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Biological Properties of Apolipoprotein A-I Mimetic Peptides

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Abstract

Apolipoprotein A-I (apoA-I) mimetic peptides resemble the physiochemical properties of the helices of apoA-I and show promise for the treatment of atherosclerotic vascular diseases and other chronic inflammatory disorders. These peptides have numerous properties, such as the ability to remodel high-density lipoprotein, sequester oxidized lipids, promote cholesterol efflux, and activate an anti-inflammatory process in macrophages, any or all of which may contribute to their antiatherogenic properties. In murine models, the 4F peptide attenuates early atherosclerosis but seems to require the addition of statins to influence more mature lesions. A recently developed method for the oral delivery of the peptides that protects them from proteolysis will facilitate further research on the mechanism of action of these peptides. This review focuses on the properties of the 4F peptide, although numerous apoA-I mimetics are under investigation and a single “best” peptide that mimics all of the properties of the antiatherogenic protein apoA-I has not been identified.

Keywords

Apolipoprotein A-I; Atherosclerosis; Mimetic peptide; Inflammation

Introduction

There is a great deal of attention devoted to examining the role of high-density lipoprotein (HDL) in the protection against development of atherosclerosis. This interest derives from epidemiologic data and animal experiments pointing toward the importance of HDL and its constituent proteins in achieving this protection. Detailed information on the regulation of HDL levels in humans is scant. The major protein of HDL is apolipoprotein A-I (apoA-I), a 243-amino acid protein made up of regularly repeating amphipathic helices that have different amino acid sequences but similar biophysical properties. Reduction or over-expression of apoA-I in animal models has provided the best evidence for protection against atherogenesis by it or by HDL, as apoA-I levels influence the amount of HDL. However, it is important to recognize that although all HDL particles contain apoA-I, the pool of HDL particles in vivo are heterogeneous in size and density and contain a large variety of lower abundance proteins, reflecting considerable degree of heterogeneity among these particles [1, 2], which likely reflects a relatively high degree of functional heterogeneity.

A large number of properties have been attributed to HDL that may conceivably contribute to its biological activity. The most important of these properties relates to reverse cholesterol transport (i.e., the transport of cholesterol from the atherosclerotic lesions to the liver for excretion), enhancement of the activity of endothelial nitric oxide synthase, enzymatic antioxidative functions (i.e., paraoxonase), and a wide array of other antioxidative, anti-inflammatory, and antithrombotic effects [3].

Because high levels of HDL, at least in experimental animals, afford cardioprotection, an apparently simple strategy is to elevate apoA-I and HDL levels by the administration of apoA-I protein. This has to be done repeatedly by injection to protect it from proteolysis in the gastro-intestinal tract [4]. Although such studies have been performed as proof of principle, this is clearly not a straightforward or easily employed treatment strategy. This review focuses on our current understanding of the properties, mechanism of action, and potential of apoA-I mimetic peptides as a treatment for atherosclerosis.

