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REVIEW

High-density lipoproteins: an emerging target in the prevention of cardiovascular disease

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High-density lipoproteins (HDLs) have been well established to protect against the development of atherosclerotic cardiovascular disease. It has become apparent that in addition to the promotion of reverse cholesterol transport, HDLs possess a number of additional functional properties that may contribute to their beneficial influence on the arterial wall. A number of exciting therapeutic strategies have been developed that target HDL and its ability to protect against the development of atherosclerotic plaque. This paper will review how the promotion of the functional properties of HDL inhibits the formation of atherosclerotic plaque and stabilises lesions in patients with established disease.

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Introduction

The last two decades has seen a large body of evidence supporting a role for aggressive modification of established risk factors in preventing clinical events attributable to atherosclerotic coronary heart disease (CHD). As a result, a number of pharmacological agents have become integral components of cardiovascular preventive strategies. This prompted two Nobel laureates to propose that CHD would be eradicated by the early stages of this century [1]. While these agents have made a substantial impact, it has become apparent that many patients continue to experience clinical events. There is an ongoing need to identify new therapeutic targets to further reduce clinical risk. High-density lipoprotein-cholesterol (HDL-C) has emerged as an attractive target in the search for new pharmacological strategies.

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What are HDLs

HDLs represent the plasma fraction of lipoproteins in the density range 1.063-1.21 mg/mL. The HDL fraction includes a wide range of circulating particles that demonstrate marked heterogeneity in terms of their shape, size, surface charge and lipid composition. The basic structure involves a lipid core surrounded by surface containing a phospholipid bilayer, free cholesterol and a number of apolipoproteins (A-I, A-II, A-IV, C, D, E and J). The heterogeneous nature of circulating HDL results from the constant remodelling of particles in response to a range of plasma factors [2].

Protective properties of HDL-C: lessons from human studies

Fifty years have passed since the initial report that patients presenting with a myocardial infarction had lower levels of the lipoprotein fraction that demonstrated α-migrating mobility on gel electrophoresis, later identified as HDL-C [3]. A number of large population studies have reported that the incidence of clinical CHD was inversely correlated with plasma levels of HDL-C [4, 5]. For every 1 mg/dL increase in plasma HDL-C, the incidence of clini-

cal events declines by 2-3%. In fact, plasma HDL-C was found to be the strongest biochemical predictor of clinical events in the Framingham Heart Study [5]. Plasma HDL-C levels were also found to be an important predictor of event rates in clinical trials testing the impact of lipid lowering therapies. In trials of both statins [6, 7] and fibrates [8], the event rate in placebo-treated patients (and therefore the relative risk reduction seen with active treatment) was greatest in those subjects with a low plasma HDL-C level at baseline.

Protective properties of HDL: lessons from animal studies

A large body of evidence has emerged from animal studies to suggest that elevation of HDL-C is protective. Badimon and colleagues were the first to report that administration of HDL-C inhibited lesion formation and promoted regression of pre-existent lesions in cholesterol-fed rabbits [9, 10]. Since that time, a number of groups have reported that elevation of plasma HDL-C by either infusion of HDL or apolipoprotein A-I (apoA-I, the predominant protein of HDL) [11-15] or transgenic expression of human apoA-I [16-18] has a beneficial impact on lesion size in animal models of atherosclerosis. In addition to a favourable effect on the early stages of atheroma formation, elevation of HDL-C by either transgenic expression of apoA-I [18] or infusion of synthetic HDL-containing apoA-I Milano (AIM) [12], a variant of apoA-I, reduced the size, lipid and inflammatory composition of established lesions.

HDL facilitates reverse cholesterol transport

While a number of functional properties of HDL have been described (Figure 1), the best characterised is its pivotal role in the promotion of reverse cholesterol transport, the process by which cholesterol is mobilised from peripheral tissue to the liver [19]. HDL is the primary acceptor of cholesterol effluxed from peripheral cells via a number of mechanisms including passive diffusion across the cellular membrane and active transport by a family of transmembrane ATP-binding protein channels [20]. The best-characterised member of this family, ABCA1, preferentially effluxes cholesterol to lipid deplete or free forms of apoA-I [21]. Patients with Tangier's disease, a syndrome characterised by low plasma levels of HDL-C and impaired cholesterol efflux, are deficient in ABCA1 [22]. It has recently been reported that other ATP-binding proteins, ABCG1 and ABCG5 [23, 24], are involved in the efflux of cholesterol to lipidated HDL particles. Immense interest has been focused on the development of pharmacologic strategies that promote the expression of these transmembrane proteins and thus facilitate reverse cholesterol transport.

