Dysfunctional HDL as a diagnostic and therapeutic target

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Abstract

The atheroprotective effects of HDL are mediated by several mechanisms including its role in reverse cholesterol transport and via its anti-inflammatory properties. However, not all HDL is functionally similar. HDL and apolipoprotein A-I may become dysfunctional or even pro-inflammatory, and thus promote atherosclerosis. ApoAI post-translational modification can have a large impact on its function. Myeloperoxidase modification of apoAI impairs it function as a cholesterol acceptor, and the molecular changes induced by myeloperoxidase have been studied in detail. These studies provide the basis for the development of an oxidant resistant form of apoAI and clinical measures of HDL modification and dysfunction, which may be useful as a treatment criterion.

Keywords

HDL; apolipoprotein A-I; reverse cholesterol transport; myeloperoxidase; anti-inflammatory

HDL-cholesterol (HDL-C) is an independent protective factor for coronary artery disease (CAD), as demonstrated by numerous epidemiological studies, such as the Framingham Heart Study[1]. In interventional settings, niacin, which effectively raises HDL levels, given as a monotherapy or combined with other drugs has been shown to reduce CAD and cerebrovasuclar events[2]. There are many proposed pathways by which HDL may protect against CAD. HDL and its major protein constituent apolipoprotein A-I (apoAI) play prime roles in mediating reverse cholesterol transport (RCT), the transfer of cholesterol from peripheral tissues, such as arterial wall cells, to the liver for excretion, and this likely plays a central role in the mechanism of HDL's atheroprotective effect[3,4]. RCT involves many steps, including the removal of cholesterol from cells (through ABCA1, which is responsible for the formation of nascent HDL, along with ABCG1, and SR-BI), the maturation of HDL through lecithin cholesterol acyltransferase (LCAT) mediated free cholesterol esterification, and the uptake and excretion of HDL cholesterol by the liver.

HDL cholesterol esters can also be transferred to apoB containing lipoproteins through the action of cholesterol ester transfer protein (CETP), and this cholesterol pool can participate in RCT by delivery to the liver through LDL receptor mediated uptake. Although CETP inhibition raises HDL-C levels, the recent failure of torcetrapib, a CETP inhibitor, in a clinical outcomes trial[5] raised the specter that CETP inhibition may lead to the production of dysfunctional HDL. However, due to the off target effects of torcetrapib on blood pressure and the plasma levels of potassium, sodium, bicarbonate, and aldosterone it is impossible to determine if the failure of this drug could be due to the formation of dysfunctional HDL[5]. In a post hoc analysis of the torcetrapib study by intravascular ultrasound, atheroma regression, rather than progression, was observed in the subjects who achieved the highest levels of HDL-C, suggesting that CETP inhibition does not lead to dysfunctional HDL[6].

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HDL has many other activities aside from its role in RCT that can also contribute to its protective mechanism. These include the removal and/or detoxification of oxidized sterols and phospholipids, anti-inflammatory activity, antioxidant activity, anti thrombotic activity, and its protective and healing activities on endothelial cells[3,7,8]. So, HDL-cholesterol is the "good cholesterol", but there are many unresolved questions: are all HDL particles equally good, does HDL from different subjects perform the same; can HDL become dysfunctional; and, can "good cholesterol" be made into "better cholesterol"? This review describes the evidence for dysfunctional HDL and its underpinnings, as well as the potential use of HDL functionality as a diagnostic tool and therapeutic target.

Evidence for and causes of dysfunctional HDL

Fogelman and colleagues developed an assay to measure the anti-inflammatory activity of HDL by determining the extent to which HDL inhibits monocyte chemotaxis induced by LDL using an in vitro reconstituted artery wall model by the co-culture of smooth muscle cells and endothelial cells[9]. Other assays for HDL function include cell free anti-oxidant activity[10], inhibition of endothelial cell adhesion molecule expression[11], and the ability of HDL to act as an acceptor of cellular cholesterol. Navab, Fogeleman, and colleagues using this artery wall model system have demonstrated several conditions in which HDL can lose its anti-inflammatory properties, including during the acute phase response when serum amyloid A partially replaces apoAI in HDL, during influenza A infection, and in apolipoprotein AII transgenic mice[12,13,14]. In a seminal study, Ansell et al. discovered that HDL from CAD subjects who had high levels of HDL had less anti-inflammatory activity, in this co-culture assay, than HDL derived from healthy control subjects, thus indicating that HDL from CAD subjects is dysfunctional[10]. In fact, HDL from many CAD patients was actually proinflammatory, thus increasing monocyte chemotaxis in response to LDL, unlike the HDL from healthy controls that reduced monocyte chemotaxis[10]. There are many possible alterations between this dysfunctional HDL and normal functional HDL.