The apoA-I Mimetic Peptides in the Treatment of Atherosclerosis

Cognizant of the regularly repeating amphipathic helices in apoA-I, Anantharamaiah et al. [5] developed an average helix prototype that shared many of the lipid-binding properties of the intact apoA-I protein. This 18-amino acid peptide, designated 18A, segregated hydrophilic and hydrophobic amino acids to different faces of the helix, as is the case for most of the naturally occurring helices in apoA-I [5]. Neutralizing the charges on the ends of 18A by acetylation and amidation increased its helicity and its biological activity [6]. Variants of this original peptide, based largely on the substitution of phenylalanine for leucine residues on the hydrophobic surface and with blocked amino and carboxyl termini, have been studied. The number of phenylalanine residues in the peptides has been used to designate particular variants. 18A has two phenylalanine residues and is therefore referred to as 2F (when its termini are blocked by acetylation and amidation), whereas 4F, the most commonly employed variant, has four phenylalanines. The properties of these mimetic peptides have been extensively reviewed [7–9], and some of the relevant biological properties are summarized in Table 1. Of these monomeric and tandem peptides, 4F has been most extensively studied with respect to its ability to attenuate atherosclerosis. Relatively low doses of 4F synthesized with D amino acids (D4F) given in drinking water can inhibit atherosclerosis initiation in low-density lipoprotein (LDL) receptor-deficient (LDLR^{-/-}) and apoE-deficient (apoE^{-/-}) mice [10]. 4F synthesized with D or L amino acids is also antiatherogenic when injected subcutaneously into wild-type rabbits, despite a greater than fourfold increase in plasma triglyceride levels [11]. In a variety of animal models, 4F either had no effect on [10, 12, 13] or reduced [11, 14] plasma cholesterol levels. Although 4F treatment is most effective on the very early atherosclerotic lesions in these models, it has been relatively ineffective in treating established atherosclerosis unless it is combined with statin treatment [15, 16•]. The basis for this synergy between peptide and statin is not clear. Most of the studies have relied largely on lesion size as the primary analysis, with some studies also looking at macrophage content. Further characterization of the lesions is needed to determine if the peptides influence other properties of the lesions, such as their lymphocyte or collagen content. In studies of 4F activity in vivo, female mice are most often used, although some studies have also employed male mice. However, no systematic study of the 4F response of males and females in mouse models has been reported thus far. In the remainder of this review, we focus on some other areas of the study of the biological properties of apoA-I mimetic peptides that require further clarification.

Use of apoA-I Mimetic Peptides for In Vivo Studies

Ideally, the peptides would be administered orally since peptide treatment is required continuously over a prolonged period of time in order to influence atherosclerotic lesion development. However, the mimetic peptides have important lysine residues and thus are potentially susceptible to trypsin proteolysis in the gastrointestinal tract. The D4F peptide is resistant to proteolysis and can be administered orally [10]. However, the exact mechanism of absorption of D4F by the gut is unclear. L4F and other peptides synthesized with L amino acids have been administered intraperitoneally or subcutaneously. When injected subcutaneously into rabbits, D4F and L4F had similar effects on reducing the inflammatory properties of LDL and HDL, decreased serum amyloid A (SAA) levels in the plasma, and reduced atherosclerosis [11], indicating that however the 4F works it does not appear to be very sensitive to the stereochemistry of the peptide. The medium in which the peptides are dissolved for intraperitoneal and subcutaneous injection may be important. Van Lenten et al. [11] have employed a buffer containing Tween 20 in some cases, especially when high peptide concentrations are used, to ensure that the peptide remains monomeric and in solution. More recently, a procedure for the oral administration of mimetic peptides, including those containing L-amino acids, has been developed that involves the apparent coupling of the peptides with niclosamide to form a complex that appears to be resistant to proteolysis [16••]. Further work with this combination of apoA-I mimetic peptides/niclosamide will be very valuable in the exploration of the function of the peptides. 4F has also been shown to modulate a variety of other chronic inflammatory diseases and some acute inflammatory disorders in experimental animals [17, 18]. For the latter group, parenteral administration may be useful because only a few injections are necessary for treatment.

The Assay for the Anti-Inflammatory Properties of Mimetic Peptides

A novel assay for assessing the inflammatory potential of lipoproteins that has been highly influential in this field was developed by Navab et al. [19]. In this assay, human aortic endothelial and smooth muscle cells are co-cultured to simulate an artery. The co-culture is then incubated with monocytes in the presence of human LDL. The LDL undergoes modification by oxidation, which promotes the chemotaxis of monocytes across the endothelial monolayer in response to increased expression of monocyte chemoattractant protein 1 (MCP-1) by the endothelial cells induced by the modified LDL. This is known as the monocyte chemoattractant assay and has been used to assess the inflammatory index of LDL and HDL. Co-incubation with HDL or apoA-I reduces the LDL inflammatory index, as long as the HDL or apoA-I is removed prior to the measurement of monocyte transit [19]. The mimetic peptides also reduce the inflammatory index of LDL, but they do not have to be removed from the incubation media in order to observe their anti-inflammatory effects. This suggests that the peptides have a higher affinity for oxidatively modified lipids than HDL or apoA-I, signifying the peptide-mediated sequestration of these lipids from the LDL or endothelial cells. This assay has been extensively employed to monitor the anti-inflammatory properties of a variety of mimetic peptides. This includes not only peptides based upon the structure of apoA-I, but also small tetrapeptides and several derived from apoJ (Table 2). Seven apoJ peptides were tested in this assay and two of these were shown to be anti-inflammatory, but apparently only one was capable of protecting against atherosclerosis [20]. This suggests that under particular circumstances, the monocyte chemotaxis assay may not be indicative of the antiatherogenic behavior of peptides in vivo. However, it must be recognized that the absence of a correlation between its anti-inflammatory and antiatherogenic properties in the case of the apoJ peptide may well be due to its pharmacokinetic properties rather than its in vivo anti-inflammatory properties. Most of the apoA-I mimetic peptides show a good correlation between anti-inflammatory effect in

