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Anti-oxidant properties of high-density lipoprotein and atherosclerosis

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SUMMARY

1. High-density lipoprotein (HDL) is one of the major carriers of cholesterol in the blood. It attracts particular attention because, in contrast with other lipoproteins, many physiological functions of HDL influence the cardiovascular system in favourable ways unless HDL is modified pathologically.
2. The best known function of HDL is the capacity to promote cellular cholesterol efflux from peripheral cells and deliver cholesterol to the liver for excretion, thereby playing a key role in reverse cholesterol transport. The functions of HDL that have recently attracted attention include anti-inflammatory and anti-oxidant activities. High anti-oxidant and anti-inflammatory activities of HDL are associated with protection from cardiovascular disease.
3. Atheroprotective activities, as well as a functional deficiency of HDL, ultimately depend on the protein and lipid composition of HDL. Conversely, these activities are compromised in many pathological states associated with inflammation.
4. The focus of the present review is on the anti-oxidant and anti-inflammatory functions of HDL and its individual components in relation to protection from atherosclerosis.

Keywords

anti-oxidant; atherosclerosis; high-density lipoprotein; inflammation; oxidative stress

INTRODUCTION

High-density lipoprotein (HDL) is a plasma lipoprotein heterogeneous in origin, size, composition and function. It is one of the major carriers of cholesterol in the blood. In contrast with other lipoproteins, many physiological functions of HDL influence the cardiovascular system in favourable ways unless HDL is modified pathologically. The protective role of HDL in cardiovascular disease has been documented in multiple clinical and animal studies. High-density lipoprotein is best known as a key player in reverse cholesterol transport. In this process, HDL and its major apolipoprotein, apolipoprotein (apo) A-I, promote cholesterol efflux from peripheral tissues; HDL then carries cholesterol in the circulation and delivers it to the liver so that it can be either reutilized for assembly of very low-density lipoprotein (VLDL) or excreted as free cholesterol or bile acids. This function of HDL is central to its atheroprotective role. The other atheroprotective functions

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of HDL that have more recently attracted attention include its anti-inflammatory, anti-oxidant and vasodilatory properties.[1–3] In addition, HDL has been shown to possess anti-apoptotic, antithrombotic and anti-infectious functions among other actions.[1] Particular attention has focused on the small dense HDL, which is the most potent promoter of cholesterol efflux and has the highest anti-oxidative and anti-inflammatory activities. Some of these multiple functions are clearly overlapping. Moreover, recent progress in the identification of minor components in HDL indicates that not all functions of HDL are currently known.

COMPOSITION OF HDL

Proteins

Apolipoprotein A-I is the most abundant protein in HDL, accounting for approximately 70% of the HDL protein mass, with apoA-II being the second most abundant protein, accounting for 15–20%.[1] The remaining protein mass is made up of minor amphipathic proteins, such as apoC, apoE, apoD, apoM and apoA-IV, enzymes such as paraoxonase (PON) 1 and platelet-activating factor acetylhydrolase (PAF-AH), glutathione peroxidase (GPx) 1, and lipid transfer proteins such as lecithin:cholesterol acyl transferase (LCAT) and cholesteryl ester transfer protein (CETP).[1] The total number of minor proteins associated with HDL reaches as many as 75, as demonstrated in recent proteomic studies.[4–7] The molar concentration of most of these proteins in the plasma is lower than that of HDL, suggesting that specific proteins may be present only in particular HDL subclasses.

Lipids

In addition to free and esterified cholesterol, a number of other lipids are detected in HDL, including various phospholipids, such as phosphatidyl choline, phosphatidyl ethanolamine, lysophosphatidyl choline, plasmalogen, free fatty acids, mono-, di- and triacylglycerols, and different sphingolipids, such as ceramide, sphingomyelin species, sphingosine-1-phosphate (S1P), lysosulphatide and sphingosylpho-sphorylcholine.[8]

