HDL-mediated mechanisms of protection in cardiovascular disease

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Low plasma levels of HDL-cholesterol (HDL-C) represent a strong and independent risk factor for cardiovascular disease. HDL particles display a wide spectrum of atheroprotective activities, which include effluxing cellular cholesterol, diminishing cellular death, decreasing vascular constriction, reducing inflammatory response, protecting from pathological oxidation, combating bacterial infection, lessening platelet activation, regulating gene expression by virtue of microRNAs, and improving glucose metabolism. It remains presently indeterminate as to whether some biological activities of HDL are more relevant for the protection of the endothelium from atherogenesis when compared with others. The multitude of such activities raises the question of a proper assay to assess HDL functionality in vivo. Together with clear understanding of molecular mechanisms underlying atheroprotective properties of HDL, such assay will provide a basis to resolve the ultimate question of the HDL field to allow the development of efficient HDL-targeting therapies.

Keywords HDL • Biological activities • Atheroprotection • Mechanisms • Cardiovascular disease

This article is part of the Spotlight Issue on HDL biology: new insights in metabolism, function, and translation.

1. Introduction

Low plasma concentrations of HDL-cholesterol (HDL-C) are firmly established to represent a strong and independent risk factor for cardiovascular disease. In contrast, elevated HDL-C does not fully protect from cardiovascular events.1 The existence of a considerable cardiovascular risk at supra-normal levels of HDL-C has led to the hypothesis of HDL function, according to which plasma levels of HDL-C alone do not reflect all facets of the atheroprotective potential of HDL.2–5 The hypothesis postulates that mechanisms of HDL-mediated atheroprotection are underlain by anti-atherogenic biological activities of HDL, broadly termed ‘HDL function’ or ‘functionality’, and do not necessarily track with HDL-C concentrations. Indeed, innumerable in vitro and in vivo studies document multiple atheroprotective activities of HDL particles, which include capacities to protect vascular endothelium via effluxing cellular cholesterol, diminishing cellular death, decreasing vascular constriction, reducing inflammatory response, protecting from pathological oxidation, combating bacterial infection, lessening platelet activation, regulating gene expression by virtue of microRNAs (miRs), and improving glucose metabolism.6–12 Intriguingly, HDL particles participate in cholesterol handling not only in blood but equally in other body compartments, such as lymph13 and brain.14

2. Cellular cholesterol efflux

A majority of the actions of HDL directly target arterial endothelium, providing potent vasoprotection. First, HDL particles remove cholesterol from arterial wall cells, primarily macrophages and macrophage-derived foam cells. This well-known and best studied biological activity of HDL (Figure 1) has long been considered to represent the first and key step in the reverse cholesterol transport (RCT) from peripheral cells to the liver for excretion into the bile. The concept of RCT is based on the dissociation between the capacity of every cell in the body to synthesize cholesterol and incapacity of many cell types to excrete this compound in significant amounts. Cholesterol efflux may thereby provide the most clinically relevant atheroprotective property of HDL that forms the basis for the association between circulating levels of HDL-C and cardiovascular risk.15 Indeed, according to the original HDL hypothesis,16 reduction of plasma HDL-C concentration may accelerate the development of atherosclerosis by impairing the clearance of cholesterol from the arterial wall. Consistent with the central role of HDL-mediated cholesterol efflux in the protection from atherogenesis, rates of cholesterol efflux from macrophages may reflect the presence of cardiovascular disease better than HDL-C levels.3,17 Further along this line, infusions of native or reconstituted HDL are capable of removing cholesterol from atherosclerotic plaques in vivo.18

To ensure cholesterol efflux, HDL must first cross the endothelium to gain access to arterial intimal cells. Endothelial cells bind, internalize, and translocate HDL from the apical to the basolateral compartment by a mechanism involving SR-BI and ABCG1.19,20 Transcytosis of lipid-free apolipoprotein A-I (apoA-I) equally occurs and involves ATP-binding cassette transporter A1 (ABCA1) as a key partner, resulting in the lipidation of apoA-I.21,22 Following transcytosis, HDL interacts with specific receptors on the cell surface, and with cellular surface lipids to induce