the monocyte chemotaxis assay and prevention of atherosclerosis. This assay has also been used to demonstrate that the inflammatory properties of LDL are reduced in animals treated with 4F and several other peptides [10, 11].

The monocyte chemotaxis assay has also been very valuable in assessing the anti-inflammatory properties of HDL. Using this assay, differences in HDL of normal individuals and those with coronary artery disease have been detected, with the latter not exhibiting the usual anti-inflammatory properties of HDL [21]. This is an important concept emphasizing that in the context of cardiovascular disease, not only the level but the functionality of HDL has to be taken into account, which is a very critical caveat in evaluating the correlation of HDL as a cardioprotective agent in population-based studies. The inflammatory index/anti-inflammatory properties of HDL isolated from cholesterol-fed animals treated with 4F and several other peptides is improved relative to the HDL from untreated animals [10, 11, 20, 22].

In Vivo Mechanism of Action of Mimetic Peptides as Anti-inflammatory or Cardioprotective Agents

In a recent review, we discussed the potential mechanisms by which mimetic peptides might function in vivo as cardiovascular protective or anti-inflammatory agents [7]. Three mechanisms were discussed: 1) the sequestration of oxidized lipids based on a high affinity of active peptides for oxidized fatty acids, oxidized sterols, and oxidized phospholipids; 2) the capacity of mimetic peptides to remodel HDL, generating pre- β HDL; and 3) the capacity to promote reverse cholesterol transport. The high affinity of peptides for oxidized lipids provides an attractive explanation for the multitude of demonstrated activities for these mimetic peptides in a variety of models of chronic inflammation. Oxidized lipids are thought to be involved in a large variety of pathologies, including atherosclerosis and inflammation. In the studies recently reported by Van Lenten et al. [23•], there was good correlation between the structure of the mimetic peptide, the relative affinity for oxidized lipids, and their relative efficacy in vivo as anti-inflammatory agents. In these studies, L4F and D4F lipid-binding characteristics were compared and it was found that for the most part they have very similar affinities. However, there was about a 10-fold difference in the affinity between L4F and D4F for 20 (S) hydroxycholesterol. This suggests that the stereochemical isomers of 4F may not have quantitatively identical function. This subtle difference could provide a valuable opportunity to probe the function of the oxysterols as mediators of chronic inflammatory states such as atherosclerosis.

Among the many oxidized lipids for which 4F has a very high affinity are the oxidized fatty acids hydroperoxy- and hydroxyl-eicosaenoic acids (HETEs) or hydroperoxy- and hydroxyl-dodecaenoic acids (HODEs) [23•]. The acute treatment of apoE^{-/-} mice with D4F resulted in a substantial reduction of peroxidated long-chain fatty acids, either free or esterified, in very low-density lipoprotein (VLDL), LDL, and HDL with lipid peroxide essentially disappearing in HDL after 4F treatment [24]. In contrast, lipid peroxides increased in the pre- β HDL fraction, suggesting that the 4F peptide remodels HDL and in doing so sequesters lipoperoxide in these pre- β particles. However, it is notable that in this model, the bulk of lipid peroxide (and cholesterol) was found in the VLDL/intermediate-density lipoprotein (IDL) fraction and not LDL. Therefore, another potential explanation for the results of this study is that 4F interacts with VLDL/IDL, extracts oxidized lipids, and sequesters them in a small/dense lipoprotein fraction that has been identified as “pre- β HDL” but that may not actually represent a metabolic product of mature HDL.

