New Drugs and Technologies

Future of High-Density Lipoprotein Infusion Therapies
Potential for Clinical Management of Vascular Disease

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The field of high-density lipoprotein (HDL) therapeutics is broadly aimed at combating the significant residual cardiovascular risk remaining after effective low-density lipoprotein (LDL) cholesterol reduction.[1] After the recent failure of 3 orally active HDL-raising agents in prospective intervention trials, including 2 in the cholesteryl ester transfer protein (CETP) inhibitor class[2,3] and niacin,[4,5] the goals of HDL therapy are currently undergoing reassessment. Key opinion leaders are advocating a shift in the target of HDL therapy from elevation in circulating HDL cholesterol levels to enhancement of the functional properties of HDL, especially HDL-mediated reverse cholesterol transport and cellular cholesterol efflux.[6,7] These events have focused the spotlight on HDL infusion therapies that transiently increase HDL particle number and enhance efflux capacity for cellular cholesterol; other defective features of HDL functionality may potentially be normalized.[8] However, evaluation of HDL infusion therapies has been limited to date to small surrogate end-point trials; indeed, large phase 3 outcome trials to test the efficacy of these agents in reducing definitive morbidity and mortality end points are eagerly awaited.

If proven effective, HDL infusion agents would have potential application across a wide range of vascular diseases but would be particularly amenable to delivery in the context of acute coronary syndromes. Coronary artery disease morbidity and mortality relate largely to either physical disruption or endothelial erosion of the fibrous cap of atherosclerotic plaques in the coronary arteries, triggering luminal or intraplaque thrombus formation.[9] An acute or unstable coronary syndrome (sudden death, nonfatal myocardial infarction, or unstable angina) results, the nature of which depends in large part on the degree of vessel occlusion. Plaques at greatest risk for rupture are characterized by a marked abundance of both extracellular and intracellular lipid (cholesterol); immunoinflammatory cells, including monocyte-macrophages and T lymphocytes; pro-oxidants; inflammatory mediators; a thin collagen fibrous cap; calcium deposits; a paucity of smooth muscle cells; and a large necrotic core.[10–12] Patients with diffuse coronary, carotid, or peripheral atherosclerosis involving vulnerable plaque[10] require efficacious treatment to reduce lipid content and intraplaque inflammation and to induce plaque stabilization. Systemic treatment, as an adjunctive therapy to acute interventions at the culprit lesion, is potentially well adapted to this therapeutic need.

HDL exerts multiple atheroprotective actions, all of which are of immediate relevance to the pathophysiology of atherosclerosis. Central among them is the potential to facilitate removal of cholesterol from peripheral tissues and the arterial wall by a number of cellular efflux mechanisms. HDL particles equally exert a wide spectrum of actions directly relevant not only to attenuation of plaque progression but also to plaque stabilization.[8,13–17] Beyond the systemic regression and stabilization of vulnerable plaques after an acute vascular event, the antiplatelet,[18] antioxidative,[14] anti-inflammatory,[15] antiapoptotic,[13] vasodilatory,[19–21] and metabolic actions[22–25] of HDL could possibly convey immediate ischemic protection for tissue downstream of the culprit lesion. HDL infusion may thus potentially protect against both acute and reperfusion ischemic injury and secondary plaque rupture events.

HDL formulations delivered via infusion represent a novel therapeutic modality, currently in clinical development, for potential reduction of the lipid content of diffuse vulnerable plaque and possess the added potential of acting efficaciously over a short time frame. This property is essential for agents targeted to acute vascular syndromes requiring rapid plaque stabilization. Infusion of reconstituted or recombinant HDL transiently raises the number of HDL acceptor particles to efflux cellular cholesterol and ultimately to eliminate it from the body; equally, such reconstituted or recombinant HDL infusions may normalize the biological function of HDL in patients at very high cardiovascular risk, including those post event.

The aim of this review is to consider the potential impact of HDL infusion therapies on the clinical management and prognosis of atherosclerotic vascular disease as distinct from oral pharmacological HDL-raising strategies involving small molecules. Discussion of the need for large outcome trials and of the relevance of the individual biological activities of HDL particles to the pathophysiology of vascular disease is an integral feature of this review. Finally, consideration is given to new therapeutic windows in which HDL infusion therapy might be evaluated for potential impact on unmet clinical needs. To assist with navigation of this review, a list of HDL terminology, including current therapeutics in clinical development, is provided in Table 1.