Once cholesterol has been effluxed to the HDL particle, it is rapidly esterified by lecithin:cholesterol acyltransferase, and subsequently stored within the core of the HDL-C particle. As the particle surface remains relatively deplete of cholesterol, this maintains the gradient driving efflux from cells to the HDL particle. Cholesterol is subsequently transported to the liver where it is taken up by the scavenger receptor SR-BI. Alternatively, esterified cholesterol is transferred to apoB-containing lipoproteins, such as very low-density lipoproteins and low-density lipoproteins (LDL), in a process facilitated by cholesteryl ester transfer protein (CETP). Following transfer to apoB-containing lipoproteins, cholesterol is either taken up by the liver via the LDL receptor or delivered to cells in the periphery. In the liver, the cholesterol is used for either lipoprotein synthesis or is excreted in the bile salts.

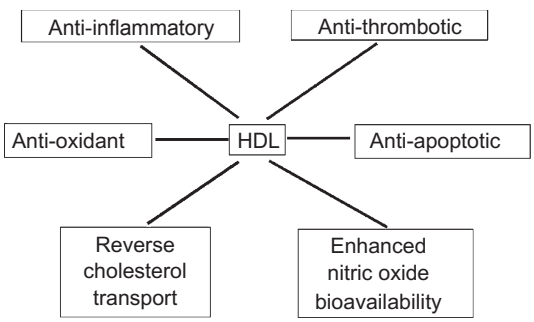

Figure 1 Potential antiatherosclerotic functional properties of HDLs.

Functional properties of HDL beyond lipids

Antioxidant properties

HDL attenuates the bioavailability of a number of prooxidant species that have been implicated in the propagation of atherogenesis. HDL inhibits the oxidative modification of LDL [25]. Non-modified forms of LDL are not proatherogenic [26]. The finding that HDLs possess a number of antioxidant factors, including paraoxonase and platelet-activating factor – acetylhydrolase [27], and are the major *in vivo* sink for lipid hydroperoxides [28], provides a number of potential mechanisms by which oxidative modification of LDL is impaired. Administration of HDL in both cellular [29] and animal [30] settings has also been reported to inhibit the generation of reactive oxygen species, such as superoxide, and restore its physiological balance to nitric oxide (NO) (Figure 2).

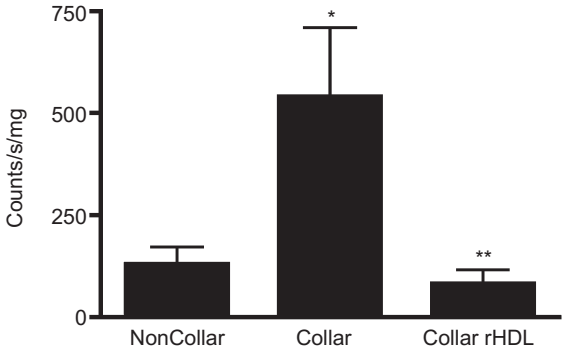

Figure 2 Expression of reactive oxygen species in the arterial wall detected by lucigenin chemiluminescence. The increased expression stimulated by application of a periarterial collar was attenuated by administration of rHDL. *p<0.01 for comparison with non-collared vessels and **p<0.001 for comparison with saline-infused animals (adapted from Circulation 2005; **111**:1543-1550).

