

## Structure and Function of HDL Mimetics

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**Abstract**—HDL mimetics have been constructed from a number of peptides and proteins with varying structures, all of which bind lipids found in HDL. HDL mimetics containing a peptide or protein have been constructed with as few as 4 and as many as 243 amino acid residues. Some HDL mimetics have been constructed with lipid but without a peptide or protein component. Some HDL mimetics promote cholesterol efflux, some have been shown to have a remarkable ability to bind oxidized lipids compared to human apolipoprotein A-I (apoA-I). Many of these peptides have been shown to have antiinflammatory properties. Based on studies in a number of animal models and in early human clinical trials, HDL mimetics appear to have promise as diagnostic and therapeutic agents. (*Arterioscler Thromb Vasc Biol.* 2010;30:164-168.)

**Key Words:** atherosclerosis ■ HDL ■ lipoproteins ■ apoA-I ■ apoA-I mimetics

### Separating HDL-Cholesterol Levels From HDL Function

Simply increasing the amount of circulating HDL-cholesterol does not reduce the risk of coronary heart disease (CHD) events, CHD deaths, or total deaths.<sup>1</sup> Heinecke<sup>2</sup> has noted that HDL-cholesterol does not define the proteins associated with HDL and suggests that the HDL proteome is a marker, and perhaps a mediator, of CHD. Zheng et al<sup>3</sup> reported that apoA-I, the major protein in HDL, is a selective target for myeloperoxidase-catalyzed oxidation, which results in impairment of the ability of HDL to promote cholesterol efflux. Singh et al<sup>4</sup> suggested that HDL could be a therapeutic target by modifying its lipid and protein cargo to improve its antiinflammatory properties. One method that has been reported to modify the lipid and protein cargo of HDL involves treatment with apolipoprotein mimetic peptides.<sup>5</sup>

### The Development of Apolipoprotein Mimetic Peptides as Therapeutic Agents

The efficacy of apoA-I in improving atherosclerosis in animal models<sup>6,7</sup> and in preliminary human studies<sup>8</sup> made it an attractive therapeutic candidate. However, human apoA-I has 243 amino acid residues, making it not only difficult and expensive to synthesize but necessitating that it be given intravenously. The initial promise<sup>8</sup> of therapeutic benefit from weekly intravenous doses for 5 to 6 weeks does not seem to have been borne out by subsequent larger clinical trials.<sup>9</sup> It is likely that longer periods of intravenous administration will be required, making this an unlikely therapy for the millions of patients with atherosclerosis.

The laboratories of Segrest and Anantharamaiah designed an 18-aa peptide that did not have sequence homology with apoA-I but mimicked the class A amphipathic helices contained in apoA-I.<sup>10-12</sup> This peptide was called 18A because it contained 18 amino acids and formed a class A amphipathic helix. When the amino and carboxyl termini were blocked by addition of an acetyl group and amide group, respectively, stability and lipid-binding properties were improved and the peptide was called 2F because of the 2 phenylalanine residues on the hydrophobic face. The 2F peptide mimicked many of the lipid binding properties of apoA-I but failed to alter lesions in a mouse model of atherosclerosis.<sup>13</sup> Using a cell-based assay, a series of peptides were tested for their ability to inhibit LDL oxidation and LDL-induced production of monocyte chemoattractant-1 (MCP-1) in response to LDL-derived oxidized lipids,<sup>14,15</sup> and peptides 4F and 5F were selected for testing in animal models.