Table 1.   Glossary of HDL Terminology

ABCA1	ATP-binding cassette transporter A1, a cell membrane transporter that in humans exports cholesterol and phospholipid to lipid-poor HDL particles, including nascent pre-βHDL particles and, to a lesser degree, HDL3
ABCG1	ATP-binding cassette transporter G1, a cell membrane transporter that in humans may promote cholesterol efflux to mature cholesteryl ester–rich spherical HDL particles
ACP-501	A recombinant human LCAT under development for human use (developed by Alphacore Pharma, which was recently acquired by AstraZeneca)
apoAI	Apolipoprotein AI, the major apolipoprotein in human HDL.
apoAI Milano	A naturally occurring mutated variant of the apoA1 protein; the commercial formulation of recombinant apoAI Milano is ETC-216, now MDCO-216 (The Medicines Company)
APP018	An oral apoAI mimetic peptide also known as D-4F (Novartis)
CER-001	An HDL mimetic made from recombinant apoAI produced in mammalian cell expression systems complexed with phospholipids (Cerenis)
CETP inhibitors	Cholesteryl ester transfer protein inhibitors, a class of drugs that inhibit cholesteryl ester transfer protein
CSL-111	An HDL mimetic manufactured from purified, authentic human plasma apoAI reconstituted with phospholipids (CSL Behring); this formulation has been discontinued
CSL-112	An HDL mimetic manufactured from purified, authentic human plasma apoAI reconstituted with phospholipids (CSL Behring); this formulation supersedes CSL-111.
D-4F	An oral apoAI mimetic peptide also known as APP018 (Novartis)
Delipidated HDL	Lipid-poor HDL produced by selective delipidation of HDL; this preparation can then be used for autologous reinfusion (apheresis)
ETC-216	Recombinant formulation of apoAI Milano produced by Pfizer until 2009 and then licensed to The Medicines Company, which renamed the product MDCO-216
HDL2	A major subfraction of native HDL containing lipid-rich, spherical HDL particles of large diameter (>8.8 nm, <12.9 nm)
HDL3	A major subfraction of native HDL containing lipid-poor, spherical HDL particles smaller in diameter (>7.2 nm, <8.8 nm) than HDL2
LCAT	Lecithin:cholesterol acyl-transferase, an enzyme that esterifies free cholesterol to cholesteryl ester; it is bound to both HDL and LDL in plasma
MDCO-216	Formerly ETC-216, a recombinant formulation of apoAI Milano currently produced by The Medicines Company in a licensing agreement with Pfizer
Pre-βHDL	A heterogeneous population of dense, lipid-poor, or nascent HDL particles, frequently diskoidal, with diameters between 5.5 nm and 7.5 nm
Recombinant HDL	HDL mimetics made from recombinant apoAI derived from cellular expression systems; apoAI is complexed with phospholipids, as for example in CER-001
Reconstituted HDL	HDL mimetics containing native apoAI derived from human plasma; apoAI is complexed with phospholipids, as for example in CSL-111 and CSL-112
RVX-208	An oral apoAI transcriptional upregulator (Resverlogix Corp)
SRB1	Scavenger receptors of class B1 identified as oxidized LDL receptors but equally involved in cholesterol transport to and from HDL particles

HDL indicates high-density lipoprotein; and LDL, low-density lipoprotein.

Therapeutic Context

The HDL cholesterol hypothesis proposes that therapeutic intervention to raise HDL cholesterol levels will translate into a decreased risk of premature cardiovascular disease. Most recently, this hypothesis has evolved in light of new data to a more dynamic concept in which emphasis is placed on the upregulation of cholesterol flux through the reverse cholesterol transport pathway, a process encompassing several biological activities of HDL particles.[7,17]

Currently available pharmacological agents that raise HDL constitute a diverse group and vary not only in the degree of their specificity for elevation of HDL levels but also in the degree to which they affect the metabolism and functionality of HDL particles.[7,17] In addition, such agents frequently exert significant effects on the circulating levels and intravascular metabolism of atherogenic lipoproteins [ie, very-low-density lipoprotein, their remnants, LDL, and lipoprotein(a)[17]], a feature that confounds the impact of any given agent on HDL itself. Among these drugs, fibrates and niacin (nicotinic acid) display low specificity for HDL elevation and equally affect atherogenic lipids and lipoproteins, in addition to multiple organ systems.[14] Two recent prospective trials involving extended-release niacin, Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglyceride and Impact on Global Health Outcomes (AIM-HIGH)[4] and Treatment of High-density lipoprotein to Reduce the Incidence of Vascular Events (HPS2-THRIVE) trials,[5] failed to demonstrate a mortality benefit in phase 3 trials, but there are valid arguments attributing these findings to issues of study design and power, low patient compliance, and non-HDL actions of these agents.[7,17,26]

