Brief Review

HDL (High-Density Lipoproteins) and Apo (Apolipoprotein) A-I Improve Stent Biocompatibility

Recent Evidence From Experimental Models and Clinical Studies

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***Abstract*—**Revascularization because of coronary artery disease is commonly achieved by percutaneous coronary intervention with stent deployment. Refinement in interventional techniques, major improvements in stent design (particularly drug-eluting stents), and adjunctive pharmacotherapy with dual antiplatelet regimens have led to marked reductions in the overall rates of stent failure. However, even with the advancements made in the latest generation of drug-eluting stents, unresolved biological problems persist including delayed re-endothelialization and neoatherosclerosis, which can promote late expansion of the neointima and late stent thrombosis. Novel strategies are still needed beyond what is currently available to specifically address the pathobiological processes that underpin the residual risk for adverse clinical events. This review focuses on the emerging evidence that HDL (high-density lipoproteins) and its main apo (apolipoprotein), apoA-I, exhibit multiple vascular biological functions that are associated with an improvement in stent biocompatibility. HDL/apoA-I have recently been shown to inhibit in-stent restenosis in animal models of stenting and suppress smooth muscle cell proliferation in in vitro studies. Reconstituted HDL also promotes endothelial cell migration, endothelial progenitor cell mobilization, and re-endothelialization. Furthermore, reconstituted HDL decreases platelet activation and HDL cholesterol is inversely associated with the risk of thrombosis. Finally, reconstituted HDL/apoA-I suppresses key inflammatory mechanisms that initiate in-stent neoatherosclerosis and can efflux cholesterol from plaque macrophages, an important function of HDLs that prevents plaque progression. These unique multifunctional effects of HDL/apoA-I suggest that, if translated appropriately, have the potential to improve stent biocompatibility. This may provide an alternate and more efficacious therapeutic pathway for the translation of HDL. **(*Arterioscler Thromb Vasc Biol*. 2018;38:00-00. DOI: 10.1161/ATVBAHA.118.310788.)**

Key Words: apolipoprotein A-I ■ coronary artery disease ■ hyperplasia ■ stents ■ thrombosis

Cardiovascular disease (CVD) is the number one cause of death worldwide. Coronary artery disease (CAD) is the largest contributor to this epidemic, causing ≈7.2 million deaths globally each year.[1] With the increasing obesity pandemic across the Western world, the incidence of CAD and its associated health concerns is expected to continually increase.

Percutaneous coronary interventions (PCI) with stenting have substantially reduced overall mortality rates from CVD.[2] However, despite significant advances in stent design and adjunctive therapies, issues still persist such as in-stent restenosis (ISR) and thrombosis. These events are triggered by aberrant vascular biological processes including global endothelial denudation, inflammation-induced smooth muscle cell (SMC) proliferation, thrombosis, and neoatherosclerosis. No treatment option is currently able to eliminate all stent-associated complications because of the complexity of controlling for the range of vascular biological processes that cause stent failure. Because of the expected rise in CVD cases in the coming decades, new and improved treatment options are required. To achieve such improvements, treatments must systematically and simultaneously address each weakness of current interventions.

One agent of interest is apo (apolipoprotein) A-I, the predominant protein constituent of HDL (high-density lipoprotein) particles. Interestingly, HDL-associated apoA-I is able to modulate key vascular processes that are associated with improved stent biocompatibility such as the suppression of SMC proliferation and neointimal hyperplasia (NIH),[3–5] inhibition of platelet activation and thrombus formation,[6] suppression of apoptosis,[7] enhancement of re-endothelialization,[8] inhibition of monocyte recruitment, and the initiation/progression of atherosclerosis.[9] Taken together, this highlights the immense potential for apoA-I/HDLs to improve stent biocompatibility. This review will discuss the accumulating evidence supporting a role for apoA-I/HDLs in the enhancement of stent biocompatibility and the associated key mechanisms.

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Nonstandard Abbreviations and Acronyms

Apo	apolipoprotein
apoA-IM	apoA-I Milano
BMS	bare metal stent
CAD	coronary artery disease
CETP	cholesteryl ester transfer protein
CVD	cardiovascular disease
DAPT	dual antiplatelet therapy
DES	drug-eluting stent
EC	endothelial cell
eNOS	endothelial NO synthase
EPC	endothelial progenitor cell
HDL	high-density lipoprotein
HDL-C	HDL cholesterol
ICAM	intercellular adhesion molecule
ISR	in-stent restenosis
LCAT	lecithin–cholesterol acetyltransferase
MAP	mitogen-activated protein
NIH	neointimal hyperplasia
PCI	percutaneous coronary intervention
PDGF	platelet-derived growth factor
rHDL	reconstituted HDL
SMC	smooth muscle cell
SR-B1	scavenger receptor B1
STARS	Stent Anticoagulation Restenosis Study
TNF	tumor necrosis factor
VCAM	vascular cell adhesion molecule

Percutaneous Stenting and the Association of HDL Cholesterol With Improved Outcomes