VARIABILITY IN HDL

Multiple, functionally distinct populations of HDL exist that differ in size, shape, protein and lipid composition. Surprisingly, although the heterogeneity of HDL particles is well known, in many pharmacological studies HDL is still regarded as a single entity whose plasma levels are reflected by HDL–cholesterol. Recent studies have addressed the hypothesis that the multiple biological functions of HDL are mediated by distinct particle subspecies defined by specific cluster(s) of bound proteins. It has been shown that proteins identified in HDL can be separated into several clusters according to their metabolic function, such as lipid transport and metabolism, markers of inflammation, protease inhibition, immune system and complement factors, coagulation and hormone binding.[6,7] In a more recent study, plasma HDL was subfractionated into five physicochemically defined particle subpopulations by isopycnic density gradient ultracentrifugation and the protein composition of the subfractions was assessed by mass spectrometry.[9] That study revealed that HDL-associated proteins were distributed in distinct patterns across the HDL particle subpopulations, including the dense HDL3 particle, known to be the most potent anti-oxidant HDL subfraction.[10] Proteins that were preferentially associated with the dense HDL3 subpopulation included apoL-I, apoF, PON1/2, phospholipid transfer protein (PLTP), apoJ, PON3, α_1-antitrypsin (A1AT), apoA-IV, apoM, apoD, serum amyloid α (SAA), albumin, fibrinogen, haptoglobin-related protein (Hrp), platelet basic protein (PBP) and transthyretin. The correlation of the relative abundance of each protein with anti-oxidative functionality of HDL revealed that apoL-I, PON1 and PON3 showed the most significant

correlations, whereas PLTP, apoM, apoJ, SAA4 and apoD showed correlations that were less significant.[9]

ANTI-OXIDANT FUNCTIONS OF HDL

The anti-oxidant properties of HDL *in vivo* can be separated into direct and indirect actions. High-density lipoprotein can directly inhibit oxidation of low-density lipoprotein (LDL; or other targets containing phospholipids). One example of a direct effect is the transfer of oxidation products from LDL to HDL so that HDL serves as a 'sink' for oxidized lipids.[11] In addition, inhibition of oxidative events and oxidative stress *in vivo* may be achieved indirectly via other functions of HDL, such as induction of cholesterol efflux and, in general, via 'anti-inflammatory' functions of HDL.[12] It has been shown that circulating HDL accumulates oxidized phospholipids, such as hydroperoxides, lysophosphatidylcholine (lyso-PC) and F2-isoprostanes.[11,13] The transfer of oxidized phospholipids serves several purposes. First, the transfer of lipid hydroperoxide from LDL, so-called 'seeding molecules', prevents the initiation of a free radical chain reaction of oxidation.[14] Second, some of the more advanced products of phospholipid oxidation serve as ligands for scavenger receptor type B (SR-B) and promote uptake of modified lipoproteins by macrophages as well as prothrombotic effects mediated by platelet scavenger receptor CD36.[15,16] Removal of these phospholipids will prevent these pathological activities. Interestingly, when oxidized phospholipids are present in HDL (in oxidized HDL), the receptors that recognize the particle may be different and the effects may be opposite to those of oxidized LDL.[17] Third, some of the products of oxidation are chemically reactive and can modify lysine residues in apoB, rendering the particle a ligand for SR-A, scavenger receptor expressed by endothelial cell (SREC) and some of the products of lipid oxidation (e.g. 4-hydroxynonenal, malondialdehyde and levuglandin).[18,19]

The transfer of oxidized phospholipids to HDL may be followed by subsequent degradation of oxidation products by HDL enzymes or by delivery to the liver for degradation. Hydrolysis of oxidized phospholipids in HDL leads to the destruction of pathological activity. In addition, in this process, chemically reactive fragments of esterified fatty acids are released from lipoproteins so that the lipoprotein is not modified and is not converted into a pro-atherosclerotic particle.[20]