### The Antiinflammatory Properties of the 4F and 5F Peptides

In contrast to the case for 2F, which was ineffective in reducing atherosclerosis in a mouse model,<sup>13</sup> the peptide 5F synthesized from L-amino acids (L-5F) and injected into mice significantly protected the mice from diet-induced atherosclerosis.<sup>16</sup> Concomitant with the reduction in lesions, the antiinflammatory properties of HDL improved in mice treated with L-5F.<sup>16</sup>

When the peptide 4F was synthesized from L-amino acids and orally administered to a mouse model of atherosclerosis, it was rapidly degraded in the gastrointestinal tract.<sup>17</sup> However, when the same peptide was synthesized from all D-amino acids (D-4F)

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and administered orally the peptide was found intact in the plasma.<sup>17</sup> Administration of D-4F in the drinking water significantly reduced atherosclerotic lesions in both LDL receptor-null mice on a Western diet and in apoE-null mice on a chow diet. In neither case was plasma cholesterol or HDL-cholesterol levels significantly altered. However, the antiinflammatory properties of HDL were significantly improved.<sup>17</sup>

Administration of the 4F peptide improved a number of pathological processes in animal models including influenza A pneumonia,<sup>18</sup> hyperlipidemia and sickle cell-induced vascular dysfunction,<sup>19,20</sup> scleroderma,<sup>21</sup> Type I diabetes,<sup>22,23</sup> Type II diabetes and obesity,<sup>24,25</sup> hepatic fibrosis,<sup>26</sup> vascular dementia,<sup>27</sup> Alzheimer disease,<sup>28</sup> arthritis,<sup>29</sup> and hyperlipidemia-induced renal inflammation.<sup>30</sup> The 4F peptide inhibited accelerated vein graft atherosclerosis in a mouse model<sup>31</sup> and was found to synergize with statins and cause regression of atherosclerotic lesions in old apoE-null mice.<sup>32</sup> A single oral dose of D-4F significantly improved the antiinflammatory properties of HDL in humans with CHD.<sup>33</sup> The antiinflammatory properties of the 4F peptides were the same whether synthesized from all L- or all D-amino acids when the peptides were administered by injection in cholesterol-fed rabbits and the improvement in atherosclerotic lesion area correlated with plasma serum amyloid A levels and lipoprotein antiinflammatory properties as measured in the cell-based assay.<sup>34</sup>

### Mechanism of Action of the Antiinflammatory Properties of Peptides Selected by the Cell-Based Assay

Because 4F is an apoA-I mimetic peptide, it was difficult to understand how concentrations of 4F of  $\approx 130$  nmol/L in animal models and  $\approx 4$  nmol/L in humans could be biologically active when the concentration of apoA-I in most of the animal models and in the humans was  $\approx 35$   $\mu$ mol/L.

Adding human apoA-I to cultures of human aortic endothelial cells at a concentration of  $\approx 35$   $\mu$ mol/L did not reduce LDL-induced MCP-1.<sup>35</sup> However, adding only 4.3 nmol/L of L-4F together with  $\approx 35$   $\mu$ mol/L of apoA-I significantly did.<sup>35</sup>

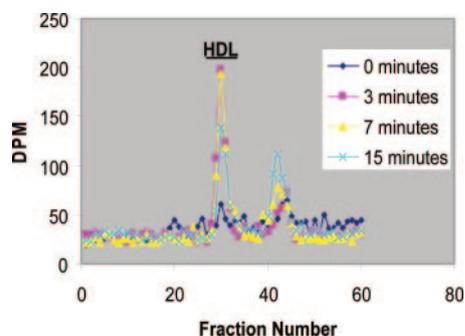
It was found that apoA-I and the 4F peptides bind nonoxidized lipids with similar affinities. The *KD* for the binding of 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (PAPC) to apoA-I was  $99\,871 \pm 14\,114$  nmol/L and for binding to L-4F it was  $192\,821 \pm 56\,505$  nmol/L. Oxidation of PAPC produced a series of oxidation products including 1-palmitoyl-2-(5,6-epoxyisoprostane E2)-*sn*-glycero-3-phosphorylcholine (PEIPC), which potently stimulates human aortic endothelial cells to produce MCP-1.<sup>36</sup> The *KD* for the binding of PEIPC to apoA-I was  $50\,720 \pm 5721$  nmol/L. In contrast, the *KD* for the binding of PEIPC to L-4F was only  $0.01 \pm 0.01$  nmol/L. Thus, the binding affinity of PEIPC for L-4F was approximately 5 million-fold greater than it was for apoA-I.<sup>35</sup>