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specific or non-specific forms of cholesterol efflux. Specific, fast, active, and unidirectional cholesterol efflux is mediated by ABCA1 and ABCG1 transporters, while the SR-BI receptor ensures a large part of the efflux of cholesterol from macrophages mediated by ABCA1 has been extensively documented in animal models. ABCG1 is a half-type ABC transporter which effluxes cholesterol to large spherical HDL2 and HDL3 particles. The transporter thereby forms a sequential pathway of HDL biogenesis with ABCA1 and LCAT. Such sequential growth of HDL particles under the action of ABCA1, LCAT, and ABCG1 can be supported by several cell types, such as macrophages and endothelial cells. The role of the ABCG1-mediated pathway of cholesterol efflux from macrophages appears to be quantitatively less important when compared with that of ABCA1. Thus, Cla-1 (human SR-BI) appears to provide a major contribution to cholesterol efflux from macrophages under non-stimulated basal conditions.

SR-BI mediates bidirectional, ATP-independent flux of cholesterol between mature HDL and plasma membranes and plays an important role in net cellular cholesterol efflux, acting primarily in hepatocytes but equally in macrophages, adipocytes, and other cell types. Thus, SR-BI appears to provide a major contribution to cholesterol efflux from macrophages under non-stimulated basal conditions. Passive receptor-independent diffusion of cholesterol from plasma membranes to HDL may be particularly important under non-stimulated basal conditions, being capable of effluxing cholesterol even more potently than SR-BI. The role of these pathways, however, becomes minor upon cholesterol loading of cells as they cannot be up-regulated.

Another mechanism that facilitates cholesterol efflux from macrophages is represented by apolipoprotein E (apoE) secretion. Indeed, production of apoE by both human and mouse macrophages enhances cholesterol efflux to HDL. As atherosclerotic plaques are only partially permeable to plasma HDL, apoE-mediated cholesterol efflux may constitute a key pathway to remove cholesterol from arterial macrophages and foam cells. This notion is particularly relevant in light of heavy biochemical and functional modifications of apoA-I in human atheroma. ApoE, in a lipid-free or lipid-poor form, can act as a primary acceptor to remove cholesterol from macrophages, acting via ABCA1. In addition, apoE can form large HDL that efflux cellular cholesterol via SR-BI.

Cellular cholesterol efflux is regulated by intracellular lipid metabolism. As macrophages accumulate cholesterol in the form of cholesteryl ester following esterification of free cholesterol by acyl-CoA:cholesterol acyltransferase (ACAT), inhibition of this enzyme stimulates cholesterol efflux. Vice versa, cholesterol mobilization from macrophages requires hydrolysis of cholesteryl ester to free cholesterol. This reaction is catalysed by neutral cholesteryl ester hydrolase; overexpression of the enzyme enhances cholesterol efflux from human macrophages and decreases atherosclerosis in mice.

Cholesterol delivery to the liver with subsequent excretion into the bile represents the final step of the RCT pathway. Both hepatic removal and biliary secretion of HDL-derived cholesterol are mediated by hepatic SR-BI.

3. Vasodilatory actions

Growing evidence exists to support the physiological relevance of other, than cholesterol efflux, anti-atherogenic actions of HDL towards the endothelium. The beneficial actions of HDLs on the endothelium involve vasodilatory activity, which primarily reflects their capacity to...
stimulate NO release by endothelial cells, but also production of prostacyclin.

Activation of NO production by HDL involves binding to SR-BI as an initiating event. HDL bound to the extracellular loop of SR-BI initiates signalling in the endothelium through interaction of the C-terminal PDZ-interacting domain of SR-BI with the adaptor PDZ-domain-containing protein PDZK1. Subsequent intracellular events are mediated by endothelial Akt and intracellular Ca\textsuperscript{2+} mobilization, increase in intracellular ceramide levels, endothelial nitric oxide synthase (eNOS)-Ser1177-phosphorylation, and eNOS-Thr495-dephosphorylation. HDL-induced activation of eNOS and Akt is dependent on AMPK activation, which in turn depends on both SR-BI and sphingosine-1-phosphate receptors.

Another pathway participating in vasodilatory effects of HDL in endothelial cells is mediated by ABCG1 and involves cholesterol efflux of cholesterol and 7-oxysterols which improves the formation of active eNOS dimers and results in decreased ROS production. Diminished cellular production of superoxide, which inactivates NO, can also increase in NO bioavailability and improved vasodilation in the presence of HDL. Indeed, HDL reduces NADPH oxidase activity and expression of its major subunits in endothelial cells.