Over the past 40 years, stent design, metal composition, coating, and strut diameter have been extensively modified and improved; however, biocompatibility issues still persist (Figure 1). The earliest stents, bare metal stents (BMS), successfully reduced vessel recoil and the incidence of restenosis compared with balloon angioplasty alone.[10] However, the inherently thrombogenic nature of the stainless steel in BMS made these devices susceptible to early stent thrombosis despite the use of anticoagulation.[11] Moreover, like balloon angioplasty alone, the process of stent deployment caused significant injury to the vessel wall, resulting in high rates of NIH and ISR, leading to late stent failure and acute coronary syndromes several months post PCI.[12]

Such problems prompted the development of drug-eluting stents (DES) which, coupled with adjunctive dual antiplatelet therapy (DAPT), have significantly reduced the need for repeat target lesion revascularization by ≈70% compared with BMS.[13] These DES incorporate a polymer coating that allows a sustained dose of an antiproliferative drug to be delivered slowly to the local tissue with the aim of overcoming NIH.[14] Early randomized clinical trials using sirolimus or paclitaxel-eluting DES showed promising results. These first-generation DES' were found to be extremely effective in suppressing SMC proliferation and reducing the incidence of ISR at 6- and 12-month postdeployment from ≈20% to 30% with BMS to 5% to 10% with DES.[15] Despite this, alternative

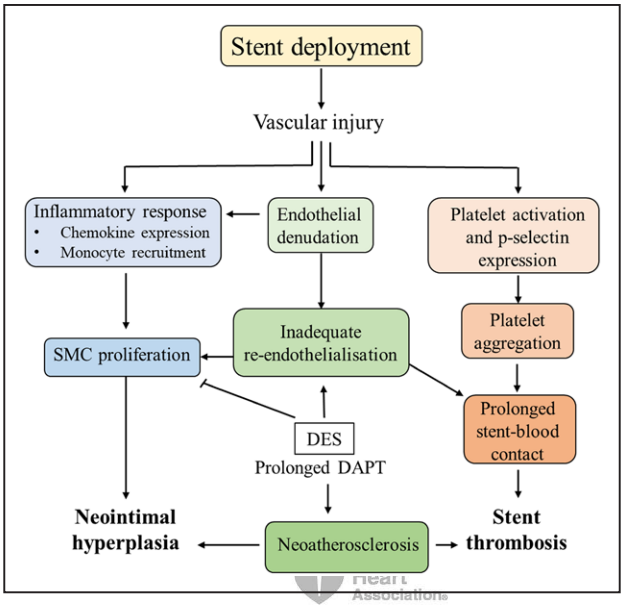

Figure 1. Downstream vascular biological processes caused by stent deployment. Stent deployment causes significant vascular injury which leads to neointimal hyperplasia, inadequate re-endothelialization, and stent thrombosis. Drug-eluting stent (DES)-induced delayed re-endothelialization leads to neoatherosclerosis which causes late expansion of the neointima and late in-stent thrombosis. DAPT indicates dual antiplatelet therapy; and SMC, smooth muscle cell.

complications emerged with the recognition that DES delayed healing and re-endothelialization at the deployment site, which could lead to increased rates of in-stent neoatherosclerosis and late stent thrombosis (occurring beyond 30-day post PCI) compared with BMS.[16] Indeed, several meta-analyses of first-generation DES showed low, though significantly higher rates of late stent thrombosis in the vicinity of 0.5% to 0.7% with DES, compared with 0.0% to 0.2% with BMS.[17] These results prompted recommendations to prolong the duration of antithrombotic DAPT, though at the expense of an increase in major bleeding events.[18]

The advent of second-generation DES incorporating novel alloy stent platforms with reduced strut thickness, more biocompatible polymer coatings, and different pharmacological agents has further reduced the incidence of ISR and stent thrombosis compared with first-generation DES.[19] The rates of target lesion revascularization, particularly for everolimus-eluting stents, are typically <5% at 12-month follow-up.[19] Second-generation DES also exhibit an improved long-term safety profile with significantly lower rates of cumulative stent thrombosis relative to first-generation DES (1.0% compared with 2.2% at 3 years in a registry study of >18 000 patients[20]). Indeed, recent studies have suggested that second-generation DES are even safer than BMS with regard to stent thrombosis.[21] Despite these vast improvements, there remains ongoing biocompatibility issues with DES such as persistent chronic inflammation, inhibited re-endothelialization, late stent thrombosis, and neoatherosclerosis. Along with the ongoing need for DAPT post-DES and its associated bleeding risks, these problems highlight a need for novel therapeutic strategies.

HDL are comprised of an amphipathic outer layer consisting of free cholesterol, phospholipid, and apolipoproteins.

