Apolipoprotein A-I mimetics and high-density lipoprotein function

Scott M. Gordon and William S. Davidson

Purpose of review
To review recently published advances in the development of apolipoprotein A-I (apoA-I) mimetic peptides as a potential treatment for cardiovascular diseases.

Recent findings
Various apoA-I mimetic peptides are currently in development and these display potent cardioprotective features that can rival or even surpass those of full length apoA-I and high-density lipoproteins (HDLs). These features include the ability to efflux cholesterol from various cell types as well as anti-inflammatory and antioxidative properties. Recent work has been aimed at identifying the structural features of these peptides that are responsible for these various functions and also for determining the operational mechanisms. There is also interesting new data suggesting that the intestine may be playing an important role in the action of these peptides.

Summary
In the last year, there have been many important advances in the relatively new field of apoA-I mimetic therapy. These findings support a strong potential for their development as treatment for not only cardiovascular disease but other disease states involving chronic inflammation and oxidation as well.

Keywords
apolipoprotein A-I, atherosclerosis, high-density lipoprotein, mimetic peptide

INTRODUCTION

The well-established cardioprotective nature of high-density lipoproteins (HDLs) has made them a popular target for potential cardiovascular therapies. Current HDL-based therapies, many of which are in the experimental stages, target a variety of metabolic pathways or use direct infusions of reconstituted HDL (rHDL) with the goal of raising circulating HDL levels. The most abundant HDL protein, apolipoprotein A-I (apoA-I), has been specifically targeted as it appears to underlie many of the cardioprotective functions attributed to HDL [1]. Although potential therapies have and are being evaluated that utilize the entire apoA-I sequence, alternative approaches have taken advantage of its most conspicuous structural feature – the amphipathic helix. Having both a hydrophilic and hydrophobic face, this motif is a critical feature that mediates apoA-I lipid binding affinity and interactions with plasma HDL remodeling factors and cell surface proteins.

The fact that all the exchangeable apolipoproteins contain varying numbers of these repeats, has led to the attractive idea that individual apolipoprotein functions can be mimicked with short peptide analogs. Recent advances in this field have generated a host of apoA-I mimetic peptides with varying structures that are capable of recapitulating HDL functions to various extents. Current research efforts are directed at: (i) optimizing their functional efficiency; (ii) determining their biological stability and optimal route of administration; and (iii) establishing clinical safety and efficacy. In this review, we will provide an update on several important developments in this field that have taken place over the last year. A more comprehensive account of the development and long-term progress of apoA-I mimetic peptides has recently been published by Osei-Hwedieh *et al.* [2▪▪].

University of Cincinnati Metabolic Diseases Institute, Cincinnati, Ohio, USA

Correspondence to W.S. Davidson, PhD, Department of Pathology, University of Cincinnati Metabolic Diseases Institute, 2180 East Galbraith Road, Cincinnati, OH 45237, USA. Tel: +1 513 558 3707; e-mail: davidswm@ucmail.uc.edu

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KEY POINTS

- apoA-I mimetics are short synthetic peptides made to mimic the amphipathic alpha helix of apoA-I, a major component of HDLs' structure and function.
- Over the last several years, studies have illustrated the capacity of apoA-I mimetics to imitate many of the protective functions associated with apoA-I/HDL.
- The last year has generated many significant developments in the study of apoA-I mimetic peptides and their potential usefulness as therapy for cardiovascular disease and other disease states involving chronic inflammation and oxidative stress, including asthma and cancer.
- Interesting new data points to the intestine as a potentially important site of action for apoA-I mimetic peptides.
- Small clinical trials have established that A-I mimetic peptides are generally well tolerated, however mixed results on efficacy in humans need to be addressed.