Normal functional HDL has high levels of anti-oxidants and active anti-oxidant proteins and enzymes with high anti-oxidant potential and has anti-inflammatory activity. However, when anti-oxidant and anti-inflammatory functions of HDL are overwhelmed by pathological processes, such as inflammation, HDL is converted into a 'dysfunctional' pro-inflammatory particle.[2,12] This dysfunctional HDL is characterized by decreased levels and activities of anti-inflammatory and anti-oxidant factors, such as apoA-I and PON1. The dysfunctional HDL contains oxidized phospholipids and lysophospholipids, as well as pro-inflammatory proteins, such as serum amyloid A and ceruloplasmin. Functionally, it cannot promote cholesterol efflux effectively or prevent LDL oxidation. Many pathological processes associated with systemic inflammation are characterized by the presensce of dysfunctional or pro-inflammatory HDL, including chronic heart disease, metabolic syndrome, chronic kidney disease, infections and some rheumato-logical diseases.[12] In addition to compositional changes to HDL that occur under pathological conditions, the protein or lipid components of HDL can be modified chemically.[21] As a result, HDL may lose its normal function and acquire pathological functions. High-density lipoprotein is susceptible to oxidation/modification *in vitro* by a variety of oxidants, such as metal ions, peroxyl and hydroxyl radicals, aldehydes, various myeloperoxidase (MPO)-generated oxidants, lipoxygenase, phospholipase A_2, elastase, non-enzymatic glycation and homocysteinylation.[21] Recent studies have shown that MPO can selectively target apoA-I as

a result of direct binding and that it catalyses apoA-I nitration and halogenation within human atheroma.[22,23] *In vitro* studies have demonstrated that MPO-catalysed oxidative modification of HDL or apoA-I leads to the loss of ABCA1-dependent cholesterol efflux function of the lipoprotein and converts HDL into a pro-inflammatory particle.[22,23]

ANTI-OXIDANT AND ANTI-INFLAMMATORY PROPERTIES OF HDL COMPONENTS

A number of *in vitro* and *in vivo* assays have been developed that allow quantification of the anti- and pro-inflammatory properties of HDL. One cell-free assay uses the fluorescent probe 2′,7′-dichlor-fluorescein, which is sensitive to oxidized phospholipids, to measure the ability of HDL to prevent the formation of oxidized phospholipids in LDL.[24] This assay can also be used to evaluate the ability of HDL to degrade oxidized phospholipids that are already formed.[24] Cell-based assays can evaluate the capacity of HDL to inhibit monocyte chemotaxis in response to oxidized LDL[25] or to prevent upregulation of cell adhesion molecules on endothelial cells.[26] Finally, the capacity of HDL to promote cholesterol efflux from cultured cells *in vitro* and reverse cholesterol transport *in vivo* can also be measured.[27]

Apolipoprotein AI

Evidence is accumulating that apoA-I is the major anti-atherogenic and anti-oxidant factor in HDL. Apolipoprotein AI is a major player in reverse cholesterol transport. However, in addition, apoA-I can remove oxidized phospholipids from oxidized LDL as well as from cells.[14,25] Human HDL can also directly reduce cholesteryl ester hydroperoxides and phosphatidylcholine hydroperoxides via Met residues 112 and 148 of apoA-I.[28] The finding that recombinant HDL containing only apoA-I and 1-palmitoyl-2-oleoyl-phosphatidyl-choline (POPC) and no enzymes was as effective in preventing LDL oxidation as complete HDL supports a key anti-oxidant role for apoA-I.[29] That interesting study did not support significant roles for PAF-AH, PON1 or LCAT in the prevention of LDL oxidation by HDL.[29] Multiple *in vivo* studies have demonstrated that apoA-I is a potent anti-oxidative, anti-inflammatory and anti-atherosclerotic agent.[30–35] Recent clinical studies have demonstrated that although very high HDL–cholesterol and large-size HDL particles are associated with a twofold increase in cardiovascular risk,[36] a high concentration of apoA-I is an independent negative predictor of cardiovascular risk.[36,37]

Apolipoprotein A-II

It has been shown that apoA-II-enriched HDL from mice transgenic for human apoA-II protected VLDL from oxidation more efficiently than control HDL.[38] Moreover, basal lipoprotein oxidation in transgenic mice was not reduced, suggesting that human apoA-II-rich HDL may maintain adequate anti-oxidant potential. High-density lipoprotein enriched in human apoA-II supports effective reverse cholesterol transport from macrophages.[39] However, apoA-II was found to displace PON1 and PAF-AH from HDL.[38] This was suggested as a mechanism underlying the increased atherosclerosis seen in dyslipidaemic mice overexpressing either human or murine apoA-II.[40,41] Conversely, a recent prospective study demonstrated that plasma apoA-II concentrations are an independent factor negatively correlated with the risk of coronary artery disease (CAD).[37]