The hydrophobic inner core of the HDL particle is made up of cholesterol esters and triglycerides. HDL precursors are discoidal particles synthesized in the liver or small intestines and predominantly consist of phospholipid and apoA-I. Lecithin–cholesterol acetyltransferase (LCAT) delivers cholesterol, mainly in the form of cholesterol esters, to discoidal HDL resulting in the formation of spherical HDL particles. HDL particles predominantly interact with 3 cell surface proteins: the cholesterol transporters ABCA1 and ABCG1 and the SR-B1 (scavenger receptor B1). These cell surface proteins mediate many of the effects of HDL/apoA-I. One of the main roles of HDL is to efflux cholesterol from tissues and then deliver the cholesterol to the liver for disposal.[22] The ability of HDL to efflux cholesterol from cells has seen it directly implicated in the prevention of atherosclerosis, through the removal of cholesterol from macrophages in the artery wall.[23] This is considered one of the main mechanisms for the inverse relationship between plasma HDL levels and CAD risk.[24]

Clinical Evidence That HDL Cholesterol Enhances Stent Biocompatibility

Although there is a strong and long-established correlation between higher HDL cholesterol (HDL-C) levels and reduced CAD risk, there is also a consistent inverse relationship between HDL-C and stent failure. Clinical evidence shows patients with higher HDL-C have improved stent patency at 1 year.[25] Epidemiological data show patients with higher HDL-C have a reduced frequency of repeat interventions and a reduction in nontarget vessel PCI.[26] Additionally, patients with low (≤40 mg/dL) HDL-C are reported to be nearly 4× more likely to develop restenosis than those individuals with higher (≥40 mg/dL) HDL-C (64% versus 17%, respectively).[27] A more recent and much larger study followed >4000 patients in the year following PCI and found low HDL-C (≤35 mg/dL) were associated with a 2-fold increase in the risk of death post-PCI.[28]

These studies provide evidence that HDL particles play a protective role after PCIs and raises the possibility that they may beneficially regulate the key vascular biological mechanisms associated with stent biocompatibility including NIH, re-endothelialization, stent thrombosis, and neoatherosclerosis.

Mechanisms of Stent Failure and the Role Native HDL, Reconstituted HDL, and apoA-I

Neointimal Hyperplasia

Stent deployment causes significant damage to the vessel wall which commonly results in the expansion of the neointima because of the uncontrolled proliferation of medial SMCs. The insult severely damages the endothelial layer and initiates a cascade of proinflammatory events. Endothelial damage induces platelet adherence and aggregation which are known to release numerous SMC proliferative growth factors such as PDGF (platelet-derived growth factor).[29] The proinflammatory environment stimulates SMC migration and proliferation along with the secretion of extracellular matrix proteins which leads to the expansion of the neointima that protrudes into the luminal space. Previous in vivo studies in apoE deficient mice have shown that apoA-I is able to suppress NIH. Adenoviral overexpression of apoA-I reduces neointimal formation after carotid artery wire injury[30] and vein grafting.[31] Interestingly, gel-embedded native HDL applied topically to the adventitial side of a vein graft inhibited neointimal area as effectively as systemically delivered apoA-I and promoted re-endothelialization.[31] This suggests that local delivery to the site of vascular injury may have therapeutic potential. apoA-I_M (apoA-I Milano), a variant form of apoA-I identified in an Italian carrier population with a proatherogenic profile but without clinical evidence of atherosclerosis[32] has also been shown to suppress NIH. In vivo studies in cholesterol-fed rabbits showed that preinfusions of recombinant apoA-I_M complexed with phospholipid significantly inhibited NIH, SMC proliferation, and macrophage content after arterial injury.[33,34] Two recent studies have found direct evidence that HDL-C/apoA-I raising can suppress in-stent NIH in animal models of stenting. Using a unique murine model of stenting,[35] it was found that alternate day infusions of apoA-I reduced in-stent neointimal area 28 days after stent deployment.[4] In a second study, it was demonstrated that raising HDL-C using CETP (cholesteryl ester transfer protein) inhibitor, des-fluoro-anacetrapib, prevented in-stent NIH in New Zealand White rabbits.[5] Similarly, administration of a commercial recombinant apoA-I_M/phospholipid complex, ETC-216, directly into the coronary vessel wall was able to reduce the development of ISR and NIH in pigs implanted with coronary stents.[36]