The lipid peroxides in question are products of 12/15-lipoxygenase. Indeed, in the characterization of the monocyte chemotactic assay, it was shown that the reduction of 12-

lipoxygenase expression in the cultured endothelial cells by an antisense to 12-lipoxygenase essentially eliminated the modification of the LDL, thus attenuating its capacity to function as a proinflammatory particle [19]. Consistent with that finding, the addition of oxidized long-chain polyene fatty acids to LDL makes that lipoprotein proinflammatory [25]. Consistent with the role of 12-lipoxygenase and the products it generates are the observations from several laboratories that the genetic ablation of this enzyme in both the apoE^{-/-} and LDLR^{-/-} murine models profoundly reduces aortic root atherosclerosis [26]. However, a recent publication reports that 12-lipoxygenase deficiency in the apoE^{-/-} model reduces atherosclerosis in a gender- and arterial site-selective fashion [27•]. The reduction of atherosclerosis was seen in the whole aorta, but not in the aortic root, when 12-lipoxygenase was ablated; however, this was only seen in female mice. A reduction of atherosclerosis in the double knockout of both 12- and 5-lipoxygenase was seen both in the aortic root and the whole aorta. In the original publication on the effect of 12-lipoxygenase on atherosclerosis, both alleles had to be removed, suggesting that more than 50% of the enzyme has to be inactivated before any effect on atherosclerosis is established [28].

Whether the *in vivo* activity of 4F and its relatives can be fully accounted for by their ability to sequester lipoxygenase products, as demonstrated *in vitro* and *in culture*, remains to be seen. However, there is at least one *in vivo* demonstration of the antioxidant activity of 4F. LDLR^{-/-} mice fed a Western-type diet exhibit insulin resistance and renal inflammation that can be reversed with a short 4F treatment [29]. This was monitored by renal immunostaining for the EO6 antibody which recognizes oxidized phosphorylcholine. Reduced staining is assumed to reflect a reduction in tissue oxidized phospholipid content.

The Importance of HDL in Relation to the Bioactivity of Mimetic Peptides

The development of apoA-I mimetic peptides derived from an analysis of the structure of apoA-I, the major apolipoprotein of HDL. HDL has been shown, like active mimetic peptides, to sequester oxidized lipids [19]. However, there is still the question of whether HDL is required for the biological activity of the peptides. At least one anti-inflammatory mechanism attributed to 4F has been shown to be apoA-I dependent: the reduction of proinflammatory HDL in LDLR^{-/-} mice fed a Western diet [30]. It is clear from the data on peptide-mediated ATP binding-cassette A1 (ABCA1)-dependent cholesterol efflux, which is performed in the absence of HDL, that HDL is not obligatory. The mimetic peptides are capable of remodeling HDL, and this implies that the peptides must interact with HDL, at least transiently. Our own experiments have shown that these peptides, especially tandem 4F peptides, are quite active in HDL remodeling [31•]. Peptides will associate with HDL when it is the sole lipoprotein in contact with the peptide, but whether the peptide obligatorily associates with HDL with high affinity is not yet fully resolved. Navab et al. [8] have reported that 4F binds to HDL, whereas we [13] have reported that 4F does not bind to the major HDL peak. There are number of methodologic issues related to the stereochemistry of the peptide, its labeling, and the dose and route of administration that could account for the differences in our results. The absence of an antibody that recognizes the peptide is a significant impediment in these studies. The major pharmacokinetic difference between D4F and L4F relates to the instability of the latter when administered orally, either by gavage or in the diet or water. L4F and D4F have similar bioactivity when presented in association with niclosamide or when injected either subcutaneously or intraperitoneally. In a recent publication, it was shown that L4F injected intraperitoneally in a reasonably large dose was sufficient to attenuate the sepsis induced in the cecal ligation and puncture model [18], despite the peritoneum in this septic model being acutely oxidative and proteolytic. Also, the administration of L5F by repeated intraperitoneal injections was sufficient to reduce atherosclerosis in wild-type mice fed an atherogenic diet [22].