HIGH-DENSITY LIPOPROTEIN FUNCTIONS MIMICKED BY PEPTIDES

To date the most well established function of HDL is its ability to promote the efflux of cholesterol and other lipids from tissues in the periphery and transport them to the liver for recycling or excretion in the process of reverse cholesterol transport (RCT). Cholesterol efflux to HDL occurs through a number of pathways that are both protein and nonprotein mediated and can involve lipid-free and lipid-bound forms of apolipoproteins. For example, lipid poor apoA-I can interact with the ATP binding cassette transporter ABCA1 to generate nascent HDL particles composed of phospholipid and cholesterol, whereas preformed HDL can take up lipids through the actions of ABCG1 and scavenger receptor B1 (SRB1). HDLs also possess anti-inflammatory properties, such as the ability to inhibit the expression of endothelial adhesion molecules and proinflammatory chemotactic factors. Other known functions include the ability to inhibit oxidation of low-density lipoproteins (LDLs) and its subsequent rapid uptake by macrophages in the vessel wall, a significant contributor to atherosclerosis. The development of small peptides which possess these functional attributes, as opposed to the entire apoA-I protein or HDL particles, would provide many advantages including: easier and lower cost of production, increased stability and the potential for oral administration. Also, by understanding the relationships between the structure and function of these mimetic peptides, it may be possible to increase their protective capacity. The most recent advances in apoA-I mimetic functionality, as related to the best-known functions of HDL, are discussed in the following sections.

CHOLESTEROL EFFLUX

The most recently published studies examining the ability of apoA-I mimetic peptides to promote cholesterol efflux focus on both single helix and bihelical peptides. Several of these are capable of effluxing cholesterol with efficiencies that rival or even surpass full length apoA-I. The bihelical mimetic peptide 5A [3] can promote cholesterol efflux via ABCA1 when lipid free but not via ABCG1. Lipidation of 5A with the synthetic phospholipid palmitoyl oleoyl phosphatidylcholine (POPC) produced a three-fold increase in cholesterol efflux *vs.* the lipid-free form with the majority of the improvement coming from a nonspecific aqueous diffusion pathway that is known to be dependent on the phospholipid content of the acceptor particles as well as a smaller contribution from ABCG1. In atherosclerosis prone apoE$^{-/-}$ mice given intravenous 5A/POPC, circulating HDL exhibited increased phospholipid and cholesterol content and efflux to whole plasma via ABCA1 and ABCG1 were increased by 20 and 47%, respectively [4]. This study also measured an increase in whole-body RCT with 5A/POPC infusion by tracking the movement of radiolabeled cholesterol from introduced macrophages to the feces. In a different study, the peptide/lipid complex known as ETC-642, consisting of the synthetic single helix peptide ESP 24218, sphingomyelin, and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) at a mass ratio of 1 : 3.75 : 3.75, exhibited a dose dependent effect on cholesterol efflux from human macrophages that was similar to lipidated full length apoA-I (apoA-I/POPC) [5].

D'Souza *et al.* [6▪▪] have probed the impact of various structural features of bihelical peptides on several of the best known functions of HDL. Many of the features examined had a significant impact on the efficiency and specificity of ABCA1 mediated cholesterol efflux including hydrophobicity, size of hydrophobic face, charge, and flexibility of the bridge joining the two peptides. They identified a mean hydrophobicity value of −0.5 to be optimal, whereas increasing the size of the hydrophobic face and maintaining a neutral charge and flexible bridge were all beneficial for cholesterol efflux. Using this information, an 'ideal' peptide was designed (ELK-2A2K2E) which proved to promote the highest cholesterol efflux capacity of the 22 peptides tested and was significantly more effective than full length apoA-I.