Over the past 15 years, more targeted approaches to HDL elevation have been pursued. The most advanced of these is CETP inhibition, the goal of which is to retain nonatherogenic cholesterol in HDL by inhibiting transfer of cholesteryl ester from HDL to triglyceride-rich lipoproteins in exchange for triglyceride. Although such action effectively increases the cholesterol content per HDL particle, the very nature of CETP activity may affect not only the composition of apolipoprotein (apo) B–containing atherogenic lipoproteins such as very-low-density lipoprotein, very-low-density lipoprotein remnants, and LDL but also their circulating levels.[27] Importantly, whether this approach translates to long-term cardiovascular benefit is uncertain after the failure of 2 phase 3 CETP inhibitor trials: torcetrapib as a result of off-target effects[2] and dalcetrapib owing to a lack of efficacy.[3] In the case of dalcetrapib, raising HDL cholesterol by 30% over

subnormal baseline levels after an acute coronary syndrome failed to demonstrate a reduction in cardiovascular events in the Dalcetrapib Outcomes (dal-OUTCOMES) trial.[3] Potential explanations for this failure include the modest but clinically significant increase in blood pressure, a feature of this drug class originally observed in the Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE) trial with torcetrapib,[2] or the possibility that the increase in HDL cholesterol of 30% was insufficient to counter the underlying very high postevent risk in these patients. Independently of the possible explanations for these confusing findings, it must be recognized that our potential to exploit the HDL hypothesis in therapeutic terms is currently in disarray.

Despite these considerable setbacks, 2 other phase 3 CETP inhibitor trials involving anacetrapib and evacetrapib are in progress.[28] Regrettably, however, the elevation in HDL cholesterol achieved by the action of CETP inhibitors in these trials is focused primarily on testing the HDL hypothesis that raising HDL cholesterol will improve cardiovascular outcome, rather than assessing their impact on the reverse cholesterol transport pathway. Agents that more directly promote reverse cholesterol transport are more suited to this task.[6,7] These include recombinant lecithin:cholesterol acyltransferase, HDL mimetics (both oral and infused), and apoAI transcriptional upregulators (eg, RVX-208); their actions have been extensively reviewed.[29] Of all these agents, HDL infusion therapies represent the most physiological approach to raising HDL particle numbers and potentially increasing the antiatherothrombotic functions of these particles.

HDL infusion agents encompass isolated, partially delipidated HDL proteins (HDL delipidation by apheresis and autologous reinfusion) and native apoAI or genetic variants such as apoAI Milano complexed with phospholipids.[30] For the purposes of this review, we refer to agents derived from apoAI purified from human plasma as reconstituted HDL and the products made by recombinant means as recombinant HDL. HDL infusion therapies are inherently targeted in their application by their intravenous mode of administration and short duration of action. This has led to a focus on indications related to revascularization, including in the context of acute coronary syndromes when intravenous access is necessary for standard clinical care.

Advantages and Limitations of HDL Infusion Therapies

HDL infusion therapies have a number of unique properties (summarized in Table 2). They induce a rapid, dose-proportional, and time-dependent elevation in apoAI and pre-βHDL particles. Such effects have been well documented for reconstituted HDL (CSL-111[15,31] and CSL-112[32]), in which plasma concentrations of apoAI have been shown to increase 2-fold[15,31] and pre-βHDL to increase >30-fold.[32] Similarly, CER-001, a recombinant HDL, induces dose-dependent increases in plasma HDL cholesterol of up to 7-fold.[33] These properties endow HDL infusion therapies with the potential to effectively improve outcome within the critical time window associated with acute vascular events. This is in contrast to oral agents that typically elevate HDL over a period of weeks to months; indeed, oral agents are more aligned with a long-term strategy to prevent or delay acute events.

HDL infusion formulations are modeled on endogenous HDL particles and after infusion are rapidly remodeled into mature spherical HDL particles.[15,31,34] In humans, infusion of reconstituted HDL induces changes in HDL size, from a population of particles before infusion with mean diameter of 8.2 nm to a new larger population with a mean particle size of 8.9 nm, together with another slightly smaller population (8.0 nm) at 4 hours after infusion. Ultimately, these major heterogeneous populations are metabolized intravascularly to a single population, the size of which is similar to that at baseline at 72 hours (8.3 nm).[15] It is also possible that the incorporation or fusion of infused HDL preparations with endogenous HDL particles is associated with acquisition of HDL-associated proteins. Such a mechanism may be highly relevant to the antiatherogenic biological activity of infusion formulations of HDL because the HDL proteome is associated with multiple functions.[16,35,36]

The integration of infused HDL formulations into the endogenous HDL pool not only results in an elevation in both plasma apoAI and HDL cholesterol concentrations but also may underlie retention of the functional antiatherogenic properties of the infused HDL preparation. Should this be the case, such an effect likely explains the clear tendency of HDL infusion agents to display fewer off-target effects than agents acting to raise HDL via less direct mechanisms. With CSL-111, liver toxicity (as indicated by transaminase elevation) has been observed at high reconstituted HDL