The inflammatory response that causes NIH after vascular injury has not been fully elucidated. However, there is increasing evidence that small inflammatory proteins called chemokines play a significant role. Recent studies have shown the direct involvement of chemokines in promoting SMC proliferation and migration culminating in excessive NIH.[37,38] In vitro studies have demonstrated that incubation of SMCs with a range of chemokines (CCL2, CCL5, and CX_3CL1) increased SMC proliferation.[39–41] The involvement of chemokines in neointimal development was supported in an in vivo study which showed that the deletion of CCL2 reduced NIH in a murine arterial injury model corroborating the potential role for chemokines.[42] In vitro studies have found that preincubation of SMCs with reconstituted HDL (rHDL), a complex of apoA-I and phosphatidylcholine, significantly decreased SMC proliferation. rHDL also concomitantly reduced the expression of 3 key chemokines, known to promote SMC proliferation including CCL2, CCL5, and CX_3CL1.[3] Mechanistically, this was found to be mediated via the inhibition of the phosphotidylinositide-3-kinase/pAkt/NF-κB signaling pathway,[3] an important driver of SMC proliferation. Using siRNA knockdown, the scavenger receptor SR-B1 was found to be critical in mediating these effects. The HDL fraction isolated from rabbits treated with the CETP inhibitor des-fluoro-anacetrapib was also found to suppress SMC proliferation when compared with HDL fraction isolated from control rabbits.[5] Similarly, this study identified that phosphotidylinositide-3-kinase/pAkt and SR-B1 were important in mediating the effects of isolated HDL from these rabbits on SMC proliferation. Consistent with these findings, another recent article found in in vitro validation studies that apoA-I, covalently immobilized to a stainless steel surface, similar to a stent surface, using a plasma-activated coating was able to strikingly reduce SMC

attachment to the stainless steel.[43] Furthermore, of the SMCs that were able to attach, their proliferation was inhibited. This demonstrates a new application for apoA-I in which it can be immobilized to a stent in its bioactive form, able to be delivered to the stent site directly.

Leukocytes have been implicated in NIH because of their ability to release inflammatory proteins and growth factors known to stimulate SMC proliferation.[44] In vitro studies have found that rHDL can inhibit monocyte recruitment into inflamed tissue and monocyte chemotaxis.[45] Furthermore, infusions of apoA-I in vivo in a murine stent model inhibited the number of circulating activated monocytes and suppressed the number of macrophages within the stented vessels, which were paralleled by a reduction in neointimal area.[4]

Taken together, these studies show that apoA-I can suppress the mechanisms by which stent deployment induces NIH including inhibition of SMC proliferation, inflammation, and leukocyte recruitment.

Inadequate Re-Endothelialization

Near-global denudation of the endothelial layer occurs during PCI because of the expansion of the balloon catheter. Re-endothelialization is an important regulator of SMC proliferation and migration. In vivo studies found that areas of early re-endothelialization had less neointimal growth after vessel injury than those areas with low re-endothelialization.[46] Early re-endothelialization also plays a critical role in reducing stent thrombosis and in DES, re-endothelialization is profoundly delayed. As a result, stent struts are exposed to circulating blood for prolonged periods of time increasing a patient's risk of developing late and very late thrombosis. Postmortem examination of patients implanted with DES found the best histological predictor of late stent thrombosis was endothelial cell (EC) coverage.[47]

In vitro studies using rHDL demonstrate a protective effect on ECs by promoting endothelial repair,[48] increasing eNOS (endothelial NO synthase) production[49] and preventing EC apoptosis.[50] rHDL increases eNOS production by activating phosphotidylinositide-3-kinase, inducing the activation of both Akt and MAP (mitogen-activated protein) kinases which are responsible for activating eNOS.[49] eNOS is the main enzyme that contributes to NO production[51] which has been shown to inhibit leukocyte adhesion, modulate vascular dilation tone, and regulate local cell growth.[52] In vitro studies have shown that ECs overexpressing eNOS inhibit platelet aggregation and SMC proliferation.[53] As such, rHDL-induced increases in eNOS, NO and re-endothelialization may be beneficial in multiple aspects of stent biocompatibility.

In vitro studies have also demonstrated that rHDL can promote EC migration and proliferation.[48,54] This is important as an in vivo study investigating re-endothelialization after stent deployment found the majority of endothelial cells involved in repopulating the injured vessel were from outside the stented area.[55] Furthermore, infusions of apoA-I in the murein stent model increased the number of ECs in stented arteries, indicating enhancement of re-endothelialization.[4] rHDL also prevents EC apoptosis. One in vitro study reported that oxidized-LDL-induced EC apoptosis was able to be reversed after incubation with either apoA-I or rHDL.[56] Supporting this, rHDL and apoA-I were found to protect against endothelial apoptosis when EC's were incubated with inflammatory cytokine TNF (tumor necrosis factor)-α.[50] Further in vitro studies have found that immobilized apoA-I on a stainless steel surface increases EC attachment, indicating the potential for stent-bound apoA-I to enhance re-endothelialization.[43]

Endothelial progenitor cells (EPCs) make significant contributions to re-endothelialization and the regulation of NIH. For example, using endothelial-specific LacZ transgenic mice, EPCs were found to contribute to re-endothelialization of the area after stent deployment.[55] Intravenous delivery of EPCs was also found to increase re-endothelialization after wire injury of the carotid artery which was associated with a decrease in NIH.[57] In a separate study, stents designed to capture circulating EPCs using a $CD34^+$ antibody demonstrated increased re-endothelialization and reduced thrombosis.[58] In vivo animal studies have shown rHDL can increase EPC mobilization and incorporation,[8,59] whereas in humans, infusions of rHDL into diabetic patients were found to increase the number of circulating EPCs.[60]