One possibility is a change in the protein composition of HDL. Human HDL particles are quite heterogeneous, encompassing a range of sizes and densities. Each HDL particle carries apoAI and may also carry other apolipoproteins, such as apoAII, apoAIV, apoE, apoCs. In addition, HDL is associated with a panoply of accessory proteins, including LCAT, phospholipid transfer protein (PLTP), paraoxonase 1 (PON1), myeloperoxidase (MPO), serum amyloid A1, (SAA1), and platelet activating factor acetylhydrolase (PAF-AH). The HDL proteome is quite large[15], and each HDL particle cannot accommodate all of the accessory proteins; thus, a minority of the HDL particles must be associated with each accessory protein. Changes in HDL protein composition due to infection, inflammation, or diabetes have been shown to be associated with decreased function[12,14,16]. An HDL proteomic study of HDL_3 from different subjects found that the levels of PON1, PON3, and apoL-I correlate with HDL's antioxidant activity[17]. Also, mouse models have been used to determine the effects of HDL protein composition changes, for example, HDL isolated from transgenic mice over expressing apoAII or PLPT show reduced function compared to control HDL[13,18].

Many studies have been performed to investigate the changes in HDL associated with inflammation. de Beer and colleagues have shown extensive HDL remodeling after treatment of mice with endotoxin, or in humans after surgery, leading to the so-called acute phase HDL, in which SAA and group IIa secretory phospholipase A2 ($sPLA_2$-IIa) are increased and apoAI levels (and CETP levels in humans) are decreased[19]. Since SAA itself can act as an acceptor for cellular cholesterol via ABCA1, SR-BI, and other pathways[20,21,22], the consequences of acute phase HDL on HDL function and RCT must be determined experimentally. Post surgical human serum containing acute phase HDL maintains most of its ABCA1 and ABCG1 dependent cholesterol acceptor activity despite decreased HDL-C and apoAI levels[23]. The in

vitro incubation of HDL with SAA, sPLA2-IIa, and CETP leads to the marked displacement of apoAI from HDL generating lipid free or lipid poor apoAI, expected to undergo more rapid catabolism, but also expected to be a good ABCA1-mediated acceptor of cellular lipids[23]. The over expression of SAA and SR-BI in mice via adenoviral vectors also leads to the production of small lipid poor apoAI particles which are more rapidly cleared[24]. Recently, Reilly's lab demonstrated that LPS treatment in mice decreases RCT, although this was mediated primarily through the inhibition of liver sterol excretion associated with decreased expression of hepatic ABCG5, ABCG8, and ABCB11[25].

Another factor that might make HDL dysfunctional is a change in the HDL associated lipids. HDL may acquire oxidized lipids from cells and by exchange with other particles, or HDL lipids may be oxidized in situ. Lipid peroxides may interfere with HDL's antioxidant, anti-inflammatory, and cholesterol acceptor activities. For example, treatment of HDL with 15-lipoxygenase, an enzyme forming lipid peroxides, reduces HDL cholesterol acceptor and anti-inflammatory activities[26,27]. HDL carries the bulk of lipid hydroperoxides in plasma[28]. These lipid hydroperoxides can be converted into less reactive lipid hydroxides through specific methionine residues of apoAI and apoAII, with the concomitant formation of methionine sulfoxide[29]. Fully functional HDL may promote lipid hydroperoxide metabolism and its uptake and clearance by the liver, which would be protective[28]. In contrast, dysfunctional HDL might promote the transfer of lipid hydroperoxides to apoB containing lipoproteins and promote VLDL and LDL oxidation[30].

