to the liver via the LDLr as part of the HDL-mediated pathway of reverse cholesterol transport. In initial studies, partial inhibition of CETP activity by either antisense oligodeoxynucleotides or anti-CETP antibodies in cholesterol-fed rabbits increased HDL and decreased aortic atherosclerosis. Administration of a CETP chemical inhibitor, JTT-705, in cholesterol-fed rabbits resulted in a 2-fold increase in HDL cholesterol, 50% decrease in non-HDL cholesterol, and 70% decrease in atherosclerosis. Initial clinical studies in humans with the JTT-705 CETP inhibitor are currently underway. Recently, clinical studies with a second CETP inhibitor, Torcetrapib, have been reported. The initial phase 1 multidose study of Torcetrapib (10, 30, 60, and 120 mg/d and 120 mg/bid) in 40 healthy normolipidemic subjects revealed no change in total plasma cholesterol (10 mg/d to 120 mg/bid) but did reveal an increase in HDL cholesterol from 16% to 91% (10 mg/d to 120 mg/bid) and a decrease in LDL cholesterol from 7% to 42% (60 mg/d to 120 mg/bid). At 120 mg/bid, apoA-I and apoE increased 27% and 66%, respectively, and apoB decreased 26%. In a single-blinded study in low HDL subjects, Torcetrapib was used alone or in combination with 20 mg atorvastatin for 4 weeks either at a dose of 120 mg/dL alone or in combination with atorvastatin, and a subset of subjects received an additional 4 weeks of 120 mg/dL alone twice daily. HDL cholesterol increased 46%, 61%, and 106%, whereas LDL cholesterol decreased 17%, 7.5%, and 17.4% with 120 mg/d, 120 mg/d plus atorvastatin, and 120 mg/d twice daily, respectively. Torcetrapib therapy was well-tolerated without major adverse events.

In contrast to partial inhibition of CETP with a CETP inhibitor, patients with a complete absence of CETP activity have large, cholesterol-enriched, dysfunctional HDL with decreased capacity to efflux cellular cholesterol, as well as decreased, polydisperse plasma LDL, and have been reported to be at risk for CVD. These results are consistent with the concept that partial CETP inhibition with a CETP inhibitor may be atheroprotective, but complete absence of CETP activity can create a proatherogenic lipid profile.

During the next several years, detailed analysis of vascular atherosclerosis using carotid intima-media thickness and coronary IVUS, as well as clinical trials, will be required to determine whether increasing HDL will decrease clinical events and which approach to increasing HDL will be the most useful. The potential for acute and chronic HDL therapy provides a new paradigm for the development of a comprehensive approach to the treatment of the patient with high-risk CVD.

References

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