To monitor association of the peptide with lipoprotein fractions in vivo, the peptides have been labeled with ^{14}C at the acetylated N-terminus by N-terminal anthranilic acid, by iodination of the single tyrosine in the peptide, or by N-terminal biotinylation. Navab et al. [8] have invariably found the 4F peptide to elute with HDL when isolated by fast protein liquid chromatography (FPLC). To identify the peptide-bound lipoproteins, Wool et al. [13] injected biotinylated peptide intraperitoneally into apoE $^{-/-}$ mice and the biotin signal was followed by avidin probe. In these experiments, also using FPLC to fractionate the lipoproteins, the peptide was not associated with HDL but rather was found in a post-HDL fraction (smaller and more dense) that does not contain much cholesterol. This same distribution was observed whether the animals had HDL (i.e., apoE $^{-/-}$ mice) or lacked HDL (i.e., apoE $^{-}$ and apoA-I deficient mice). Importantly, the same distribution was observed when plasma from these animals was incubated ex vivo with biotinylated peptide. On the other hand, when the biotinylated tandem 4F-Pro-4F was studied in the same fashion, a different distribution was observed. The peptide associated with all lipoproteins, including a specific association with HDL. In addition, biotinylated 4F, when examined by surface plasmon resonance, did not exhibit high affinity for HDL. Of course, it is possible that by following the biotin signal we may not be following the intact peptide. It is also possible that biotinylated peptide has properties different than those of the nonbiotinylated peptide. It is worth noting however that Van Lenten et al. [23••] showed that biotinylated L4F peptide had the same capacity to bind oxidized lipids as the nonbiotinylated peptide. Further studies are clearly needed to resolve these issues.

A mechanism for the atheroprotective effects of apoA-I and perhaps mimetic peptides is the promotion of reverse cholesterol transport. Synthetic amphipathic helical peptide can promote ABCA1-dependent cholesterol efflux in vitro (Table 1). 4F has also been shown to promote reverse cholesterol transport in vivo using cholesterol-loaded J774 cells injected into the peritoneum of mice, in a context in which the atheroprotective influence of the peptide was demonstrable [24]. A number of variants of the 18-amino acid mimetic peptides have been studied. These include symmetric tandem peptides with proline, alanine, or an interhelical seven-amino acid sequence (derived from the sequence between apoA-I helices 4 and 5) between the two 4F helices. These tandem peptides are more potent than monomeric 4F in promoting ABCA1-dependent efflux in vitro [31•]. The efflux assay is performed in the absence of HDL and thus it is unlikely that the efflux capabilities of the peptides are dependent on HDL remodeling to produce a lipid-free pool of apoA-I. A similar enhanced cholesterol efflux has been demonstrated for an asymmetric tandem peptide, designated 5A. This tandem peptide contains 4F as one helix linked via a proline to a less hydrophobic sequence as the second helix [32].

The interaction of apoA-I and apoA-I mimetic peptides with ABCA1 promotes the efflux of cholesterol and also an ABCA1-dependent activation of JAK2 [33]. JAK2 is a tyrosine kinase that activates the STAT family of transcription factors, and recently apoA-I had been shown to activate STAT3 in an ABCA1-dependent process [34••]. Activation of STAT3 is anti-inflammatory in macrophages, involving the suppression of induction of proinflammatory cytokines. Because the mimetic peptides have been shown to activate JAK2, it is likely that they also activate STAT3, but this has not yet been demonstrated. Thus, it remains possible that a critical antiatherogenic effect of the peptides may be the activation of an ABCA1-dependent anti-inflammatory effect in macrophages rather than acting solely as a cholesterol efflux agent. However, it is not clear whether the promotion of an anti-inflammatory pathway is dependent on the removal of cholesterol or is a cholesterol-independent aspect of ABCA1 ligation. Further work is required to resolve this.