Taken together, rHDL and apoA-I show potential in inducing the mechanisms that lead to an enhancement of re-endothelialization post-PCI, a key determinant of stent biocompatibility. In support of this, in vivo studies have found that rHDL infusions increase re-endothelialization after endothelial denudation in mice.[61] Furthermore, in the murine stent model, apoA-I–infused mice had significantly more endothelial cells present in stent aortae 28-day post-stent deployment, as measured by flow cytometry, an indication of enhanced re-endothelialization.[4]

Stent Thrombosis

Because of the thrombogenic nature of BMS, stent thrombosis was a major clinical problem that, in early procedures, necessitated oral anticoagulation with heparin or warfarin.[19] However, the rates of major bleeding complications with these therapies became unacceptably high, leading to trials of antiplatelet agents such as aspirin to reduce stent thrombosis post PCI. Randomized controlled trials in the 1990s demonstrated DAPT regimens to be markedly more effective in preventing death, myocardial infarction, or repeat coronary interventions compared with anticoagulation regimes, while also causing much less incidences of hemorrhagic complications.[62] Moreover, DAPT was more effective than single antiplatelet regimens. The STARS (Stent Anticoagulation Restenosis Study) showed that the incidence of death, target lesion revascularization, vessel thrombosis, or myocardial infarction after 30 days was 3.6% when patients were treated with aspirin alone; however, this was significantly reduced to 0.5% with DAPT (aspirin+ticlopidine).[46] The more favorable side effect profile of clopidogrel saw it replace ticlopidine in DAPT regimens,[63] while more recently, prasugrel[64] and ticagrelor[65] have been favored particularly in patients with ACS because of their greater potency, superior efficacy in reducing ischemic events and less interindividual response variability compared with clopidogrel.[19]

Although DAPT is necessary to reduce thrombosis risk, they are not without significant issues. It is estimated that ≈4% to 8% of patients require noncardiac surgery within the first

year after stent deployment.[66] Patients relying on DAPT to reduce their thrombotic risk are then faced with the dilemma of continuing DAPT and risking both minor and major bleeding during surgery and postoperatively. Alternatively, patients can choose to stop DAPT which increases their risk of a thrombotic event.[67] As DES are susceptible to late and very late thrombosis,[68] it is recommended that DAPT continues for 12 months.[69] However, the optimal duration of DAPT post-DES insertion is still debated as longer durations are recognized to reduce the incidence of stent thrombosis but increase the rate of clinically significant bleeding, though there are recent suggestions that patients with second-generation DES may not derive significant benefits from DAPT beyond 6 months.[70] Nevertheless, because of the necessity for prolonged use, some patients experience mild to severe side effects from the medications. For example, continued use of aspirin can cause gastrointestinal upset and tinnitus, whereas clopidogrel can cause gastrointestinal upset, diarrhea, and rash.[71] Alternate strategies that reduce the thrombogenicity associated with stent deployment are still required.

HDLs can inhibit multiple steps in the coagulation cascade and prevent thrombus formation in many diseases. rHDL reduces platelet aggregation in vitro via a decrease in the expression of p-selectin. Platelet-rich plasma isolated from individuals infused with rHDL have less platelet aggregation.[6] Furthermore, rHDL has been shown to downregulate plasminogen activator inhibitor-I, a protein involved in the inhibition of fibrinolysis resulting in a decrease of thrombus formation.[72] ApoA-I and rHDL immobilized onto stainless steel surfaces is able to significantly reduce in vitro thrombosis in both static thrombosis assays and underflow conditions using the chandler loop.[43] Many studies have also reported antithrombotic effects with HDLs in clinical settings. Although not in the context of CVD, infusions of rHDL reduced the development of a procoagulant state in healthy patients injected with low doses of endotoxin.[73] Furthermore, in diabetic patients a single rHDL infusion reduced platelet activity and platelet aggregation ex vivo by >50%.[74] Finally, a case study investigating definite stent thrombosis found diabetic patients with higher HDL-C (≥40 mg/dL) had a 12% lower risk of developing stent thrombosis, compared with those patients with lower HDL-C (≤40 mg/dL).[75]

Harnessing the antithrombotic actions of HDLs/apoA-I, therefore, represents a promising prospect for the development of novel agents to reduce thrombosis post-stent deployment. This may mitigate the need for excessive antithrombotic therapy which can increase bleeding risk.

Neoatherosclerosis

In-stent neoatherosclerosis is emerging as an important contributing factor to the late vascular complications that occur post-stent deployment, particularly with DES. In-stent neoatherosclerosis in the neointima has similar characteristics to native atherosclerosis and contains macrophage foam cells, cholesterol clefts, areas of calcification, and necrotic cores. However, it occurs within 6 months to 5 years post-stent deployment, whereas native atherosclerosis develops over a lifetime. Neoatherosclerosis accelerates late expansion of the neointima a key cause of stent failure. There is evidence that these plaques are unstable and the primary cause of late in-stent thrombosis.[76] Although the cause of neoatherosclerosis is not entirely elucidated the higher occurrence of neoatherosclerosis in DES may be the result of drug resistance, a reaction to the DES polymers or DES-induced delayed re-endothelialization.