A prominent factor that can lead to HDL dysfunction is the post-translational modification of apoAI. Copper mediated oxidation of HDL leads to altered HDL migration on an agarose gel, apoAI proteolysis, and decreased ability of HDL to unload cholesterol esters from cholesterol loaded macrophages[31]. Malondialdehyde modification of HDL leads to similar changes in regard to HDL structure and function, and is associated with the loss of lysine and tryptophan residues, and apoAI polymerization[32]. HDL from diabetic subjects has evidence of glycated apoAI and apoAII, and this glycated apoAI has altered structure and lipid binding activity[33, 34]. Similarly, HDL incubated with high glucose results in a reduction in paraoxonase activity and decreased anti-inflammatory activity[35]. Lipid peroxides added to human plasma can covalently modify apoAI[36]. Also, incubation of HDL with activated neutrophils was shown to render apoAI more negatively charged and decrease its cellular cholesterol acceptor activity[37,38].

Myeloperoxidase modification of apoAI

Myeloperoxidase (MPO) is an enzyme found in neutrophils, monocytes, and some macrophages that uses hydrogen peroxide to generate chlorinating and nitrating oxidants, which play an important role in killing microorganisms. However, these same reactive species can also modify host proteins and lipids. MPO is enriched in human atheroma [39], and its presence may promote lesion progression, by increasing LDL oxidation, and block plaque regression, by modifications of apoAI/HDL that impair RCT. Bergt et al. demonstrated that incubation of HDL with MPO, in a chlorinating reaction ($MPO/H_2O_2/Cl^-$) that produces HOCl (chlorine bleach), or with reagent HOCl (at a very high 100:1 ratio of HOCl:apoAI) leads to a loss of unsaturated fatty acids in phospholipids and cholesterol esters, and the loss of cholesterol acceptor activity[40]. Bergt et al. also showed that modification of lipid-free apoAI at an HOCl:apoAI ratio of 25:1 led to the loss of apoAI methionine residues[40], consistent with the high sensitivity of methionine to $MPO/H2O2/Cl^-$[41]. Subsequently, it was shown that selective apoAI methionine oxidation does not disrupt its structure, and actually leads to an increase in lipid binding and cellular lipid acceptor activities[42]; although apoAI Met-148 modification by MPO is associated with loss of LCAT activation[43]. Bergt et al. also used mass spectroscopy to identify specific alterations caused by reagent or MPO generated HOCl and they confirmed

the disappearance of the methionine-containing tryptic peptides with concomitant production of methionine sulfoxide-containing peptides44. The cholesterol acceptor activity of recombinant HDL (rHDL) made by cholate dialysis using apoAI and phosphatidylcholine is much more sensitive to the HOCl treatment, with loss of activity starting at an HOCl:apoAI ratio of 5:140. In addition to decreased lipid acceptor activity, Panzenboeck et al. also showed that MPO or HOCl modified HDL was more susceptible to uptake and degradation by macrophages, thus turning HDL from a lipid accepting lipoprotein to a lipid loading lipoprotein45. Although methionine and cysteine residues (apoAI has no Cys residues) are the most sensitive to MPO generated oxidants, tyrosine, lysine, tryptophan, and other residues are also targets, with 3-chlorotyrosine being used as a fingerprint for MPO oxidants, as HOCl uniquely forms this adduct46,47.

Since 2004, the combined Smith, Hazen, and Kinter labs at the Cleveland Clinic, and the combined Heineke and Oram labs at the University of Washington have each examined the MPO induced molecular alterations in apoAI and their functional significances. Both teams fundamentally agree that MPO modification of apoAI is physiologically relevant by demonstrating that: 1) apoAI in plasma and more so in arterial lesions is a selective target of MPO modification that leads to the nitration and chlorination of specific apoAI tyrosine residues; 2) plasma apoAI nitro-and chloro-tyrosine levels are higher in coronary artery disease patients than in control subjects; and 3) apoAI tyrosine chlorination, whether in endogenous plasma or after in vitro MPO-mediated modification, is associated with the specific loss of ABCA1-mediated cholesterol acceptor activity, such that apoAI cholesterol acceptor activity was inversely correlated with apoAI nitro- and chloro- tyrosine levels48,49,50,51,52,53. Wu et al. demonstrated LCAT binding to apoAI at residues 159–180 in rHDL; and, that Tyr166 is required for LCAT activation, such that MPO induced loss of LCAT activation correlates with loss of the Tyr166 containing parent peptide54. Additionally, Zheng et al. demonstrated direct binding of MPO with apoAI, and identified residues 190–203 in apoAI helix 8 as the region that bind to MPO, thus providing a mechanism for the selective modification of apoAI in plasma48. However, the precise mechanism by which MPO modification renders apoAI dysfunctional remains controversial.