Anti-inflammatory properties

HDLs modulate a number of inflammatory events that participate in the formation of atherosclerotic plaque and its evolution to clinical ischemia. HDLs inhibit the expression of proinflammatory adhesion molecules [31-33] and chemokines by endothelial cells and subsequent monocyte chemotaxis [34], key early events in atherogenesis. These *in vitro* properties have been reported to differ markedly between HDL isolated from different subjects [35, 36]. The finding that altering the phospholipid composition of reconstituted HDL influenced their ability to inhibit adhesion molecule expression provides one potential mechanism for this heterogeneity [37]. These benefits have been extended to the *in vivo* setting, where elevation of HDL-C resulted in a reduction in the vascular expression of adhesion molecules and chemokines and infiltration of inflammatory cells into the arterial wall [12, 14, 18]. Given that these findings are derived from studies involving hypercholesterolaemic animals, it has been proposed that the anti-inflammatory properties are simply a consequence of cholesterol efflux from the arterial wall.

HDL was recently demonstrated to possess anti-inflammatory properties in a normocholesterolaemic model of acute vascular inflammation [30]. The vascular infiltration by neutrophils and endothelial expression of adhesion molecules stimulated by application of a periarterial collar was markedly attenuated by infusion of rHDL to chow-fed rabbits (Figure 3). Given that the animals had low systemic cholesterol levels, it is unlikely that there was a substantial degree of cholesterol efflux from the arterial wall, supporting a primary anti-inflammatory role of HDL *in vivo*.

Inflammatory factors play a major role in determining the propensity of established atherosclerotic plaque to rupture and promote thrombus formation. Elevation of HDL-C, via infusion of HDL [12] or transgenic expression of apoA-I [18], results in a reduction of macrophages and chemokines within established atherosclerotic lesions. This was further supported by the recent report that infusion of small amounts of reconstituted or native HDL, without an increase in systemic HDL-C levels, rapidly reduces lesion size (Figure 4) and increases the ratio of smooth muscle cells to macrophages (Figure 5) in a model of established atherosclerotic plaque, induced by aortic balloon denudation in the cholesterol-fed rabbit [38]. This suggests that the quality of HDL that is administered, rather than the systemic level of HDL-C achieved, is of particular importance in potential remodelling of established atherosclerotic plaque and its proclivity to result in clinical ischaemia.

Other properties

HDL has been reported to possess a number of potentially antithrombotic properties including inhibition of platelet activation and promotion of endogenous fibrinolytic and anticoagulant systems [39]. The finding that HDL increases

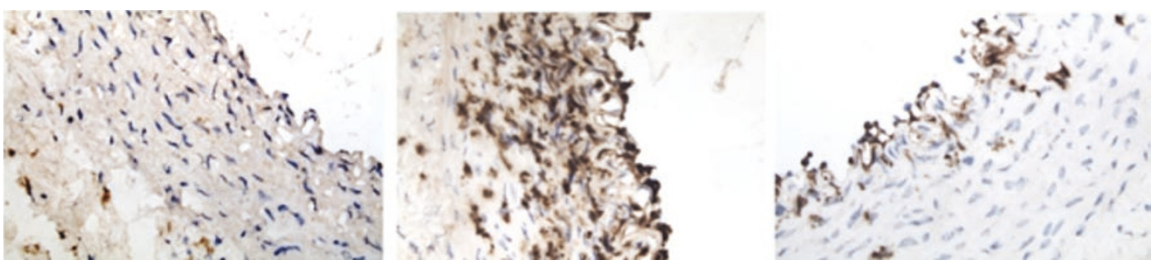

Figure 3 Infiltration of neutrophils into the vascular wall of carotid arteries without (left panel) and with application of a periarterial collar (middle panel) in animals infused with saline. The collar-induced neutrophil influx is markedly attenuated by infusion of animals with reconstituted HDL (right panel) (adapted from Circulation 2005; **111**:1543-1550).