Table 2. Advantages and Limitations of HDL Infusion Therapies

Property	Advantages	Limitations
Pharmacokinetics	Increase in HDL particle number: large, rapid, dose-proportional, time-dependent elevation of apoAI and rapid generation of high levels of preβHDL	Short apoAI half-life and duration of action Steady-state increases in particle number are not possible (but may not be necessary)
Intravenous route of administration	Suitable for administration during short-term vascular interventions	Long-term dosing not possible on a large scale Multiple doses required
HDL functionality	High, eg, cholesterol efflux, glucose uptake, vasodilatation, and inhibition of inflammation, adhesion, and platelet reactivity	
Safety profile	Generally good	
Ease and cost of production	Reconstituted HDL derived from human plasma is relatively inexpensive to produce	Recombinant HDL and apoAI mimetic peptide production is more complex and expensive

apoAI indicates apolipoprotein AI; and HDL, high-density lipoprotein.

infusion concentrations in early-phase trials,[37] although this effect has since been attributed to the excipients rather than the apoAI component of the infusion. Such effects are being addressed in reformulation in the case of CSL-112, and phase 1 results have been encouraging in terms of the safety profile.[38] Similarly, infused recombinant HDL (CER-001) has not induced changes in liver function in preliminary reports of early-phase clinical trials.[33]

The intravenous route of administration of HDL infusion therapies renders them suboptimal for long-term treatment regimens. The half-life of apoAI in these treatments is ≈48 to 72 hours, so the effect duration is relatively short. Whether the efficacy of a short-term HDL infusion is sufficient to compensate for the short duration of action in terms of plaque regression/stabilization and clinical outcomes is yet to be determined. Studies to date suggest that multiple infusions would likely be required to induce clinically meaningful effects. Animal studies suggest, however, that HDL therapies have the potential to induce significant plaque regression in as little as a week and, in this respect, are far more rapid than LDL-lowering strategies, which have proven efficacy on plaque but over longer time periods (months to years).[39,40]

A final distinction should be made between recombinant HDL/apoAI mimetics and reconstituted HDL. The apoAI component of reconstituted HDL is typically derived from a fraction of the plasma that is normally discarded, thereby reducing the cost of production. For recombinant or apoAI mimetics, production or isolation of the apolipoprotein constituents can be complex. For example, purification of the apolipoprotein constituents from host cell components for recombinant products presents technical challenges, can be costly, and may represent a potential barrier to widespread use. Cerenis, however, has developed a commercially viable production process involving mammalian cell expression systems that secrete apoAI, thereby resulting in the avoidance of bacterial endotoxin, fewer purification steps, and an increase in the final yield of purified protein far beyond *Escherichia coli* systems.

HDL Infusion Clinical Trials

Small intravascular ultrasound (IVUS) clinical trials (47–60 patients) over the past 10 years have demonstrated that infusions of recombinant HDL (apoAI Milano) in patients with coronary artery disease significantly reduce coronary plaque volume (1%–2% relative to placebo), albeit modestly, and that reconstituted HDL (CSL-111) exerts favorable effects on plaque morphology.[37,41,42] These studies were randomized, placebo-controlled trials and involved 4 to 5 weekly HDL infusions in the setting of acute coronary syndrome. In comparison, in a study of >1000 patients, high-dose atorvastatin and rosuvastatin induced percent atheroma volume changes of a similar magnitude (0.99% and 1.22%) but over a much longer time period (104 weeks).[43] More recently, autologous reinfusion of selectively delipidated HDL was studied in a randomized, placebo-controlled trial of 28 acute coronary syndrome patients.[44] In this study, 7 weekly infusions of delipidated HDL showed a trend to reduce carotid atheroma volume, although this outcome did not attain statistical significance.[44]

Thus, the effects of HDL infusion formulations to date have been modest and have been examined over relatively short (2–6 weeks) follow-up periods. This contrasts with the robust evidence base for statin therapy, for which multiple large outcome trials supported by strong mechanistic evidence of plaque regression are available.[45] Interestingly, in a post hoc analysis combining raw data from 4 prospective, randomized trials, the minor degree of statin-induced elevation in HDL cholesterol contributed significantly to plaque regression when it exceeded 7.5%.[46] On the other hand, the end point of coronary atheroma volume does not provide insight into other aspects of plaque vulnerability that may be modulated by infused HDL.

The complex biology underlying plaque stability is difficult to capture with current clinical techniques. IVUS is the current gold standard approach for assessing plaque properties in phase 2 clinical trials of HDL infusion therapies. IVUS improves greatly on conventional angiography by providing far more detail of plaque burden and, importantly, permits assessment of remodeling characteristics, including plaque geometry and volume. IVUS software correlating radiofrequency spectral analysis with plaque characteristics in tissue characterization software is beginning to address the issue of plaque biology. Approaches combining optical coherence tomography, gray-scale, and integrated backscatter IVUS appear promising in allowing quantification of parameters, including lipid volume and fibrous cap thickness.[47] To date, however, these techniques are insufficiently validated to be used as primary end points in therapeutic trials involving HDL infusion. Primary end points focused on plaque size have therefore been the norm and likely represent a limitation in assessing the true effects of HDL infusions on plaque vulnerability.