In addition to engineering peptides to be more effective at a specific function such as cholesterol efflux, efforts have been made to increase their biological stability and availability. One example is the stabilization of the helical structure of an apoA-I mimetic peptide by a technique known as hydrocarbon stapling. Here, a covalent linkage is introduced between two turns of the helical structure. This linkage is targeted to specific sites along the helix preventing its unwinding and stabilizing it against thermal and proteolytic denaturation. This modification was not only found to increase a given peptide's cholesterol efflux capacity [7▪], it also represents a potential means to reduce intestinal proteolysis and thereby increase the bioavailability of orally administered peptides. This is an important obstacle that must be overcome if oral delivery of these peptides is going to be pursued. Another approach applied to several of the existing mimetic peptides has been to synthesize them with D isomer amino acids, rather than naturally occurring L isomers. This trick allows them to escape intestinal proteolytic enzymes although preserving the biophysical characteristics as well as most biological efficacies [8].

ANTI-INFLAMMATORY FUNCTION

HDL can inhibit inflammatory processes by various mechanisms (see [1] for a recent review) and it has been shown that mimetic peptides can recapitulate many of these activities. A recent example is the apoA-I mimetic peptide/lipid complex ETC-642. In vitro, ETC-642 treated human carotid artery endothelial cells (HCAECs) exhibited reduced TNF-α stimulated THP-1 monocyte adhesion and reduced VCAM-1, MCP-1, and nuclear factor-κB mRNA [9]. In vivo, these investigators utilized a vascular inflammation model in New Zealand white rabbits where a nonocclusive carotid collar was used to induce inflammation characterized by increased ICAM-1 and VCAM-1 expression. Previous studies have clearly shown that both lipid-free apoA-I and HDL can mitigate cell adhesion molecule expression in this system [10]. Interestingly, ETC-642 administered intravenously at 30 mg/kg could also reverse collar induced increases in both adhesion molecules [9].

Recent work also demonstrates that the single helix peptide 4F can influence inflammation that occurs as a result of disease states other than cardiovascular disease. In a small clinical study, patients infected with HIV and receiving combination antiretroviral therapy were found to have a higher HDL inflammatory index (HII), indicating specific defects in the HDL of these patients in terms of modulating inflammation. However, treatment with 4F restored the anti-inflammatory activity of HDL in these patients [11]. In a rat model for chronic kidney disease (CKD), subcutaneous administration of 4F decreased vascular lipid accumulation and inflammation that occurred as a result of 5/6 nephrectomy [12]. These reports support a broad anti-inflammatory function for apoA-I mimetic peptides and invite further study into their potential use for treatment of non-CVD related inflammatory diseases.

The structural features of apoA-I mimetic peptides that can influence anti-inflammatory activity have also been examined. Asymmetry of the two helices was found to be the most important factor affecting the ability of bihelical peptides to inhibit CD11b expression by monocytes. For example, when the second helix of the symmetrical peptide ELK was changed such that it was no longer identical to the first helix, the ability of the peptide to inhibit CD11b expression was increased. In fact, of the 22 peptides examined, three were asymmetrical and these reduced CD11b expression greater than any of the other peptides. Maintaining a neutral or negative net charge and a hydrophobic face of less than 180 degrees was also beneficial [6▪▪]. Interestingly, it was found that structural features that can affect anti-inflammatory responses in one cell type can be different from those in another cell type. For example, in contrast to CD11b in macrophages, helical asymmetry had only a marginal impact on a peptide's effect on VCAM-1 expression in endothelial cells. In this case, increasing the size of the hydrophobic face as well as addition of a net positive charge to the peptides was found to be beneficial. This observation underscores the remarkable potential of carefully designed mimetic peptides as selective mediators of inflammation [6▪▪].