Both groups using mass spectroscopy studies identified apoAI Tyr192, within helix 8, as the most sensitive site to MPO mediated chlorination 51,52. Furthermore, Bergt et al. demonstrated, using short synthetic peptides, that tyrosine chlorination is maximally stimulated when the tyrosine is three residues away from a lysine residue, which is the case for the sensitive Tyr192 53. The University of Washington group claimed that MPO impairment of apoAI's ABCA1-mediated cholesterol acceptor activity is through methionine oxidation and site-specific chlorination of Tyr192, as treatment of MPO modified apoAI with the enzyme methionine sulfide reductase could partially restore apoAI's cholesterol acceptor activity, particularly in a recombinant apoAI (r-apoAI) isoform with Try192 replaced by Phe43. On the other hand, the Cleveland Clinic group made r-apoAI with all seven Tyr residues replaced by Phe (7YF isoform), and found that it is equally susceptible to MPO or HOCl loss of cholesterol acceptor activity as the wild type isoform55. Furthermore, replacement of the three Met residues in apoAI with Val residues actually increased susceptibility to low doses of H_2O_2 in the complete MPO chlorination system, showing that apoAI Met residues can absorb HOCl oxidation without loss of function, hence protecting the other residues56, and in agreement with the prior finding42. Thus, the site directed substitution and cholesterol efflux data indicate that neither methionine nor tyrosine serve as the oxidant sensitive residue involved in MPO-induced apoAI inactivation. Thus, the levels of plasma apoAI nitro-and chloro-tyrosine may serve as physiological useful and specific markers of MPO activity in vivo, even though tyrosine modification does not appear to be responsible for turning the "good" HDL-cholesterol "bad".

Discovery of MPO-resistant apoAI

The Cleveland group extended and confirmed several prior observations on the effects of MPO or HOCl on apoAI structure, specifically the loss of alpha helix content, and the loss of tryptophan and lysine residues. MPO treatment of apoAI leads to the transient conversion of apoAI lysine residues to lysine ε-chloramine that spontaneously decompose to α-aminoadipic acid, a product that is enriched in apoAI recovered from atheroma vs. plasma[55,56]. However, apoAI with lysine residues modified and rendered less reactive by reductive methylation is not protected from MPO induced loss of function[57,56]. Thus, lysine modification by MPO is not likely to be the initiating event in formation of dysfunctional HDL. The Cleveland group used mass spectrometry to identify mono- and di-hydroxytryptophan at all four Trp positions in apoAI isolated from human atheroma[56]. Thus, Peng et al. created r-apoAI with all four Trp residues replaced. The replacement by four Leu residues leads to an inactive 4WL isoform, while the replacement by four Phe residues creates an active 4WF isoform[56]. Thus, apoAI activity appears to require both hydrophobic and aromatic residues in the Trp positions. When the 4WF and wild type apoAI isoforms were treated with increasing oxidant stress from MPO or HOCl, the 4WF isoform is markedly resistant to loss of cholesterol acceptor activity[56]. Thus, the 4WF apoAI isoform is MPO resistant, and may be a better therapeutic reagent to promote the regression of atherosclerotic plaques, an environment where MPO and MPO-derived oxidants are abundant. If this strategy is successful, then "good" cholesterol can be made "better".

Diagnostic use of dysfunctional HDL

Figure 1 summarizes the central role that inflammation plays in creating dysfunctional HDL and reduced RCT. Biomarkers associated with dysfunctional HDL may be useful for identifying subjects at most risk for a coronary event. Hazen and colleagues reported that plasma MPO levels are positively associated with CAD and the risk of a subsequent major adverse cardiac event [58,59]. MPO levels are predictive of events, but are not a specific marker of dysfunctional HDL. In a mixed cohort of cardiovascular disease subjects and controls, Zheng et al., using mass spectrometry, demonstrated that the level of apoAI chlorotyrosine was a better predictor of cardiovascular disease than the levels of total plasma chloro-or nitro-tyrosine [48]. This type of assay is more specific for dysfunctional HDL, but is too laborious for routine clinical use. The identification of specific modified residues in apoAI, such as chlorotyrosine and hydroxytryptophan make it possible to develop novel and scalable immunoassays specific for oxidized/dysfunctional HDL. Through the development these new assays, it will be possible to perform observational and interventional studies to determine the association of dysfunctional HDL with CAD, cerebrovascular events, and mortality. Just as the recent JUPITER trial demonstrated the benefit of statin treatment for subjects with elevated CRP and normal LDL levels[60], in the future, dysfunctional HDL levels may be used as a criterion for treatment to prevent CAD.