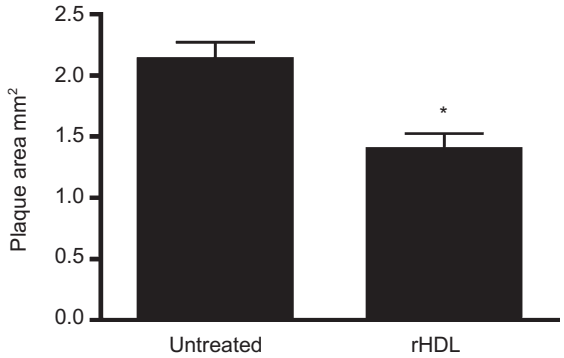

Figure 4 Atherosclerotic lesion size in the abdominal aorta in rabbits that received no treatment or infusions of reconstituted HDL. *p<0.05 for comparison with untreated animals (adapted from Arterioscler Thromb Vasc Biol 2005; **25**:2416-2421).

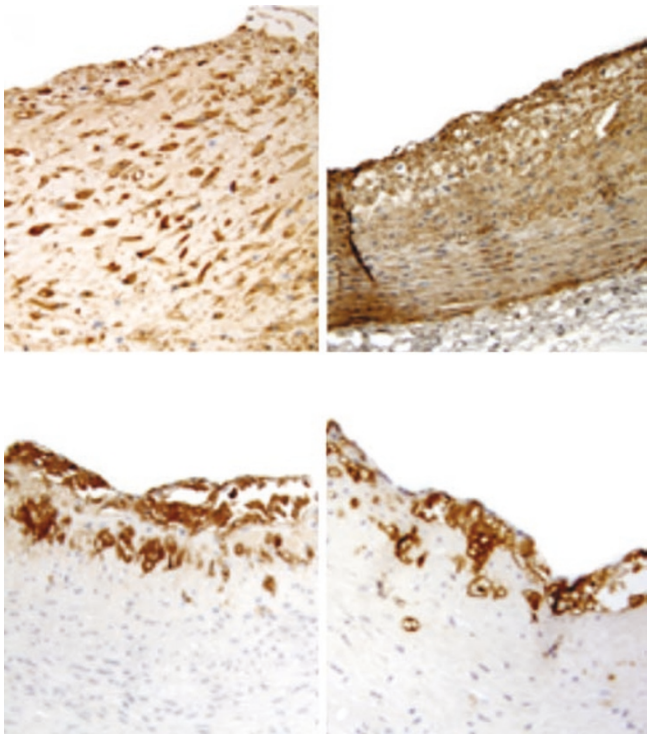

Figure 5 Representative staining of atherosclerotic lesions for smooth muscle cells (upper panels) and macrophages (lower panels) in animals that received no treatment (left panels) or infusions of reconstituted HDLs (right panels) (adapted from Arterioscler Thromb Vasc Biol 2005; **25**:2416-2421).

NO synthesis [40] may also contribute to an antithrombotic environment. Enhanced NO production results from interaction of HDL with the scavenger receptor in caveolae resulting in increased NO synthase activity [41]. Reports that HDL improves reactivity of vascular rings in organ bath studies [42, 43] was extended to the human setting where infusion of rHDL restores endothelial function in subjects with hypercholesterolaemia [44] or low levels of HDL-C in the setting of heterozygous ABCA1 deficiency [45]. Reduced tissue injury in models of ischaemia-reperfusion [46, 47] and shock in the setting of both hypovolemia [48] and sepsis [49] is likely to result from the combination of antioxidant, anti-inflammatory and NO-enhancing activities of HDL. HDLs have also been reported to possess antiapoptotic activity [50-53], although the underlying mechanism for this property remains unresolved.

Dysfunctional forms of HDL-C

The finding that many subjects with plasma HDL-C levels that are considered to be normal or elevated have CHD [54] highlights an interesting paradox. Is it possible that not all HDL particles are protective? A number of groups have reported that when HDL particles contain apoA-II, its cholesterol efflux, antioxidant and anti-inflammatory properties are less than that seen with apoA-I-only-containing particles [55]. As a result, it has been reported that apoA-II may be either less atheroprotective or potentially proatherogenic [55, 56]. Further, the ability of HDL to inhibit *in vitro* monocyte chemotaxis following stimulation with oxidised LDL is impaired in the setting of the acute phase response to influenza in mice [57]. It has also been reported that anti-inflammatory properties of HDL vary widely among different human subjects [35]. In fact, it was demonstrated that HDL isolated from subjects with elevated plasma HDL-C levels and CHD actually promoted, rather than inhibited, monocyte chemotaxis [36].