Vasodilatory activity of HDL may translate into improved endothelial function; clinical evidence to support this hypothesis is accumulating.

4. Cytoprotective actions

HDL particles display potent cytoprotective actions. Thus, HDL protects both macrophages and endothelial cells from apoptosis induced by loading with free cholesterol or by oxidized LDL. HDL also protects endothelial cells against cell death induced by chylomicron remnants. TNF-alpha, proteins of the complement system, and growth factor withdrawal.

HDL-mediated protection from apoptosis induced by loading with free cholesterol or oxidized LDL is mediated by cellular efflux of oxidized cholesterol, primarily of 7-ketosterol, which include reduced cellular generation of superoxide anion and/or hydrogen peroxide secondary to down-regulation of superoxide production by NADPH oxidase and/or mitochondrial electron transport chain.

Major intracellular mechanisms underlying the anti-apoptotic actions of HDL include preservation of mitochondrial integrity, abrogation of caspase cascade activation, reduced fragmentation of nuclear DNA, blockage of cytotoxic calcium signalling pathways, and activation of the survival Akt pathway. Furthermore, stimulation of NO synthesis by HDL may significantly contribute to its cytoprotective effects. Regulation of the signalling function of proteins concentrated in caveolae, such as eNOS and SR-BI, may underlie this activity. Another HDL receptor, ABCG1, is critical to the anti-apoptotic effects of HDL in macrophages and endothelial cells.

Another cytoprotective activity of HDL relevant for vasoprotection involves inhibition of apoptosis, cell detachment, and extracellular matrix degradation induced by elastase in human vascular smooth muscle cells. Such an anti-elastase action can be related to the presence in HDL of serpin peptidase inhibitors, including alpha-1-antitrypsin.

5. Anti-inflammatory and antioxidative actions

HDL particles display multiple anti-inflammatory actions which collectively may contribute to suppression of a chronic inflammatory response in the arterial wall which evolves in response to LDL-derived cholesterol deposition. HDL potently decreases adhesion molecule expression on endothelial cells activated by cytokines and thereby inhibits monocyte adhesion to the endothelium both in vitro and in vivo. HDL also directly inhibits monocyte activation, reducing expression of chemokines and chemokine receptors via the modulation of nuclear factor kappa B (NFkB) and PPAR gamma. HDL actions on monocytes include decreased myeloid cell proliferation and reduced monocytophagocytosis as observed in LDL receptor-deficient mice.

HDL particles also inhibit stimulation of T-cells by antigen-presenting cells and activation of monocytes by stimulated T-cells, thereby inhibiting the production of proinflammatory cytokines and chemokines induced by T-cell contact with monocytes. Interaction of HDL with T-lymphocytes, which blocks subsequent activation of monocytes by lymphocytes, can account for this effect. HDL can also restore the migratory process of macrophages and monocyte-derived dendritic cells and thus result in the resolution of inflammatory reactions in atherosclerotic plaques. In addition, HDL potently reduces neutrophil activation in vivo and in vitro. Collectively, these effects constitute an important facet of HDL action on the innate and adaptive immunity.

The multiple effects of HDL on the immune system suggest that several mechanisms of action may be operative. Cellular efflux of non-oxidized and oxidized lipids may form a mechanistic basis for the capacity of HDL to decrease adhesion molecule expression, for direct inhibitory actions of HDL on monocyte and neutrophil activation, and for reductions in myeloid cell proliferation and monocytophagocytosis.

Removal of cellular cholesterol down-regulates the inflammatory phenotype of macrophages with consequent attenuation of signalling via Toll-like receptors. Macrophage ABCA1 is pivotal for this effect. Anti-inflammatory effects of HDL in adipocytes are equally mediated by ABCA1 but also by ABCG1 and SR-BI. In contrast, apoA-I interaction with ABC transporters may induce anti-inflammatory signalling independently of cholesterol efflux. Anti-inflammatory reprogramming of macrophages by HDL occurs via the transcriptional regulator ATF3, leading to the down-regulation of Toll-like receptor-mediated pathways. The action of HDL on adhesion molecule expression in endothelial cells involves inhibition of NFkB activation induced by oxidized LDL and TNF-alpha, with subsequent reduction of inflammatory signalling. This effect is dependent on the up-regulation of 3-beta-hydroxysteroid-delta-24-reductase (DHCR24) and requires interaction with SR-BI on the cellular surface. In addition, HDL-induced inhibition of adhesion molecule expression is dependent on AMPK activation. Up-regulation of transforming growth factor beta may also contribute to the anti-inflammatory activity of HDL.