There is an emerging literature investigating the potential effects of HDL infusions on plaque stability, thrombotic risk, hemodynamics, and metabolism, although much of this evidence is based on a single HDL infusion at a relatively high dose. Therefore, significant potential exists for future investigation of end points related to plaque stability (including sound- and light-based imaging modalities) rather than simple plaque geometry. However, ultimately, longer-term morbidity and mortality outcome trials are required to bring these therapies to the market because none of the currently available imaging techniques are surrogates for cardiovascular outcome.[48] In the meantime, mechanistic studies will continue to play an important role in elucidating which aspects of HDL functionality have relevance to long-term clinical outcome.

Mechanisms of HDL Infusion Action in Humans

The long-term clinical relevance of the wide spectrum of HDL actions is yet to be established. There is clinical evidence from small mechanistic trials of single infusions that HDL exerts biological actions that may provide protection from both acute ischemic events and recurrent plaque rupture (Figure 1). Obtaining direct clinical evidence with regard to the effects of HDL infusion therapies on plaque regression and stabilization is challenging, particularly in the setting of coronary disease. In a parallel-design study, a single high-dose infusion
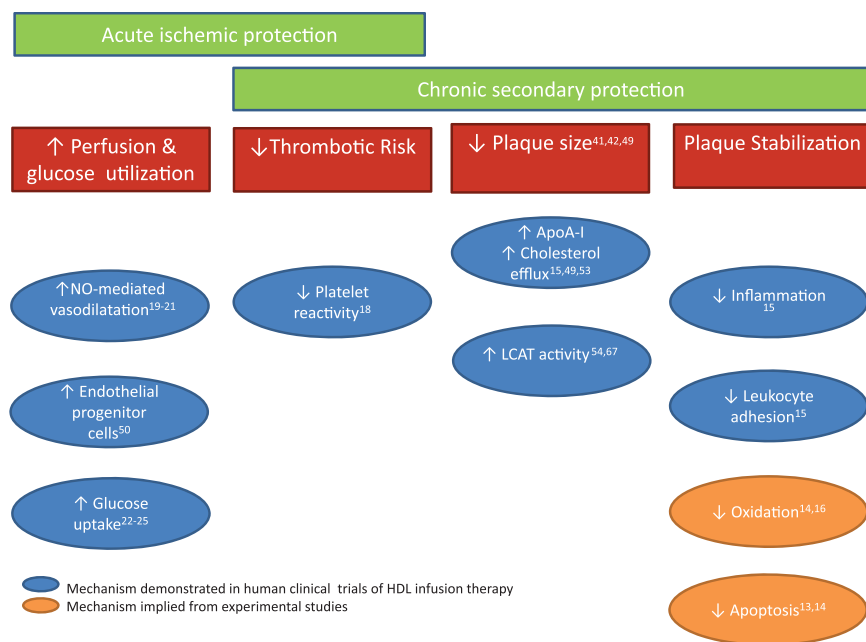
Figure 1. Potential mechanisms of high-density lipoprotein (HDL) action on infusion. HDL infusion therapies may induce both acute and chronic mechanisms that mediate atheroprotection. In the context of acute vascular events, such therapies have the capacity to reduce thrombotic risk and to attenuate tissue ischemia through increased blood perfusion and enhanced tissue glucose uptake and use. In the high-risk, postevent period, HDL therapies may provide protection through reduced platelet reactivity and through plaque regression and stabilization. Many of these actions are secondary to HDL-mediated cholesterol efflux at both the intracellular and extracellular levels. ApoA-I indicates apolipoprotein A-I; LCAT, lecithin:cholesterol acyltransferase; and NO, nitric oxide.

of reconstituted HDL (CSL-111) in patients with peripheral artery disease has been associated with a robust reduction in plaque lipid content. Although the study was small, it provides preliminary evidence that HDL infusion promotes cholesterol efflux from human plaque. Even though there are no atherectomy data for the new CSL formulation (CSL-112), recent data indicate potent cholesterol efflux activity to human plasma after CSL-112 infusion.[32]

Femoral atherectomy specimens have also displayed a reduction in macrophage size and measures of inflammation after reconstituted HDL infusion compared with placebo.[49] The reduction in inflammatory indexes is likely attributable to plaque lipid removal and has been substantiated by other studies showing downregulation of the expression of endothelial adhesion proteins for inflammatory cells such as monocytes and lymphocytes (Figure 1).[15] HDL-mediated protection of cells against apoptosis induced by oxidized lipids[13,14] could potentially confer plaque fibrous cap stability, although this has not been demonstrated directly in human studies. Finally, an increase in the number of circulating endothelial progenitor cells could contribute to improved endothelial function.[50] It remains to be tested whether these actions collectively contribute to protection from recurrent vascular events (Figure 1).