ANTI-OXIDATIVE FUNCTION

Oxidized LDLs (oxLDLs) are a potent trigger of inflammation and atherosclerosis development in the vessel wall. ApoA-I, HDL (from healthy individuals) and apoA-I mimetic peptides can inhibit or prevent the oxidative modification of LDL lipids and proteins by various mechanisms, either (a) acting as a direct scavenger of reactive oxygen species and oxidized lipids, (b) inhibition of oxidation initiating species such as 12-lipoxygenase, or (c) action of HDL associated enzymes such as PON1 and PAF-AH. The rat CKD model, discussed above with regard to the anti-inflammatory properties of apoA-I mimetic peptides, also gives insight into the antioxidative functions of apoA-I mimetics. Impairment of renal function results in a state of increased systemic

oxidative stress, in this model, treatment with 4F reduced the abundance of the protein oxidation product nitrotyrosine in the aorta and also decreased plasma and urine concentrations of malondialdehyde, a lipid oxidation product [12]. The 4F peptide also reduced oxidative stress by affecting antioxidant enzymes. In an ovarian cancer cell line, treatment with 4F increased expression levels and activity of manganese superoxide dismutase (MnSOD), an antioxidant enzyme which catalyzes the formation of water and hydrogen peroxide from the superoxide anion. [13].

D'Souza *et al.* [6▪▪] have identified structural features that can influence the antioxidative capacity of apoA-I mimetic peptides. The inclusion of Cys or His amino acids increases the ability of bihelical mimetic peptides ELK and 5A to inhibit Cu^{2+} mediated oxidation of LDL lipids. Several other changes to the structure of these peptides such as decreasing the flexibility of the peptide linker by replacing the proline with an alanine residue or switching to either G or Y type helices also appeared to improve this function, increasing antioxidant capacity [6▪▪].

EMERGING AREAS

Inflammation and oxidation are common features of many disease states in addition to cardiovascular disease. Thus the strong anti-inflammatory and antioxidative effects of apoA-I mimetic peptides may prove useful in the management of other conditions as well. One group has published several reports in the last year demonstrating the beneficial influence of apoA-I and its mimetic peptides on tumor development in a mouse model of ovarian cancer. They first found that apoA-I transgenic mice survive longer and have decreased tumor development compared to wild type mice when injected with an ovarian epithelial adenocarcinoma cell line (ID8 cells). This positive outcome led to investigation with apoA-I mimetic peptides. In vitro, ID8 cells treated with 4F and 5F displayed decreased viability and proliferation. In vivo, subcutaneous injection of 4F reduced tumor mass and volume. The authors demonstrated that this effect may be a result of increased binding of lysophophatidic acid (LPA) (a lysophospholipid known to promote tumorigenesis) by HDL in the apoA-I mimetic treated animals [14▪]. In 2011 this group reported an additional pathway which may contribute to the antitumorigenic effects of the 4F peptide by inducing expression and the activity of the antioxidant enzyme MnSOD [13], mentioned in the previous section. The 5F peptide could be acting via an additional mechanism to prevent tumor development by inhibiting angiogenesis [15].

Asthma is a chronic disease characterized by wheezing, coughing, and shortness of breath as a result of excessive inflammation in the airways of the lungs. Because of the known anti-inflammatory properties of apoA-I mimetic peptides, Yao *et al.* hypothesized that they may act as an effective therapeutic approach to asthma. Treatment with the 5A peptide in a house dust mite-induced asthma model in mice prevented the development of asthma associated features including airway hyperresponsiveness and airway remodeling [16▪]. 5A treatment also reduced the number of inflammatory cells found in bronchoalveolar lavage fluid, likely a result of the decreased cytokine and chemokine expression detected in the treated animals.

An LPS induced sepsis model in rats leads to hypotension due to activation of nuclear factor-κB signaling resulting in increased expression of the nitric oxide generating enzyme NOS2. Injections of 4F improved vascular contractility in this model by promoting the sequestration of LPS by HDL, effectively neutralizing it [17]. The ability of HDL to sequester LPS has been known for some time but the 4F peptide appears to greatly enhance this effect. Using surface plasmon resonance, van Lenten *et al.* have demonstrated that 4F has a dramatically higher (four to six orders of magnitude) binding affinity for proinflammatory oxidized lipids than apoA-I [18]. This is likely to be a major factor contributing to the potent anti-inflammatory properties of this peptide.