The peptide 3F<sup>14</sup> contains one more phenylalanine residue at position 14 compared to the parent peptide 2F and was ineffective in the cell based assay.<sup>13</sup> In contrast, another peptide with 3 phenylalanine residues on the nonpolar face of the peptide, 3F-2 was found to be very effective in the cell-based assay.<sup>37</sup> Consistent with the cell-based assay measuring the ability of the peptides to bind and remove oxidized

lipids from LDL, 3F<sup>14</sup> failed to scavenge lipid hydroperoxides from LDL whereas 3F-2 was highly effective.<sup>37</sup> Administration of 3F-2 to apoE-null mice significantly reduced lesion area whereas administration of 3F<sup>14</sup> did not.<sup>38</sup> It was determined that the binding affinity of oxidized PAPC and oxidized forms of arachidonic acid to 3F-2 was similar to the binding affinity of these oxidized lipids to L-4F; the binding affinity of these oxidized lipids to 3F<sup>14</sup> was similar to their binding affinity for apoA-I.<sup>35</sup> It was hypothesized that apoA-I mimetic peptides which bind proinflammatory oxidized lipids with much higher affinity than is the case for apoA-I will be found to be antiinflammatory, and those that bind oxidized lipids similar to apoA-I will not.<sup>35</sup>

### Single Versus Tandem 4F Peptides

Wool et al<sup>39</sup> reported that in vitro tandem 4F-based peptides exceeded a single 4F peptide in HDL remodeling and cholesterol efflux but not in protection against LDL oxidation. Only the single 4F peptide was able to slow copper-mediated LDL oxidation, and all tandem peptides increased the rate of lipoxygenase-mediated LDL oxidation. These apoA-I mimetic peptides increased cellular cholesterol efflux by both ABCA1 and ABCG1 pathways.<sup>39</sup> In a subsequent study in mice, Wool et al<sup>40</sup> compared 4F to the tandem peptide 4F linked by a proline (4F-P-4F). In these studies biotinylated 4F synthesized from all L-amino acids was compared to the tandem peptide (4F-P-4F) which was also synthesized from all L-amino acids and biotinylated. These peptides were injected intraperitoneally into wild-type or apo-null or double knockout mice that were deficient in both apoE and apoA-I. Wool et al<sup>40</sup> concluded that in contrast to previous studies<sup>41,42</sup> the single 4F peptide does not associate with HDL in vivo, whereas the tandem peptide 4F-P-4F associates with all lipoprotein classes including HDL. Wool et al<sup>40</sup> further concluded that the 4F peptide binds to hemoglobin in the post HDL FPLC fractions. A major difference in the studies of Wool et al<sup>40</sup> and the previous studies<sup>41,42</sup> was the use of peptides synthesized from all L-amino acids. The previous studies<sup>41,42</sup> used 4F peptide synthesized from all D-amino acids, which is resistant to degradation in mammals.<sup>17</sup> In the study by Wool et al<sup>40</sup> (see their Figure 1) injection of biotinylated 4F into apoE null mice produced a maximum plasma signal 3 hours later. Eight hours after injection the plasma signal had only declined by one-third, and at 24 hours after injection the signal in plasma was  $\approx$  one half of the peak seen 3 hours after injection. In the previous studies in mice<sup>41,42</sup> with D-4F and in humans,<sup>33</sup> the 4F peptide was cleared from the circulation dramatically faster. As expected for a peptide synthesized from L-amino acids, and as shown in Figure 1 in this article, intravenous injection of <sup>14</sup>C-4F peptide rapidly resulted in degradation products in the post-HDL FPLC fractions. The persistence of the biotin signal in plasma for 24 hours in the studies of Wool et al<sup>40</sup> may indicate that degraded fragments of the peptide were associating with proteins with longer plasma half-lives such as hemoglobin. Consistent with this possibility are the observations by Handattu et al<sup>38</sup> that the 3F-2 peptide which has binding and bioactivity characteristics very similar to 4F has a plasma  $T_{1/2}$  of only 0.54 hours. Wool et al<sup>40</sup> also reported