A potential mechanism underlying the heterogeneity in the functional properties of HDL is the degree of oxidative modification. Oxidative modification of HDL, either directly [58] or in the setting of glycation [59, 60], has been reported to be associated with impaired cholesterol efflux capacity. It has been speculated that oxidation of various phospholipid species on HDL may contribute to its degree of functional activity [61]. In addition, it has been recently reported that apoA-I is a selective target for modification by myeloperoxidase (MPO)-generated oxidants in the *in vivo* setting [62-66]. ApoA-I isolated from serum of patients with CHD contained greater amounts of nitrotyrosine and chlorotyrosine, oxidative products of MPO, than circulating apoA-I from healthy controls. ApoA-I isolated from atherosclerotic plaque demonstrated substantially greater amounts of these oxidative products, suggesting that modification occurs preferentially within the arterial wall. Subsequent studies revealed that increasing oxidative

modification of apoA-I is accompanied by an increasing impairment of ABCA1-dependent cholesterol efflux from macrophages [65, 66].

The demonstration that HDL function varies raises the possibility that it might potentially be modified in response to a range of interventions. It has recently been reported that the ability of HDL to inhibit proinflammatory adhesion molecule expression by endothelial cells is enhanced following consumption of a polyunsaturated fat-rich meal. In contrast, consumption of a saturated fat decreases this activity of HDL [67]. Further, statin therapy is associated with an enhanced ability of HDL to inhibit monocyte chemotaxis [36]. As statin therapy has been reported to attenuate MPO expression by macrophages and reduces systemic levels of the MPO product, nitrotyrosine [68, 69], it is also possible that this would result in a reduction in oxidative modification of apoA-I and subsequently enhanced cholesterol efflux activity.

Existing therapeutic strategies to raise HDL

A number of established interventions have been demonstrated to promote an increase in plasma HDL-C levels. Lifestyle modifications, with regard to diet, exercise and smoking cessation, result in a modest elevation in the plasma concentration and particle size of HDL-C. In fact, it was reported that substantial levels of exercise result in a 10% increase in HDL-C levels at best [70].

Several established pharmacologic agents that are primarily directed at lowering levels of atherogenic lipoproteins also raise HDL-C levels. While statins raise HDL-C by 5-10% [71], the greatest efficacy in terms of clinical event reduction was seen in those subjects with the lowest levels of HDL-C [6, 7]. Fibrates raise HDL-C by 5-15% [72, 73] and have been demonstrated to reduce clinical events in studies of both primary [72] and secondary [73] prevention. Despite the ability of fibrates to lower triglyceride levels, their effect on clinical events was attributed more to their ability to elevate HDL-C [8]. Fibrates act primarily as agonists of the nuclear receptor peroxisome proliferator-activated receptor-alpha (PPAR-α) [74]. In addition to regulating expression of a number of factors promoting reverse cholesterol transport, PPAR-α stimulation has also been reported to be anti-inflammatory [75]. Therefore, fibrates might potentially exert their atheroprotective properties via both their promotion of an antiatherogenic lipid profile and a direct protective influence on the arterial wall.

Nicotinic acid is the most potent HDL-C-raising agent (15-30%) currently available [76, 77]. Long-term administration results in a reduction in clinical events [78], and when used in combination with statin therapy, promotes angiographic regression [79]. However, nicotinic acid is limited by a high incidence of intolerance, primarily related to flushing. The use of an extended release formulation, which appears to be free of these adverse events, was demonstrated to halt progression of carotid intimal-medial thickness, a measure of subclinical atherosclerosis, in association with HDL-C elevation [80]. Given that raising HDL-C with established therapies is typically modest, there is currently no defined target for HDL-C elevation. As a result, HDL-C elevation remains a secondary goal in the lipid management guidelines of the National Cholesterol Education Programme [81].