The 4F peptide may also be useful against the autoimmune disease systemic lupus erythematosus (SLE), treatment in a mouse model for SLE with accelerated atherosclerosis reduced autoantibody production, osteopenia and glomerulonephritis that occur as a result of the disease [19]. These beneficial effects were attributed largely to known protective mechanisms of apoA-I/HDL/mimetic peptides including inhibition of VCAM-1 and sequestration of oxidized phospholipids. Aortic lesions in these animals were larger with 4F treatment, but displayed a more stable composition profile, having more smooth muscle area and decreased macrophage area.

IMPACT ON CARDIOVASCULAR DISEASE

The studies listed above show clearly that many of the cardioprotective effects of HDL can be recapitulated with remarkable specificity using mimetic peptides. Additionally, there do not appear to be major toxicity issues with apoA-I mimetic peptide therapy, at least thus far. Thus, many investigators are now beginning to relate these functional capacities with cardiovascular outcomes and direct measurements of cardiovascular health in various

animal models of atherosclerosis and in clinical studies. In a hyperlipidemic rabbit model, biweekly injections of ETC-642 over a 12-week-period were found to prevent a 10% increase in aortic plaque burden, assessed by intravascular ultrasound (IVUS) [5]. This effect may be related to a treatment associated decrease in pro-inflammatory negatively charged LDL. In the apoE knockout mouse, injections of mimetic peptide 5A complexed with phospholipid reduced aortic plaque surface area by up to 53% with increased cholesterol efflux cited as the likely mechanism [4].

The 4F peptide has also been shown to be efficacious against atherosclerosis in diabetic mice. ApoE knockout mice given streptozotocin to induce hyperglycemia develop three-fold greater atherosclerotic lesion area compared to apoE knockout mice. However, this increase was reduced by almost 50% upon treatment with 4F [20]. 4F has also been demonstrated to increase insulin sensitivity [21] and to prevent the development of cardiac myopathy which occurs as a result of the diabetic state [22].

One study has compared the efficacy of oral vs. subcutaneous routes of apoA-I mimetic delivery in apoE knockout mice on a Western diet. The same dose of 4F given by either method reduced plasma levels of the acute phase protein serum amyloid A (SAA), improved the HII, and inhibited aortic atherosclerosis by about 50%. It is very interesting that although plasma levels were ~1 000-fold higher with subcutaneous administration, 4F efficacy with respect to the measured parameters of cardiovascular stress was similar when administered by either method. These data suggest that it is the dose given and not the method of administration or the plasma concentration achieved that determines the effectiveness of the peptide. Additionally, the amount of peptide found in the feces was the same for the two delivery methods. This study may point to an important role for the gastrointestinal tract in the action of the 4F peptide, and raises an important question about the mechanism(s) and location(s) where this peptide might be acting to provide these benefits [23▪▪].

CONCLUSION

The last year has generated many significant developments in the study of apoA-I mimetic peptides and their potential usefulness as therapy for not only cardiovascular disease but other disease states involving chronic inflammation and oxidative stress. This recent work has highlighted the extraordinary potential of these agents to be targeted to specific metabolic pathways. A more complete understanding of how the sequence and biophysical properties impact these functions may allow for the augmentation of specific HDL cardiovascular benefits without altering other aspects of lipoprotein metabolism. However, key questions remain with regard to the specific mechanism of action of these effects, the impact of route of peptide delivery on efficacy including the role of the intestinal tract, and the optimal methods for boosting effective concentrations of peptides at the correct site of action. In two phase 1 clinical trials intravenous and subcutaneous administrations of L4F were well tolerated however, measures of HDL function were not significantly improved [24] as was seen previously in animal models and in a previous clinical study where D4F was administered orally [25]. It is clear that significantly more research is needed before these peptides find their way into mainstream clinical use.

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Conflicts of interest

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 145).

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