Beyond plaque regression and stabilization, HDL infusion delivered at the time of a vascular event has the potential to ameliorate the acute ischemia and consequent downstream tissue damage associated with plaque rupture/erosion (Figure 1). Anti-ischemic effects could be mediated by a combination of HDL actions, including antiplatelet,[18] vasodilatory,[19–21] and metabolic actions.[22–25] The antiplatelet effects of HDL infusion therapies could reduce thrombus formation and arterial occlusion in the acute setting. Such actions have been demonstrated with a single reconstituted HDL infusion that inhibited the heightened reactivity of diabetic platelets, partly through a reduction in the cholesterol content of platelet membranes.[18]

Clinical trial evidence substantiates the fact that HDL infusions might protect against ischemia through direct effects on blood flow.[19–21] Such evidence relies on techniques that assess vascular function in accessible peripheral regions rather than in the coronary or cerebral territories. For example, reconstituted HDL infusions increase forearm nitric oxide–mediated, endothelium-dependent vasodilatation in patients with hypercholesterolemia,[21] familial hypoalphalipoproteinemia,[19] and type 2 diabetes mellitus.[21] Two of these studies showed that HDL infusion resulted in an enhanced contribution of nitric oxide to basal blood flow,[19,20] a finding that may have relevance to protection from myocardial and cerebrovascular ischemia and claudication.

In conjunction with blood flow mechanisms that would increase oxygen and substrate delivery to critical tissues, there is emerging clinical literature suggesting that HDL infusions stimulate tissue glucose uptake and utilization.[24] The key clinically relevant observation is that an acute reconstituted HDL infusion can reduce blood glucose concentration in individuals with type 2 diabetes mellitus.[23] The underlying mechanism is insulin independent and involves intracellular signaling via ABCA1 and phosphorylation of the AMP-activated protein kinase.[23] Emerging evidence also suggests that HDL elevation may increase insulin sensitivity[17] and modulate pancreatic insulin secretion.[23] Taken together, these data suggest that HDL could contribute directly to increasing tissue ATP levels through increased glucose uptake. The evidence to date is focused on skeletal muscle glucose uptake, suggesting that HDL infusion could potentially elevate claudication threshold and increase walking time in patients with peripheral artery disease. In addition, there is circumstantial evidence that HDL promotes glucose uptake in the myocardium and that this effect is important during the acute phase of myocardial infarction.[51] Potential effects of HDL on energy substrate metabolism certainly warrant further investigation and could serve the dual purpose of lowering blood glucose and contributing to vascular protection while improving tissue glucose delivery and providing protection from ischemic damage.

Current HDL Infusion Clinical Trials

Although a number of early-phase clinical trials have been conducted using HDL infusion therapies, development has stalled for many because of issues with manufacturing (ETC-216, apoAI Milano)[41,42] and bioavailability (APP-018, d4F) and prohibitive production expenses. Currently, there is an ongoing phase 2a trial assessing the safety, tolerability, and pharmacokinetic profiles of a reconstituted HDL preparation, CSL-112 (CSL Behring), the successor to CSL-111, which showed promise in early IVUS[37] and mechanistic trials (Table 3).[15,18,20,21,23,31,49,52–54] There are also 2 trials underway with CER-001, an engineered pre-β–like HDL particle from Cerenis Therapeutics (Table 3). The first CER-001 trial (Can HDL Infusions Significantly Quicken Atherosclerosis Regression, CHI-SQUARE) is in acute coronary syndrome and uses IVUS measures of coronary plaque volume as the primary end point.[55] Although this study is not powered to detect a change in clinical outcome, it will nonetheless represent the fourth HDL infusion trial that has moved beyond safety end points with primary outcome related to plaque volume. The sample size is an order of magnitude greater than the apoAI Milano study[42] (47 versus 504 patients) and is powered to show change from baseline in the active versus the placebo group and to explore the dose range for CER-001. The second of the Cerenis trials (MODE) is in the setting of familial hypercholesterolemia with the percentage change in carotid plaque volume measured by magnetic resonance imaging as the primary end point.[56] Finally, acute partial HDL delipidation remains a viable HDL infusion therapy, but there are currently no trials in progress.

Cholesterol Removal From Vulnerable Plaques

How rapidly HDL infusion therapies can act to promote cholesterol and lipid efflux on the one hand and meaningful effects on plaque size and stability on the other is a key question. Statins have well-documented efficacy in reducing cardiovascular disease risk over a period beyond 4 months, but meta-analyses of statin efficacy in the first 14 days after an acute coronary syndrome indicate little impact.[39,40] However, HDL infusion therapies raise HDL particle number rapidly and, by virtue of their elevated cholesterol efflux capacity, have the potential to accelerate stabilization of vulnerable plaques in the first days to weeks after an acute plaque rupture.