Emerging therapeutic strategies to raise HDL

The fact that LDL-C reduction is the primary goal of lipid management results from the unequivocal evidence of the efficacy of LDL-C reduction by statins in numerous clinical trials [6, 7, 82, 83]. In contrast, the relative paucity of effective therapeutic options for HDL-C elevation has contributed to the lack of consensus on what would be an appropriate treatment goal. As a result, HDL-C elevation remains a secondary target for cardiovascular prevention. A number of novel strategies that promote the direct administration of HDL or influence its metabolic remodelling are in various stages of development.

Infusion of synthetic HDL

A number of reports have emerged to support the concept that directly infusing synthetic forms of HDL has a beneficial impact on the vasculature in humans. A single infusion of reconstituted HDL has been demonstrated to restore endothelial function in subjects with either hypercholesterolaemia [44] or low HDL-C levels in the setting of heterozygous ABCA1 deficiency [45]. In addition, infusion of reconstituted HDL is followed by an increase in faecal sterol excretion, a surrogate index of reverse cholesterol transport [84]. Given that these findings suggest that administration of HDL stimulates NO bioavailability and reverse cholesterol transport, it seems plausible that a therapeutic consequence might involve a favourable influence on atherosclerotic plaque. An exciting proof-of-concept study subsequently reported that administration of reconstituted particles containing the apoA-I variant, AIM, and phospholipid promoted regression of coronary atheroma in humans [85] (Figure 6). Forty-seven subjects, within 2 weeks of an acute coronary syndrome, received infusions of saline or rHDL containing either low (15 mg/kg) or high (45 mg/kg) dose AIM weekly for 5 weeks. Serial intravascular ultrasound analysis of a coronary arterial segment revealed a 4.2% reduction in atheroma volume in subjects who received infusions of rHDL. This extends the finding that

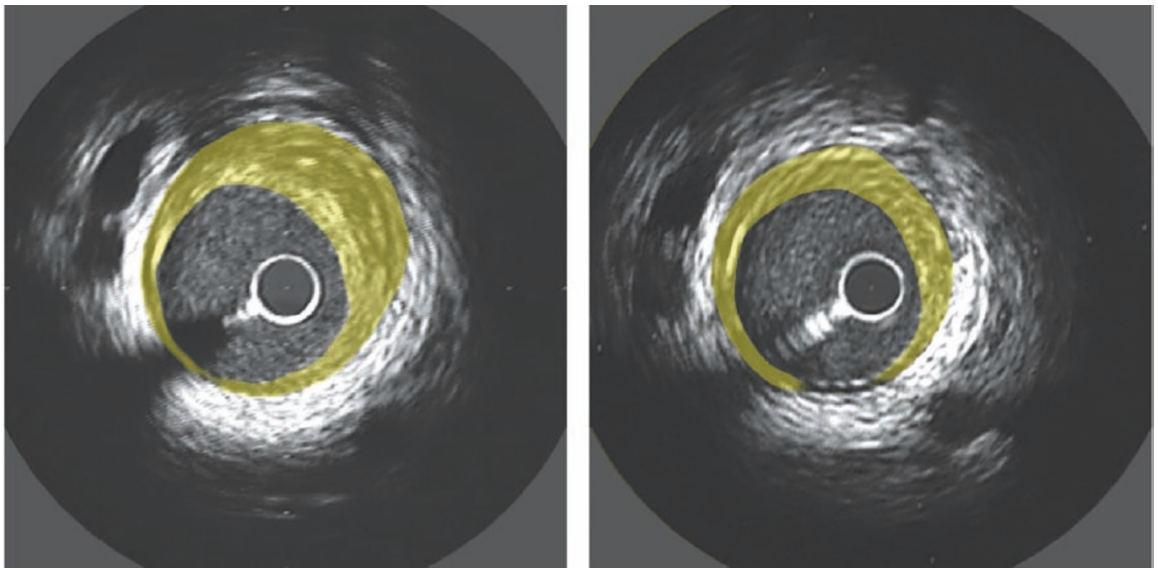

Figure 6 Representative example of regression of coronary atherosclerotic plaque (shaded areas) at a matched site imaged by intravascular ultrasound performed before (left panel) and following (right panel) treatment with reconstituted HDL particles containing AIM.

a single infusion of rHDL-containing AIM has a profound impact on atherosclerotic plaque in animal studies [12]. The potential significance of this result is further highlighted by the fact that in previous studies of antiatherosclerotic interventions, benefit was demonstrated only after a much longer time of follow-up [86, 87]. A larger trial is required to investigate this phenomenon further and to assess the potential impact on clinical events.