Conclusions

It is clear that the apoA-I mimetic peptides have a number of biological effects, including HDL remodeling, sequestration of oxidized lipids, promotion of cholesterol efflux, and an ABCA1 dependent anti-inflammatory effect. These peptides also have the capacity in certain contexts to attenuate atherosclerosis. The antiatherosclerotic effects of these peptides do not seem to be dependent on a reproducible change in plasma lipoprotein levels. In fact, in rabbits, 4F is atheroprotective despite a large increase in triglyceride-containing lipoproteins [11]. The atherosclerosis data for 4F are diverse but seem to show more robust atheroprotective effects in less mature lesions.

What is not clear is which of these peptide actions are the critical ones for atheroprotection. It has to be borne in mind that more than one of these mechanisms may operate cooperatively, or even synergistically, in protecting against the development of atherosclerosis. It is also necessary to more clearly establish the role of HDL in achieving this atheroprotection.

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Table 1

Apolipoprotein A-I mimetic peptides

Peptide	In vitro effects		Peptide-lipoprotein association and vascular effects		Study
	Cellular cholesterol efflux	Monocyte chemotactic assay		Effect on atherosclerosis ^a	
Monomeric apoA-I class A mimetic peptides					
2F Ac18ANH ₂	No effect	<i>b</i> Weak	Associates with plasma HDL and free protein	No effect	Navab et al. [8] Mendez et al. [35] Garber et al. [36]
3F-2	N/A	<i>b</i>	Associates with HDL > VLDL > IDL/LDL; binds oxPL at higher affinity than apoA-I	↓ AR lesion in 10-wk apoE ^{-/-} mice treated for 6 wk with peptide	Navab et al. [8] Handattu et al. [37]
3F14	N/A	No effect	Associates with VLDL/IDL/LDL > HDL; binds oxPL with similar affinity as apoA-I	No effect, AR lesion in 10-wk old apoE ^{-/-} mice treated for 6 wk with peptide	Van Lenten et al. [22] Navab et al. [8] Handattu et al. [37]
4F	<i>b</i> Similar to level of effect of L2F, D2F, or 37pA	<i>b</i> ↓ LOOH in lipoproteins, but ↑ in pre-β HDL	No effect on plasma lipids levels	↓ AR lesion in apoE ^{-/-} and LDLR ^{-/-} mice	Van Lenten et al. [23••] Navab et al. [10]
			↓ Plasma TC 40% by oral D4F but not IP D4F	No effect AR lesion in 20-wk apoE ^{-/-} mice fed Western diet (oral or IP D4F)	Li et al. [14]
			↓ Proinflammatory HDL (apoA-I dependent)	N/A	Ou et al. [12]
			↓ apoA-I-associated MPO		
			↑ Vasodilatation in LDLR ^{-/-} mice on HFD		Ou et al. [30]
		↓ LDL oxidation	Associates with late HDL and late LDL	N/A	Wool et al. [13]
		D and L 4F bind oxPL with much higher affinity than apoA-I	↓ SAA levels (subcutaneous D4F/L4F)	↓ <i>en face</i> aortic lesion in cholesterol-fed NZW rabbits	Van Lenten et al. [11]
			↑ Plasma TG 4–8×, ↓ plasma TC 25%	↓ AR apoE ^{-/-} mice (oral L4F with niclosamide and pravastatin for 26 wk)	Navab et al. [16••]
			N/A	Dose-dependent ↓ AR in apoE ^{-/-} mice (oral D4F)	Navab et al. [15]