One of the most characterized properties of HDL is its atheroprotective effects via its ability to efflux cholesterol from plaque macrophages. This along with its other properties such as inhibition of LDL oxidation,[7] reduction in cell adhesion molecules (VCAM [vascular cell adhesion molecule]-1, ICAM [intercellular adhesion molecule]-1),[7] suppression of chemokine expression[45] and reduction in monocyte infiltration lend it to be an ideal agent for the prevention of the initiation and progression of in-stent neoatherosclerosis. No study to date has specifically determined the relationship between HDL-C and the incidence of in-stent neoatherosclerosis; however, data showing an inverse relationship between HDL-C and neointimal area are consistent with a reduction in neoatherosclerosis by HDL.

HDL-C and apoA-I Raising Strategies: Limitations in Their Translation and Future Options

rHDL and apoA-I have shown benefit in a vast number of in vitro studies and in preclinical models; however, large-scale clinical trials aimed at raising HDL-C in patients with established atherosclerosis have shown very little effect on cardiovascular outcome.[77–80] These clinical trials highlight that if HDL/apoA-I were to be used to improve stent biocompatibility, the translational strategy would need to be well targeted, so the multifunctional properties of HDL/apoA-I were used in such as way as to maximize its potential and be effective.

Several approaches seeking to raise HDL-C and modify apoA-I have been investigated. These can be broadly categorized into chronic therapies such as niacin and CETP inhibitors, and acute infusion therapies such as rHDL particles composed of apoA-I complexed with phospholipid, or recombinant LCAT.

Despite effectively raising HDL-C by at least 50%, the first CETP inhibitor torcetrapib was stopped prematurely because of higher all-cause mortality rates and increased incidence of myocardial infarction, heart failure, and hypertension in patients with high-risk CVD.[77] This has been attributed to off-target effects of torcetrapib in inducing the secretion of aldosterone,[81] as well as impairing endothelial function via reduced eNOS and increased endothelin-1.[82] Subsequent clinical trials of dalcetrapib[78] and evacetrapib[79] found that these CETP inhibitors did not induce the same off-target effects, but nevertheless lacked efficacy in reducing cardiovascular end points despite increasing HDL-C. More recently, the REVEAL clinical trial demonstrated for the first time that the addition of anacetrapib to statin therapy in patients with atherosclerotic vascular disease led to a significant reduction of major adverse CAD outcomes, associated with higher HDL-C and lower non–HDL-C.[83] It remains unclear as to why the clinical benefit of anacetrapib differs from that of dalcetrapib

and evacetrapib. Proposed explanations include the lack of a non–HDL-C lowering effect particularly with dalcetrapib and insufficient duration of follow-up in earlier clinical trials to detect significant differences.[84,85] Moreover, the functionality of endogenous HDL may be disrupted in disease or perhaps by the action of CETP inhibitors themselves,[86] though HDL from anacetrapib-treated patients has been previously found to induce enhanced cholesterol efflux from foam cells via ABCA1 and ABCG1 in vitro.[87] More studies are clearly needed to better understand how CETP inhibitors may modulate the functionality of HDL particles or apoA-I as this may facilitate the improvement of stent outcomes. Indeed, a recent study has found that treatment of rabbits implanted with an iliac artery stent using an analog of anacetrapib reduced the incidence of NIH by increasing HDL-C, leading to reduced vascular inflammation and SMC proliferation.[5]

Extended-release niacin has also been trialed with the aim of increasing HDL-C to improve cardiovascular outcomes in patients with CAD. The first clinical trial raised HDL-C by a modest 11% and no changes were found between treatment groups. Concerns were then raised over study design and that it was underpowered (3414 patients).[88] A subsequent clinical trial addressed these design issues, achieved a 16.9% increase in HDL-C and was much larger (>25 000 patients), but was halted early because of increases in side effects such as myopathy and gastrointestinal issues, and there was no observed cardiovascular benefit.[80]

A proposed reason for the failure of these trials is that HDL particles in CAD patients have been rendered dysfunctional, likely via modifications of apoA-I. Several studies have found that the beneficial vascular effects of HDL isolated from CAD patients is impaired relative to healthy individuals.[89–91] In a murine model of arterial injury, injections of HDL from healthy patients promoted endothelial repair, whereas in mice receiving HDL from patients with CAD, re-endothelialization was not enhanced.[90] Consistent with this, another study found HDL from CAD patients did not reduce EC apoptosis in vitro or in vivo but rather stimulated proapoptotic pathways.[91] Indeed, a body of evidence indicates that apoA-I within human atherosclerotic plaque is selectively targeted for site-specific oxidation by myeloperoxidase, leading to profound functional impairment of apoA-I in relation to ABCA1-dependent cholesterol efflux.[92,93] Similarly, injections of apoA-I–deficient mice with isolated human apoA-I oxidized by myeloperoxidase ex vivo significantly impaired reverse cholesterol transport in vivo compared with injections of native human apoA-I.[94] Oxidative modifications of apoA-I by myeloperoxidase also suppress the anti-inflammatory and antiapoptotic activities of HDL on endothelial cells in vitro, while also inducing proinflammatory functions.[95] Taken together, these studies suggest that simply raising HDL-C by increasing functionally defective HDL particles is unlikely to be an effective therapeutic strategy and that potential stent systems incorporating apoA-I will need to overcome issues with oxidative modification and dysfunction. Strategies that involve the targeting of functional apoA-I to the site of deployment may need to be devised.