Apolipoprotein E

The anti-atherosclerotic activity of apoE related to receptor-mediated uptake of lipoprotein is well known. However, both murine and human apoE can inhibit the development of atherosclerosis in mice without any significant effect on hypercholesterolaemia.[42–44] Moreover, apoE has been shown to have allele-specific anti-oxidant activity.[45]

Apolipoprotein E2 stimulates endothelial nitric oxide (NO) release and has anti-inflammatory activities.[46] In contrast, apoE4 is pro-inflammatory.[47]

Apolipoprotein J

Apolipoprotein J is associated with a subset of small HDL containing PON1. It has been reported that HDL-associated apoJ can inhibit LDL oxidation by artery wall cells.[48] In addition, at low physiological levels apoJ has been reported to be cytoprotective.[49]

Apolipoprotein A-IV

Apolipoprotein A-IV has multiple activities related to lipid and lipoprotein metabolism. It can participate in reverse cholesterol transport by promoting cholesterol efflux via ABCA1[50] and by activating LCAT.[51] In addition, it has been demonstrated that ApoA-IV has anti-oxidant, anti-inflammatory and anti-atherosclerotic actions *in vivo*.[52–54]

Paraoxonases 1 and 3

Paraoxonases 1 is an HDL-associated enzyme with anti-oxidant properties. There are data suggesting that the direct anti-oxidant effect of HDL on LDL oxidation, measured as a reduction in lipid peroxides, is likely mediated by PON1.[55–57] However, it should be noted that the role of paraoxonases in the direct protection of LDL from oxidative stress is not firmly established at present.[1] Interestingly, it has been shown that PON1 enhances cholesterol efflux from macrophages by promoting HDL binding mediated by ABCA1.[58] This effect of PON1 can indirectly reduce pro-inflammatory signalling in cells *in vivo* and contribute to the anti-atherosclerotic effects of PON1.[59,60] Multiple *in vivo* studies support the hypothesis that PON1 protects against atherosclerosis. For example, in mice deficient in PON1 and on a high-fat diet, the average lesion area in the proximal aorta was almost twice that in control mice;[59] however, overexpression of human PON1 confers significant protection against the development of atherosclerosis in mice.[60] Moreover, signs of oxidative stress, vascular inflammation and thrombotic tendencies have been observed in PON1-deficient mice.[61] In human studies, higher PON1 activity is associated with a lower incidence of major cardiovascular events.[62] However, it remains uncertain whether this relationship is causal or correlational. A recent prospective study failed to demonstrate a causal relationship between PON1 activity and the risk of future CAD.[63] Pathological conditions associated with oxidative stress, such as chronic renal failure, rheumatoid arthritis and Alzheimer's disease, are frequently associated with reduced activity of PON1.[62] Furthermore, a pro-atherosclerotic high-fat diet leads to reduced PON1 activity.[62] In general, it has been shown that multiple factors (genetic, pathological, physiological, pharmacological and lifestyle) can change the PON1activity. Taking into account the growing evidence of the protective role of PON1 in CAD, this enzyme could be an important target for pharmacological regulation.

Paraoxonase 3 is also associated with HDL and has been shown to prevent the oxidation of LDL *in vitro*.[64,65] Furthermore, human PON3 transgenic mice have been shown to be protected from the development of atherosclerosis, without any significant changes in plasma lipoprotein cholesterol, triglyceride or glucose levels.[66] However, additional studies are needed to determine the role of *PON3* gene polymorphisms in cardiovascular disease.