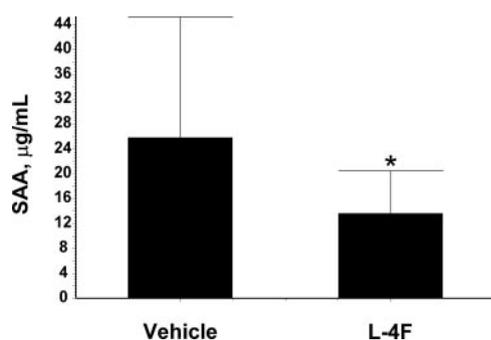


**Figure 1.** The 4F peptide when synthesized from L-amino acids undergoes degradation after injection. 40 000 dpm of  $^{14}\text{C}$ -L-4F (specific activity 4mCi/mg peptide) was injected intravenously into a wild-type C57BL/6 mouse. The mouse was bled at the indicated time points, and the plasma was fractionated by FPLC, the cholesterol content of the fractions determined (data not shown), and the radioactivity in the fractions counted and graphed as shown in the Figure. The HDL-containing fractions and the post-HDL fractions were then analyzed by reverse phase HPLC, and the results were confirmed by mass spectrometry. Intact  $^{14}\text{C}$ -L-4F was found in the HDL-containing FPLC fractions. No intact  $^{14}\text{C}$ -L-4F was found in the fractions eluting after the HDL-containing fractions at any time point.

that the 4F peptide did not reduce serum amyloid A (SAA) levels in apoE-null mice, whereas the tandem peptide did. As shown in Figure 2 of this article, despite rapid degradation of 4F synthesized from all L-amino acids, subcutaneous injection of the peptide into apoE-null mice resulted in a significant reduction in SAA levels as was previously the case for rabbits.<sup>34</sup> These differing results, which are likely attributable to methodological differences, will need to be resolved by future studies.

### Other HDL Peptide Mimetics

Sethi et al<sup>43</sup> observed that a tandem peptide with 5 alanine residues in the second helix was particularly potent in stimulating ABCA1-mediated cellular efflux. Jia et al<sup>44</sup> found that cysteine-free peptides were weak inhibitors of lipid peroxidation, but peptides based on the helical segment of apoA-I<sub>Milano</sub> that contains its cysteine variants were strong inhibitors of lipid peroxidation.



**Figure 2.** The 4F peptide significantly reduced serum amyloid A (SAA) levels in the plasma of apoE-null mice. The 4F peptide synthesized from all L-amino acids was injected subcutaneously at a dose of 1 mg/kg into apoE-null mice ( $n=19$ ), or the mice were injected with the vehicle alone ( $n=14$ ). Six hours later the mice were bled and serum amyloid A (SAA) levels in plasma were determined by ELISA. The values shown are mean $\pm$ SD; \* $P=0.0317$ .

Gupta et al<sup>45</sup> reported that a dual-domain peptide with a class A amphipathic helix linked to the receptor-binding domain of apoE (Ac-hE-18A-NH<sub>2</sub>) reduced plasma cholesterol levels in WHHL rabbits and improved arterial endothelial function. These changes were associated with reduced plasma lipid hydroperoxide levels, an increase in paraoxonase-1 activity, and a reduction of superoxide anion levels.

A 10-aa residue from apoJ containing a G\* helix was reported to render HDL antiinflammatory in mice and monkeys and reduced atherosclerosis in apoE-null mice.<sup>42</sup> A group of even smaller peptides that are not capable of forming a helix were also found to be able to improve HDL antiinflammatory properties in mice and monkeys and reduced atherosclerosis in apoE-null mice.<sup>46</sup> Busseuil et al<sup>47</sup> reported that in rabbits on a cholesterol-enriched diet supplemented with vitamin D<sub>2</sub> infusion of a class A amphipathic peptide containing 22 amino acid residues without terminal blocking groups but complexed with egg sphingomyelin and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine led to regression of aortic valve stenosis.