In vitro, cholesterol-loaded J774 cells (mouse monocyte/macrophage cell line) exposed to 50 µg/mL HDL3 export cholesterol at the rate of 2.5 to 3.1 µg cholesterol per 1 mg of protein per hour.[57] By calculation, foam cells containing equal parts cholesterol and protein should efflux half of their cholesterol content in 1 week. In mice, raising apoAI, but not lowering apoB, causes plaque regression within 1 week.[58] In this study, apoAI was necessary for regression and even caused regression in the presence of high levels of non-HDL cholesterol. In rabbits, apoAI Milano (75 mg/kg) delivered twice, 4 days apart, reduced aortic cholesterol content by half.[59] Aortic plaque size was reduced by up to 32% in high-fat–fed LDL receptor–knockout mice treated with 5 to 10 doses of recombinant HDL (CER-001) on alternate days.[60] These animal data are consistent with reported human data showing a reduction in the femoral plaque lipid area and macrophage size after a single infusion of reconstituted HDL (80 mg/kg).[49]

The rapid removal of cholesterol from plaque by HDL may relate to the multiple pathways by which cholesterol can exit the cell. The predominant pathway is via the ABCA1 transporter to nascent pre-βHDL particles and, to a lesser extent, HDL3.[61,62] SRB1 and ABCG1 may also promote cholesterol efflux to mature cholesteryl ester–rich spherical HDL particles (Figure 2).[61,63] The ABCA1 pathway is particularly important given that this transporter is expressed at high levels in atherosclerotic plaque[64,65] and that some HDL therapies[15,31,32] preferentially increase numbers of pre-βHDL particles that target efflux via ABCA1.[66]

The role of lecithin:cholesterol acyltransferase in esterifying cholesterol is also a key process in reverse cholesterol transport. Reconstituted HDL has been associated with increased cholesterol esterification in plasma,[54] and CSL-112 has recently been reported to rapidly activate lecithin:cholesterol acyltransferase and to increase the rate of cholesterol esterification by 2-fold after infusion into rabbits.[67] The immediate relevance of cholesterol efflux capacity to the atheroprotective effects of HDL has also been documented in studies in which the efflux capacity of plasma in vitro was a stronger inverse predictor of atherosclerotic severity than plasma HDL cholesterol.[68] This important observation is consistent with the thesis that cholesterol efflux has relevance beyond macrophage foam cell formation, inhibition of inflammation, and plaque regression and likely underpins other beneficial actions of HDL, including a reduction in platelet activation and the

Table 3.   Summary of HDL Infusion Trials in Progress*

HDL Formulation	Phase	Intervention	Condition	Patients, n	Primary Outcome	Estimated Completion
CER-001 CHI-SQUARE study	2	Placebo vs low-, mid-, and high-dose CER-001, 6 infusions over 5 wk	Acute coronary syndrome	504	Total coronary plaque volume (IVUS)	2013
CER-001 MODE study	2	Placebo vs biweekly infusion of CER-001 for 24 wk	Homozygous familial hypercholesterolemia	30	Percent change in total carotid plaque volume (MRI)	2013
CSL-112	2a	Placebo vs CSL-112 (single escalating intravenous doses)	Stable atherothrombotic disease	40	Safety	2013

HDL indicates high-density lipoprotein; IVUS, intravascular ultrasound; and MRI, magnetic resonance imaging.

*Registered at http://www.clinicaltrials.gov.
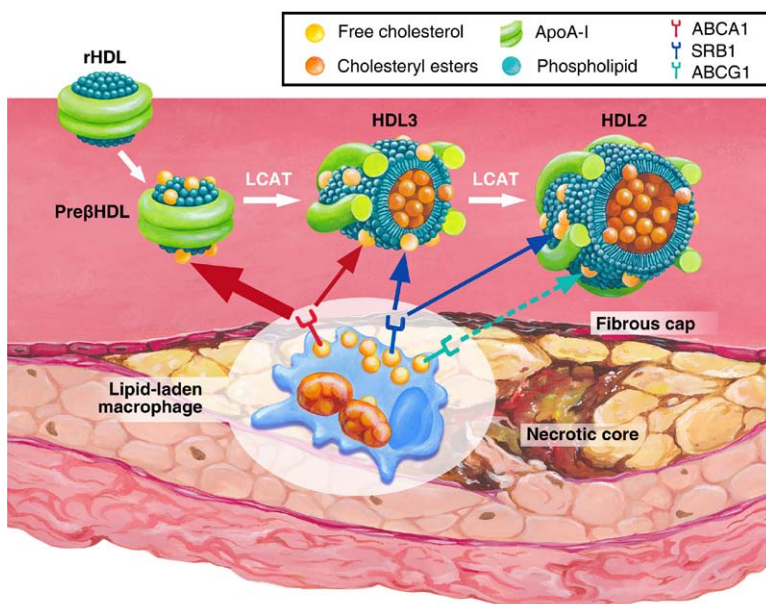
Figure 2. Schematic diagram of proposed mechanisms of cholesterol removal from an unstable plaque by high-density lipoprotein (HDL) infusion therapies. The removal of free cholesterol from lipid-laden macrophages (magnified) within atherosclerotic plaques by HDL and apolipoprotein A-I (ApoA-I) is thought to be pivotal to atheroprotection. HDL and apoA-I mediate reverse cholesterol transport from plaque through binding to a number of different, but functionally similar, proteins on the macrophage cell membrane. Among these, the ABCA1 transporter is central in modulating cholesterol removal to nascent pre-βHDL particles and, to a lesser extent, HDL3. The ABCG1 transporter and SRB1 may facilitate cholesterol uptake by more mature spherical HDL particles. Maturation of HDL particles from pre-βHDL to HDL3 and then larger HDL2 occurs under the intravascular action of lecithin:cholesterol acyltransferase (LCAT), which catalyzes conversion of free cholesterol to cholesteryl ester. In this way, cholesterol is irreversibly removed from peripheral tissues and the arterial wall. Mature HDL2 particles may subsequently transport their cholesterol cargo either directly to the liver or indirectly on transfer of cholesteryl esters via cholesteryl ester transfer protein to very-low-density lipoprotein and low-density lipoprotein (LDL), which are taken up by hepatic LDL receptors. HDL infusion therapies (reconstituted or recombinant HDL [rHDL]) comprise lipid-poor nascent HDL particles (resembling pre-βHDL) that integrate into the endogenous HDL pool where they can contribute to cellular cholesterol efflux and reverse cholesterol transport. Therefore, it is likely that these therapies influence reverse cholesterol transport primarily through the actions of the ABCA1 transporter. Note that, to aid interpretation, triglycerides have not been depicted in HDL particles.