ApoA-I mimetic peptides

The development of short peptides, with similar structure and function to that of apoA-I, presents an alternative option to the intravenous infusion of rHDL. When synthesised with D-type amino acids, these peptides are resistant to hydrolysis by stomach acid and can therefore be administered orally [88]. One such peptide, D-4F, has been demonstrated to elevate levels of lipid-deplete HDL [88]. D-4F has been reported to enhance cholesterol efflux, enhance NO bioavailability, inhibit superoxide formation and inhibit monocyte chemotaxis in the *in vitro* and *ex vivo* setting [89]. Accordingly, D-4F has been reported to inhibit lesion formation in animal models of atherosclerosis [88-92].

Phospholipid vesicles

Phospholipid is the alternative component of nascent HDL that can be potentially administered. Phospholipid does not circulate in a free form and therefore anything that is introduced into the systemic circulation is rapidly incorporated into lipoproteins. This results in an increase in circulating forms of nascent forms of HDL containing only apoAI and phospholipid. Given that lipid-deplete forms of HDLs possess efficient cholesterol efflux and anti-inflammatory activity, it is possible that the generation of these particles would be beneficial. It has been reported that administration of phospholipid inhibits lesion formation and promotes regression of existing plaque in animal models of atherosclerosis [93]. It remains to be determined whether administration of phospholipid vesicles exerts a beneficial impact on the vasculature in humans.

Enhanced PPAR agonists

PPARs are a family of nuclear transcription factors that have been demonstrated to play pivotal roles in the regulation of a range of processes regulating metabolic homeostasis [94]. Activation of PPAR- promotes an antiatherogenic lipid milieu, characterised by increases in HDL expression and function, and lowering of plasma triglyceride. PPAR-γ activation improves insulin sensitivity. In addition, it appears that PPAR agonists might have a direct effect at the level of the arterial wall. In particular, both PPAR-α and PPAR-γ inhibit a number of critical steps in the inflammatory cascade, which may contribute to vascular protection. Established therapies including fibrates [74] and thiazolidinediones [95] have been demonstrated to act as agonists of PPAR-α and PPAR-γ, respectively. However, currently available agents are relatively weak agonists. A number of experimental agents that are currently in development are

either stronger pharmacologic agonists or interact with both classes of receptors and therefore have the potential to result in a more comprehensive influence on the arterial wall.

CETP inhibition

Considerable debate has focused on whether CETP exerts an influence on atherogenesis. Once esterified cholesterol is transferred to apoB-containing lipoproteins, it can be taken up, via the LDL receptor on the liver surface. This would provide an alternative route for reverse cholesterol transport and have a potentially beneficial impact on atherogenesis. On the other hand, given that apoB-containing lipoproteins might alternatively deliver esterified cholesterol to peripheral tissues, including the arterial wall, this process might promote atheroma formation. Conflicting data have emerged from epidemiological studies. Populations with a high incidence of CETP deficiency appear to be relatively protected from CHD in some, but not all, studies [96]. The development of a number of experimental approaches to inhibit CETP activity has produced consistent results in rabbit models of atherosclerosis. Administration of antisense oligonucleotides [97], a vaccine against CETP [98], and chemical inhibitors of CETP [99] have each been demonstrated to inhibit lesion formation. Administration of oral CETP inhibitors in humans has been reported to elevate HDL-C and lower LDL-C [100]. The influence of these agents on atherosclerotic plaque progression and clinical events is currently being investigated in large-scale human clinical trials.

Conclusion

HDLs possess a number of biological activities that potentially modulate pathologic events that contribute to all stages of atheroma formation and its clinical complications. As a result of an enhanced understanding of factors that promote HDL levels and function, a number of exciting therapeutic strategies are currently in development, which have the potential to have a major impact on the prevention and treatment of atherosclerotic cardiovascular disease.

Acknowledgments

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