Cormode et al<sup>48</sup> reported that the peptide 37pA (2 18A peptides joined by a proline residue) could be used to construct nanoparticles for delivering MRI contrast agent into arteries to elucidate atherosclerotic plaque composition in a mouse model of atherosclerosis. In vitro particles made with 37pA or 18A gave similar excellent contrast for the detection of atherosclerotic macrophages even though the larger peptide demonstrated better lipid-binding properties.<sup>49</sup>

### Other HDL Mimetics

The lipid components of HDL have also been used. Studies with apoA-I<sub>Milano</sub><sup>8</sup> and recombinant HDL<sup>9</sup> have already been mentioned above. Anderson et al<sup>50</sup> reported that infusion of large unilamellar vesicles into patients with coronary atherosclerosis did not significantly improve brachial artery flow-mediated dilation but may have improved nitroglycerin-mediated dilation. Patel et al<sup>51</sup> found that infusions of recombinant HDL into 13 male patients with type 2 diabetes mellitus improved HDL inflammatory properties and reduced peripheral blood monocyte CD11b expression and neutrophil adhesion to fibrinogen matrix. After infusion of the recombinant HDL the plasma from the subjects also demonstrated increased ability to receive cholesterol from THP-1 macrophages. In another study, Drew et al<sup>52</sup> reported that infusion of recombinant HDL into 13 diabetic subjects with type 2 diabetes mellitus reduced plasma glucose levels by increasing plasma insulin and activating AMP-activated protein kinase in skeletal muscle.

### Using Binding Characteristics to Allow Oral Delivery of Apolipoprotein Mimetic Peptides Synthesized From all L-Amino Acids

When D-4F and L-4F were directly compared after subcutaneous injection in cholesterol-fed rabbits, the peptides were found to be equivalent in their ability to inhibit atherosclerotic lesions.<sup>34</sup> Therefore, it appeared likely that D-4F and L-4F only differed in their ability to resist enzymatic degradation. Based on the work of Garber et al,<sup>53</sup> one would predict that resistance to enzymatic degradation would be an advantage for oral administration of a peptide synthesized from

D-amino acids, but after absorption by any route, it also would likely remain undegraded in tissues for prolonged periods. Quite by accident it was discovered that at acid pH L-4F binds niclosamide (a drug that has been in clinical use for more than 3 decades for the treatment of parasitic infections and which has very low toxicity for mammals), forming a very tight association that protects the peptide from trypsin digestion allowing L-4F absorption and biological activity after oral administration.<sup>54</sup> A class G\* amphipathic peptide synthesized from all L-amino acids that also binds oxidized lipids and inhibits lesions in mouse models<sup>42</sup> was likewise able to be given orally with niclosamide suggesting that the binding characteristics of the mimetic peptides may allow a novel method for oral administration of peptides synthesized from all L-amino acids.<sup>54</sup>

### Use of HDL Mimetics in the Acute Versus the Chronic Setting

The use of HDL mimetics that require intravenous infusion is likely well suited to the diagnosis and treatment of acute coronary events. Because reconstituted HDL particles<sup>9,10,47,49,51,52</sup> and large unilamellar vesicles<sup>50</sup> appear to require intravenous infusion, these agents are likely to be of most use in the acute setting or in a diagnostic test.<sup>48,49</sup> In the chronic setting the use of smaller particles that can be given by subcutaneous injection<sup>34</sup> or orally<sup>17,20,21,27,28,30–33,42,46,54</sup> would seem to be more likely. The 18-aa residue containing peptide 4F has been shown to synergize with statins<sup>32</sup> in mice. Early human studies suggest that 4F may have additional benefit in patients taking statins<sup>33</sup> suggesting that such peptides maybe a useful addition to a chronic treatment regimen.

### Summary

HDL mimetics have been made from a variety of peptides, lipids, and proteins. They have been studied in a number of animal models and in early human studies, and the results suggest that they may prove useful as diagnostic and therapeutic agents.

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G.M.A., M.N., S.T.R., and A.M.F. are principals in Bruin Pharma, and A.M.F. is an officer in Bruin Pharma.

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