Antioxidative properties of HDL are closely linked to its anti-inflammatory potential. Indeed, oxidative modifications of cholesterol-rich lipoproteins, primarily LDL, retained in the arterial wall result in the formation of highly proinflammatory oxidized phospholipids, such as 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine and 1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphocholine. The response-to-retention hypothesis of atherosclerosis postulates that these and other products of LDL oxidation, acting together, induce local inflammatory response. HDL-mediated protection of LDL from pathological oxidation may therefore result in the inhibition of inflammation.

HDL can protect LDL and other lipoproteins from oxidative stress in vitro induced by various oxidants, which include one- and two-electron species. Removal of oxidized lipids from LDL or cells represents the first step of HDL-mediated protection from oxidative damage induced
by free radicals. Indeed, phospholipid hydroperoxides (PLOOHs) are rapidly transferred from oxidized LDL to HDL upon their co-incubation.\textsuperscript{113} Inactivation of oxidized lipids associated with HDL particles represents the second step in this antioxidative pathway.\textsuperscript{114} Depending on their structure, oxidized lipids can be reduced (LOOHs) by apo-A-I and other redox-active HDL components\textsuperscript{113,115–118} or hydrolysed (short-chain oxidized phospholipids, lysophosphatidylcholine) by HDL-associated hydrolytic enzymes.\textsuperscript{119}

As a consequence, HDL particles constitute a major transport vehicle of LOOH in human plasma and may therefore function as a ‘sink’ for oxidized lipids\textsuperscript{120,121} which can accumulate in the particle when the LOOH-inactivating capacity of HDL is overwhelmed. Subsequently, CEEOHs and their corresponding hydroxides can be rapidly removed from HDL via selective uptake by the liver mediated by SR-BI.\textsuperscript{122}

HDL also inhibits generation of reactive oxygen species (ROS) and decreases intracellular oxidative stress both in vitro\textsuperscript{69,72–74,123} and in vivo.\textsuperscript{124} Such antioxidant actions of HDL require interaction with surface receptors, including SR-BI and ABCG1,\textsuperscript{75,123} but do not necessitate direct contact between HDL and prooxidative agents in the extra-cellular compartment. Diminished cellular production of superoxide and/or hydrogen peroxide may be implicated in the antioxidative effect of HDL in endothelial cells.\textsuperscript{75}

8. Reduction of platelet activation

HDL exerts several anti-thrombotic effects which primarily include reduction of platelet activity as directly demonstrated ex vivo in intervention trials.\textsuperscript{141,142} In vitro anti-thrombotic activities of HDL are expressed as dose-dependent inhibitory actions on agonist-stimulated platelet aggregation, fibrinogen binding, granule secretion, and thromboxane A2 and 12-hydroxy-eicosatetraenoic acid production.\textsuperscript{7,142–144} HDL decreases platelet aggregation mediated by glycoprotein IIb/IIIa in response to thrombin, collagen, ADP, and adrenaline.\textsuperscript{143,145–148} In addition to its effects on platelets, HDL exerts anti-thrombotic effects on endothelial cells.\textsuperscript{149}

Mechanisms of the antiplatelet actions of HDL include enhanced production of NO triggered by the interaction of HDL with platelet SR-BI.\textsuperscript{150,151} The anti-thrombotic effects of HDL towards endothelial cells equally depend on the stimulation of cellular NO production.\textsuperscript{149}

Interaction of HDL with the platelet SR-BI receptor may trigger intracellular signalling cascades, which encompass intracellular release of diacylglycerol from plasma membrane phosphatidylcholine, activation of protein kinase C, stimulation of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange, and reduction of platelet activity as directly demonstrated ex vivo in experimental trials.\textsuperscript{141,142}

9. Regulation of gene expression by miRs

Recently, HDL has been shown to transport small non-coding miRs.\textsuperscript{152} Multiple copies of several miRs are transported by circulating HDL in man.\textsuperscript{153} miRs are key intracellular regulators of gene expression which post-transcriptionally control cellular cholesterol homeostasis, including cholesterol efflux.\textsuperscript{154} HDL carries miRs that control cholesterol metabolism;\textsuperscript{155} miR-33 down-regulates expression of ABCA1 and ABCG1 and reduces HDL biogenesis in mice.\textsuperscript{155–160} As a corollary, both antisense\textsuperscript{161} and deficiency\textsuperscript{162} of miR-33 raise circulating HDL-C levels, enhance macrophage cholesterol efflux, and prevents progression of atherosclerosis. Such anti-atherosclerotic effects suggest antagonism and down-regulation of miR-33 as a novel strategy for atheroprotection.