To date, arguably the most promising mode of translating HDL-associated therapies has been via acute infusions of high-dose clinical grade rHDL.[96] Clinical studies have found small but significant reductions in coronary atheroma volume[97] and adverse plaque characteristics based on intravascular ultrasound and coronary angiography after rHDL infusion in patients with ACS.[98] In patients with femoral atherosclerotic plaque, a single high-dose rHDL infusion was also found to reduce plaque lipid content, macrophage size, and inflammatory mediators such as VCAM-1.[10] The infusion of functional rHDL particles, rather than an increase in endogenous HDL in a diseased patient (as with CETP inhibitors and niacin), may explain why infusion strategies tend to have improved effectiveness. There is also recent interest in developing recombinant LCAT infusion therapies as LCAT is recognized to play a role in reverse cholesterol transport by esterifying free cholesterol, thereby creating a concentration gradient for further efflux and facilitating the maturation of small, discoidal pre-beta HDL into larger spherical HDL particles that have a longer circulating half-life.[99] The delivery of recombinant LCAT to mice with LCAT deficiency effectively raised HDL-C and reduced non–HDL-C with a concomitant increase in cholesterol efflux seen in vitro.[100] A recent phase I trial of recombinant LCAT, ACP-501, in patients with stable CAD showed dose-dependent elevations in HDL-C and apoA-I levels with an acceptable safety profile.[101] Further trials to assess the efficacy of recombinant LCAT in modulating atherosclerotic plaque parameters and CVD outcomes are awaited.

The therapeutic use of apoA-I_M has also generated much interest.[102,103] Infusions of apoA-I_M/phospholipid complexes in rabbit models of atherosclerosis have been found to induce rapid plaque regression when assessed by intravascular ultrasound or magnetic resonance imaging.[104,105] These findings were corroborated in a landmark clinical trial in patients with ACS, in whom 5 weekly infusions of ETC-216 led to small though significant regression of coronary atheromas based on intravascular ultrasound assessment.[97] Despite this, there is conflicting in vitro and in vivo data as to whether apoA-I_M more effectively induces cholesterol mobilization and efflux from macrophages relative to wild-type apoA-I.[106] Compared with infusions of wild-type apoA-I in rabbits with aortic atherosclerosis, infusions of apoA-I_M induced a similar extent of plaque regression but demonstrated significant reductions in the expression of plaque inflammatory markers and superior plaque-stabilizing properties in vitro.[107] This uncertainty coupled with only modest effects on plaque regression have led to somewhat diminished interest in apoA-I_M. Subsequent clinical trials with ETC-216 were also hampered by adverse events; in particular, a patient who developed multiorgan failure from a systemic inflammatory response thought to be related to host cell protein contaminants introduced during the manufacturing process.[108] Further refinement saw the reintroduction of recombinant apoA-I_M as MDCO-216, which has since demonstrated safety and efficacy in a phase I trial of healthy volunteers and patients with stable CAD, producing a dose-dependent increase in pre-beta HDL particles and ex vivo ABCA1-mediated cholesterol efflux.[109] To date, no studies have investigated the translational incorporation of apoA-I_M

onto stents, and this may be explored once its efficacy is better defined in clinical trials.

In addition to issues with dysfunctional apoA-I, it is probable that the biology of an advanced plaque composed of a large necrotic core with macrophage debris and extracellular lipid may not be conducive to active cholesterol efflux and plaque regression. In an advanced plaque, a large proportion of the plaque macrophages may be necrotic, and thus cholesterol efflux via apoA-I and the ABC cholesterol transporters is likely compromised. This raises the question as to whether advanced atherosclerotic disease, as was the selection criteria in all HDL-raising clinical trials, is the ideal pathology to target. The vascular biological processes that regulate stent biocompatibility may, in fact, be better suited to improvement by apoA-I and other HDL-associated therapies. Accordingly, several potential options exist for translation of these therapies to improve stent outcomes. Acute infusion therapies of rHDL immediately post-stent deployment could be explored, whereas the development of bioresorbable stents incorporating rHDL particles could also provide local antirestenotic and antithrombotic effects. Our group recently showed that the bioactivity of apoA-I covalently attached to a stent surface could be preserved using a novel plasma-activated coating technology.[43] Further studies will assess whether local delivery of apoA-I to the stent site can prevent restenosis. Indeed, other groups have explored novel approaches such as the coating of stent surfaces with anti–apoA-I antibodies to capture endogenous apoA-I, though the performance of this stent in vivo was not significantly better than a BMS with regard to luminal stenosis or NIH in a rabbit iliac injury model.[110] The efficacy of this stent was perhaps limited by the efficiency of apoA-I capture as well as the possibility that the captured endogenous apoA-I was dysfunctional. This highlights the need for ongoing research into novel and improved translation strategies for apoA-I.