Peptide	In vitro effects		Peptide-lipoprotein association and vascular effects		Effect on atherosclerosis ^a	Study
	Cellular cholesterol efflux	Monocyte chemotactic assay				
5F	N/A	<i>b</i>	↑ In vivo RCT in apoE ^{-/-} mice		N/A	Navab et al. [24]
Tandem apoA-I mimetic peptides						
37pA	<i>b</i>	N/A	Associates with plasma HDL and free protein		N/A	Navab et al. [8] Garber et al. [22]
4F-IHS-4F	<i>b</i>	N/A	N/A		N/A	Mendez et al. [35] Garber et al. [36]
4F-Ala-4F	<i>b</i>	N/A	Increases LDL oxidation		N/A	Wool et al. [31•]
4F-Pro-4F	<i>b</i>	N/A	Binds HDL specifically, as compared to L4F		N/A	Wool et al. [31•]

^a Analyzed in female mice unless noted

^b Promotes cholesterol efflux or is anti-inflammatory in the monocyte chemotaxis assay

ApoA-I apolipoprotein A-I, *AR* aortic root, *HDL* high-density lipoprotein, *HFD* high-fat diet, *IDL* intermediate-density lipoprotein, *IP* intraperitoneal, *LDL* low-density lipoprotein, *LDLR*^{-/-} low-density lipoprotein receptor deficient, *LOOH* lipid hydroperoxide, *MPO* myeloperoxidase, *N/A* not available, *NZW* New Zealand white, *oxPL* oxidized phospholipid, *RCT* reverse cholesterol transport, *SAA* serum amyloid A, *TC* total cholesterol, *TG* triglyceride, *VLDL* very low-density lipoprotein

Table 2

Peptides that mimic other apolipoproteins

Name	In vitro effects		Peptide-lipoprotein association and vascular effects	Effect on atherosclerosis ^a	Study
	ABCA1 cholesterol efflux	Monocyte chemotactic assay			
apoJ fragment peptide					
[336–357]apoJ	N/A	<i>b</i>	N/A	No effect	Navab et al. [8]
[113–122]apoJ	N/A	<i>b</i>	Associates with late HDL	↓ AR (oral D-peptide) 30-wk apoE ^{-/-} mice on chow diet treated for 24 wk	Navab et al. [8]
		Either L- or D-amino acids	Increases PON activity		Navab et al. [16••]
SAA fragment peptides ^c					
Functional as L-amino acid-based peptides					
mSAA1.1 (1–20)	↑ Efflux 4×	N/A	N/A	N/A	Tam et al. [38]
mSAA2.1 (1–20) ^d	↑ Efflux 8×	N/A	No effect	↓ <i>en face</i> lesion (IV peptide/liposome) in 14-wk apoE ^{-/-} mice on HFD + cholate treated for 2 wk	Tam et al. [38]
mSAA2.1 (74–103) ^d	↑ Efflux 8×	N/A	No effect	↓ <i>en face</i> lesion in 14-wk apoE ^{-/-} mice on HFD + cholate treated for 2 wk	Tam et al. [38]
hSAA1.1/2.1 (1–23)	↑ Efflux 8×	N/A	N/A	N/A	Tam et al. [38]
Tetrapeptides					
Functional as D- or L-amino acid-based peptides					
KRES	N/A	<i>b</i>	Associates with HDL Increases PON activity	↓ AR lesion after 12 wk (oral peptide) treatment in 18-wk apoE ^{-/-} mice	Navab et al. [39]
KERS	N/A	No effect	No HDL binding	No effect	Navab et al. [39]
			No effect on HDL levels or PON activity		
FREL	N/A	<i>b</i>	Associates with HDL Increases PON activity and HDL cholesterol	↓ AR lesion after 12 wk (oral peptide) treatment in 18-wk apoE ^{-/-} mice	Navab et al. [39]

^a Analyzed in female mice unless noted

^b Promotes cholesterol efflux or is anti-inflammatory in the monocyte chemotaxis assay

^c Only active in vitro or in vivo when administered as part of POPC/cholesterol liposome

^d Synergistic effects seen for both efflux and atherosclerosis prevention when using mSAA2.1 (1–20) and mSAA2.1 (74–103) together

ABCA1 ATP binding-cassette A1, *apo* apolipoprotein, AR aortic root, HDL high-density lipoprotein cholesterol, HFD high-fat diet, IV intravenous, N/A not available, PON paraoxonase, POPC palmitoyl oleoyl phosphatidyl choline