10. Cholesterol handling in the brain

Brain relies heavily on cholesterol supply which is essential for cell membrane synthesis and myelin production. Strikingly, while the central nervous system accounts for only 2.1% of body weight, it contains 23% of the whole body cholesterol pool.\textsuperscript{163} Cholesterol transport mediated by lipoproteins has long been thought to underlie the proper functioning of the brain. Indeed, all major types of neuronal cells can bind and internalize lipoproteins present in the extracellular fluid.\textsuperscript{164}

Brain lipoproteins appear to be similar to those of cerebrospinal fluid,\textsuperscript{165,166} as a consequence of the passage between the two
compartments. Human cerebrospinal fluid primarily contains spherical lipoproteins of approximately 10–22 nm in diameter with a density of 1.063–1.25 g/mL, which corresponds to the density of HDL and very-HDL of human plasma.14,165 Lipoprotein concentrations in the cerebrospinal fluid are, however, several hundred-fold lower when compared with the plasma compartment.166,167 ApoE and apoA-I are the major apolipoproteins in human CSF, with slightly higher concentration of the former.167–169 ApoE- and cholesterol-rich lipoproteins secreted by astrocytes appear to ensure a continuous supply of cholesterol required for normal functioning of neurons.170 In addition, HDL obtained from cerebrospinal fluid efficiently effluxes cholesterol from neuronal cells.169 Similar to the plasma compartment, metabolism of brain lipoproteins is regulated by ABCA1, ABCG1, and other proteins known to participate in plasma lipoprotein metabolism.171–173 Available data indicate that metabolism of brain lipoproteins can be impaired in Alzheimer’s disease,14,165 as occurs in the presence of apolipoprotein E4, the primary genetic risk factor for the sporadic disease.

11. Concluding remarks

HDL particles display multiple biological activities which may contribute to the protection from atherosclerosis, diabetes, inflammatory diseases, and neurodegenerative disorders. Mechanisms underlying such protection may widely differ; the capacity to efflux cellular cholesterol may, however, underlie several effects of HDL, including anti-inflammatory, cytotoxic, vasodilatory, anti-thrombotic and, anti-diabetic actions. This observation may reflect distinct physicochemical properties of apoA-I, the major HDL apolipoprotein and a typical amphipathic protein that binds avidly to lipids. Similar mechanisms involving modulation of cell membrane lipid composition with subsequent effects on membrane-associated proteins may therefore form a basis of several HDL-mediated effects in vivo.

The multitude of HDL functions may also reflect a wide variety of protein and lipid components carried by HDL.174–179 Such compositional heterogeneity is consistent with a high level of functional heterogeneity among HDL particles. Indeed, despite the fact that all HDL subpopulations display multiple biological activities, many of such activities are enriched in small, dense, protein-rich HDL.178,180,181 The multiple biological functions of HDL may therefore be mediated by distinct particle subspecies defined by specific clusters of proteins and lipids.174,179

It remains indeterminate as to whether some biological activities of HDL are more relevant for atheroprotection when compared with others. The multitude of such activities though raises the question of the assessment of HDL functionality ex vivo. Measurement of any individual HDL activity will obviously provide only limited information on the total quality of the circulating HDL pool. An integrative assay is therefore needed which would ideally reflect all major facets of the functionality of circulating HDL. Such a single common denominator reflecting major HDL functions may not, however, be attainable and evaluation of specific biological activities of HDL will most probably remain the main approach to assess HDL quality in the near future.182 Numerous issues related to standardization, reproducibility, and reliability will have to be resolved in order to apply biological assays of HDL function to clinical practice. Identification of analytical biomarkers of multiple HDL functions that can serve as readily measurable surrogates for functional assays may therefore represent a viable alternative to biological activity assays and remains particularly attractive.60 When developed, such biomarkers will require rigorous evaluation in epidemiological studies and in clinical trials of HDL-targeted therapies.

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