optimization of pancreatic β-cell function and insulin secretion.[69] In addition, a recent study suggests that the cholesterol efflux capacity of HDL particles is reduced by inflammation,[70] a fact that also would explain why cholesterol efflux relates to atherosclerotic severity to a greater degree than HDL cholesterol concentration.

Future Therapeutic Windows

The potential spectrum of clinical application of HDL infusion therapies spans all vascular territories susceptible to atherothrombotic disease, from the coronaries to carotid/cerebral beds to the abdominal aorta to lower-limb arteries. The initial intervention should focus on improving short-term outcomes in the context of acute coronary syndromes and critical limb ischemia. Revascularization procedures, including coronary angioplasty and bypass surgery, carotid endarterectomy, and peripheral endovascular interventions, provide suitable clinical opportunities for HDL infusion. These settings are all associated with high recurrent risk and are compatible with an initial intravenous HDL infusion.[71] Emerging clinical and experimental evidence suggests that the rate of abdominal aortic aneurysm growth may equally be ameliorated by HDL therapies, although oral HDL-raising agents, if proven effective, would be more appropriate for this indication.[72,73]

Although the essential large outcome trials will certainly be conducted in patients with acute coronary syndromes,

proof-of-concept trials focusing on highly clinically phenotyped patient populations at elevated risk could prove a productive strategy for primary prevention. These include patients with familial hypercholesterolemia, type 2 diabetes mellitus, or HIV who exhibit low levels of HDL (which may in all likelihood be dysfunctional), premature atherosclerosis, or both and post–cardiac transplantation patients or individuals with a high systemic inflammatory burden as indicated by elevated levels of plasma high-sensitivity C-reactive protein.

Conclusions

The HDL infusion therapies in development are currently at a crossroad. The decision of whether to progress directly to phase 3 versus intermediary-phase 2b trials will depend on the perceived risk-to-benefit ratio. Ultimately, a decision will be required on the nature of HDL infusion regimens to be tested in a large morbidity-mortality outcome trial. This includes definition of the optimum dose and the number and timing of doses, with due consideration of patient compliance, duration of action, and timing of follow-up.

Large phase 3 trials will likely be "all-comer" trials that evaluate the impact of HDL infusion regimens on cardiovascular events in the months after an acute coronary syndrome using 1:1 randomization with placebo on a background of current best practice management. An ≈15% reduction in

relative risk is considered clinically meaningful and would require an estimated enrollment of ≈15 000 patients at a cost of US $250 to $300 million. Although this is a considerable investment, the market is relatively large, and the potential benefit to patients could extend beyond the coronary circulation to cerebral and peripheral vascular territories. The introduction of HDL-based therapies for short-term treatment of atherosclerotic disease would be a welcome addition to the current HDL therapeutic candidates that are focused on long-term risk reduction. The potent CETP inhibitors currently in phase 3 trials for chronic cardiovascular risk reduction are expected to report in 2015 and 2017 for evacetrapib and anacetrapib, respectively.

While options regarding the next steps are considered, small mechanistic proof-of-concept studies will continue to be important in providing insight into the broad spectrum of HDL actions. Such studies will inform the development of the necessary large outcome trials, which may potentially include end points beyond the coronary circulation to encompass cerebral and peripheral arterial diseases and potentially type 2 diabetes mellitus.

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