Platelet-activating factor acetylhydrolase

Platelet-activating factor acetylhydrolase is another enzyme that is found in HDL that can hydrolyse oxidized phospholipids. There are data suggesting that PAF-AH, rather than PON1, is the major hydrolase in HDL responsible for the hydrolysis of oxidized phospholipids.[67,68] Lipoproteins isolated from mice expressing human PAF-AH are more

resistant to oxidative stress.[69] Moreover, murine HDL with human PAF-AH has been shown to inhibit foam cell formation and facilitate cholesterol efflux in macrophages.[69] Adenoviral gene transfer of human PAF-AH in apoE$^{-/-}$ mice increased circulating PAF-AH activity by 50% and was associated *in vivo* with reduced monocyte adhesion to the endothelium.[70] The b-migrating (b) VLDL isolated from these mice had a low capacity to induce monocyte adhesion *ex vivo*. In experimental atherosclerosis, gene transfer of *PAF-AH* inhibited lesion formation in apoE-deficient mice.[71] In arteries of non-hyperlipidaemic rabbits, local expression of PAF-AH reduced the accumulation of oxidatively modified LDL without changing plasma levels of PAF-AH and reduced the expression of endothelial cell adhesion molecules.[72] In humans, PAF-AH deficiency is associated with increases in cardiovascular disease; for example, missense mutation of the gene of the PAF-AH gene is an independent risk factor for coronary artery disease in Japanese men.[73] At the same time, circulating levels of PAF-AH serve as an independent marker of the risk of CAD.[74] Whether PAF-AH plays a causal role in cardiovascular disease or simply is increased in response to the chronic inflammatory environment needs to be established. It is believed that PAF-AH in the necrotic core of coronary lesions may contribute to inflammation and plaque vulnerability. For example, prolonged inhibition of PAF-AH with the oral inhibitor darapladib prevented necrotic core expansion in patients with CAD.[75] In conclusion, even though the data from animal studies suggest that PAF-AH is anti-atherosclerotic, the role of PAF-AH in human physiology is far from being completely understood.[73]

Glutathione peroxidase 1

Glutathione peroxidase 1, another enzyme detected in HDL, can reduce lipid hydroperoxides to corresponding hydroxides and thereby detoxify them.[76,77] Multiple clinical studies suggest an atheroprotective role for GPx-1.[78–82] Furthermore, two studies in apoE$^{-/-}$ mice have demonstrated that GPx-1 deficiency leads to increased atherosclerosis when mice are further challenged by a Western-type diet or made diabetic.[83,84] However, it should be noted that no protection from atherosclerosis has been found in GPx-1$^{-/-}$ mice fed a high-fat diet.[85] It seems that the role of GPx-1 in the development of atherosclerosis is particularly prominent under conditions of significant oxidative stress.

Lecithin: cholesterol acyltransferase

Lecithin:cholesterol acyltransferase has been shown to directly hydrolyse oxidized polar phospholipids.[86]

Sphingosine-1-phosphate

High-density lipoprotein is the most prominent plasma carrier of S1P. Moreover, many biological effects of HDL, such as the induction of endothelial NO production, vasodilation, survival and cardioprotection, are partially mediated by S1P. Approximately half the effects of HDL on the induction of endothelial NO synthase occur via S1P receptors.[87] In general, multiple effects of HDL on endothelial cells, such as migration, proliferation, endothelial integrity and angiogenesis, are mediated in part by S1P in HDL.[8] Furthermore, S1P in HDL inhibits pro-inflammatory responses, such as the generation of reactive oxygen species, activation of NAD(P)H oxidase and the production of monocyte chemoattractant protein-1, in vascular smooth muscle cells and the aorta.[88] In endothelial cells, S1P in HDL inhibits tumour necrosis factor-α-induced expression of cell adhesion molecules and promotes prostacyclin production.[89] It should be noted that in its free form (i.e. not associated with HDL), S1P is also involved in pro-inflammatory processes.[88]

In summary, high anti-oxidant and anti-inflammatory activities of HDL are associated with protection from cardiovascular disease. These activities depend on the protein and lipid composition of HDL and are the highest in small dense HDL. These activities are

compromised in many pathological states associated with inflammation. The functional deficiency of HDL is intimately associated with changes in HDL composition. The search is continuing to find the best approaches for the prevention of the loss or the restoration of the anti-oxidant and anti-inflammatory potential of HDL.

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