Concluding Remarks

Native HDL and apoA-I beneficially modulate the key vascular biological pathways associated with stent failure (Figure 2). Studies show they decrease inflammation and SMC proliferation and recently it has been shown that CETP inhibition and apoA-I infusion reduces in-stent NIH in animal models of stenting. Both p-selectin and platelet aggregation are decreased by apoA-I infusions in in vitro and ex vivo studies and clinical data show HDL-C is inversely associated with the incidence of in-stent thrombosis. Finally, native HDL and rHDL increase EC migration, proliferation, re-endothelialization after vessel injury and EPC mobilization/incorporation into injured vessels. Although not reported to date, it is highly likely that HDL/apoA-I may inhibit the formation of in-stent neoatherosclerosis. Taken together, HDL/apoA-I seem to be unique agents that simultaneously regulate all key vascular biological processes associated with enhanced stent biocompatibility. However, novel translation strategies are likely to be required so the multifunctional biological benefits of native HDL and apoA-I are maximized.

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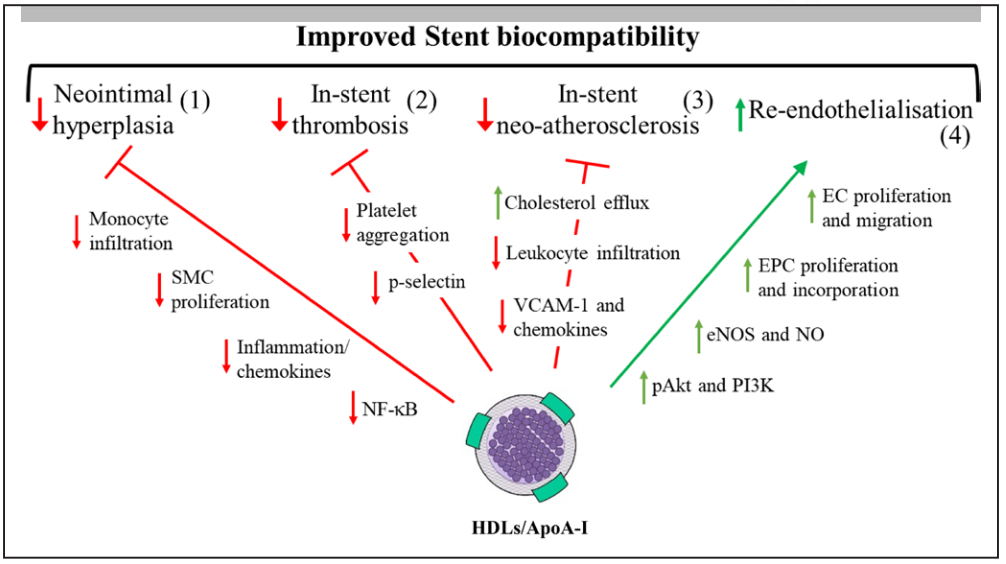

Figure 2. HDLs (high-density lipoproteins) and apo (apolipoprotein) A-I regulate biological processes associated with enhanced stent biocompatibility by (1) suppressing neointimal hyperplasia via decreasing NF-κB and inflammation/chemokine expression leading to the suppression of smooth muscle cell (SMC) proliferation; (2) decreasing both p-selectin and platelet aggregation and reducing in-stent thrombosis; and (3) are likely to suppress in-stent neoatherosclerosis via its well-established effects of inhibiting VCAM (vascular cell adhesion molecule)-1, chemokines, and leukocyte infiltration as well as through macrophage cholesterol efflux. (4) HDLs/apoA-I also promote re-endothelialization by increasing eNOS (endothelial NO synthase) activation and NO production (via pAkt/phosphotidylinositide-3-kinase) leading to an increase in endothelial cell (EC) proliferation and migration as well as endothelial progenitor cell (EPC) proliferation and incorporation.

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Highlights

- HDL (high-density lipoprotein)/apo (apolipoprotein) A-I has recently been shown to reduce in-stent restenosis in animal models of stenting.
- HDL and apoA-I exhibit multifunctional properties that include modulation of key mechanisms associated with enhanced stent biocompatibility including inhibition of inflammation-induced smooth muscle cell proliferation, enhanced re-endothelization and endothelial progenitor cell mobilization, and suppression of platelet activation and thrombosis.
- HDL has the potential to improve stent biocompatibility, presenting as an alternate and efficacious pathway for the translation of HDL.

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Laura Z. Vanags, Nathan Kum Pang Wong, Stephen James Nicholls and Christina A. Bursill

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