Acknowledgments

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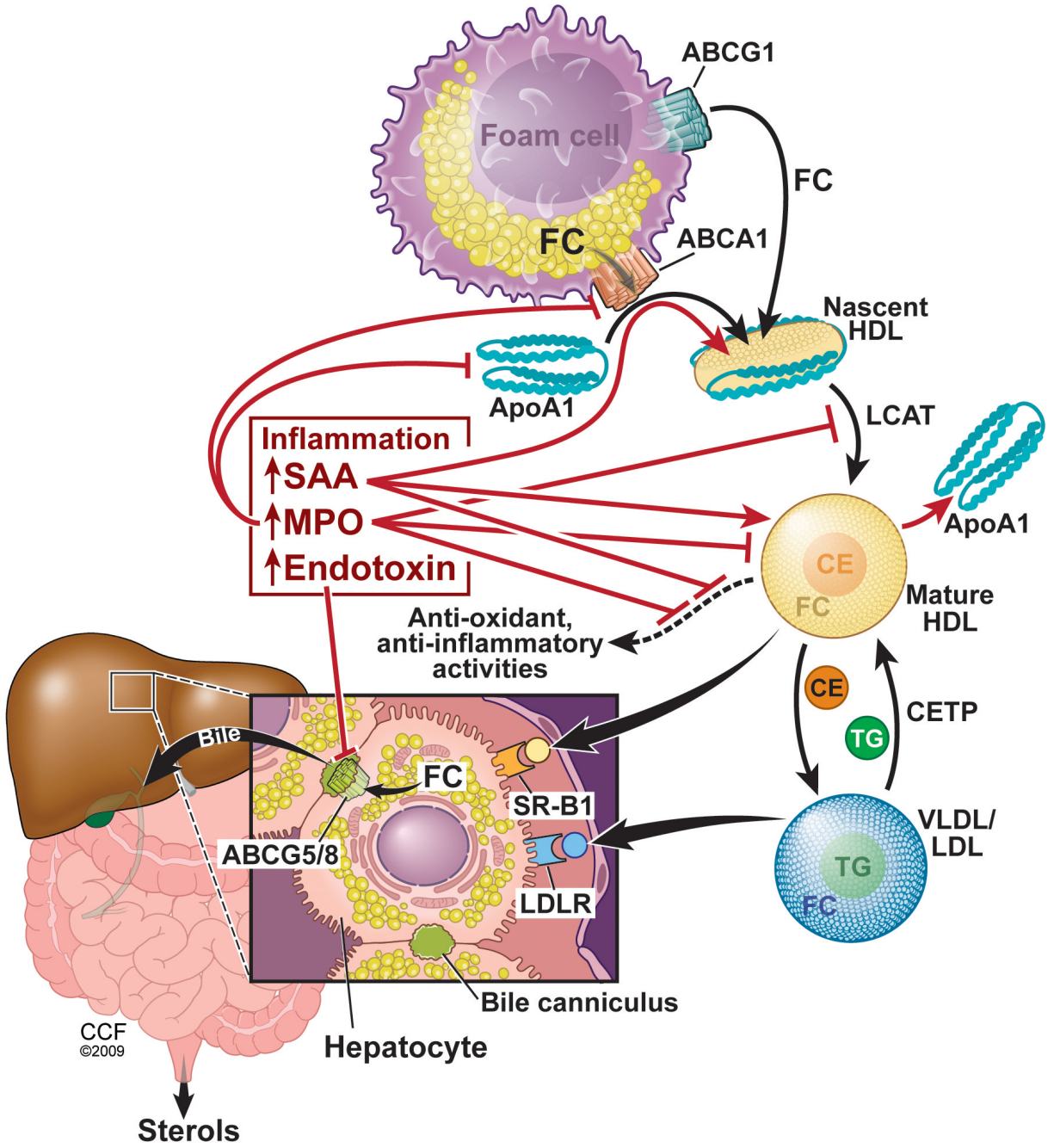
Figure 1.
Central role of inflammation in creating dysfunctional HDL and disrupting RCT. Cholesterol/ sterol trafficking from the foam cell to their excretion is shown in black arrows. The effects of inflammation are shown in red. FC, free cholesterol; CE, cholesterol ester; TG, triglycerides.