

Apolipoprotein A-I Mimetic Peptides for the Treatment of Coronary Artery Disease

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Abstract: High density lipoprotein (HDL) plays a critical physiological role in protecting the body against atherosclerosis by facilitating reverse cholesterol transport and the removal of oxidized phospholipids from artery walls. Apolipoprotein A-I (apoA-I) is the principal protein component of HDL that is responsible for these atheroprotective effects. We have developed an apoA-I peptide analog called D-4F, which mimics the properties of apoA-I. D-4F is synthesized from D-amino acids and thus can be administered orally. In murine models of atherosclerosis, D-4F markedly reduced atherosclerotic lesions in the absence of significant changes in total plasma cholesterol or HDL cholesterol levels. These results suggested that raw values of lipoprotein *quantity* are not the only indicators of atherosclerotic risk; the *quality* of the circulating lipoproteins is also important. As atherogenesis has been shown to be strongly linked to the presence of pro-inflammatory HDL, D-4F suggests therapeutic potential for treating atherosclerosis by improving the quality of patients' HDL (i.e. converting pro-inflammatory HDL to anti-inflammatory HDL *in vitro*). ApoA-I mimetic peptides may also prove helpful in treating other symptoms of atherosclerosis, such as endothelial dysfunction and abnormal vasorelaxation. ApoA-I mimetic peptides like D-4F indicate much promise for becoming yet another part of achieving greater cardiovascular health.

Keywords: Atherosclerosis, apoA-I, HDL, LDL, cholesterol, coronary artery disease.

High density lipoproteins (HDL) are most well-known for their role in reverse cholesterol transport. Aided by the ATP-binding cassette protein A1 (ABCA1) transporter, HDL particles remove deposited cholesterol and other lipids from artery walls and carry them back to the liver for degradation and excretion. Thus, HDL particles play a critical physiological role in protecting the body against the buildup of fatty deposits in artery walls and the ultimate development of atherosclerosis. HDL also combats atherogenesis by removing the "seeding molecules" that are responsible for the oxidation of low-density lipoprotein (LDL) [1,2]. Apolipoprotein A-I (apoA-I) is the principal protein component of HDL that is responsible for these atheroprotective effects. Like plasma levels of HDL, plasma levels of apoA-I are inversely associated with risk for coronary heart disease [3]. In fact, some studies have suggested that ApoA-I levels are a comparable or even stronger predictor of coronary heart disease than HDL levels [4-8]. From the early stages of HDL formation, apoA-I is closely associated with the phospholipid core of nascent HDL particles, and the protein is integral to the activation of lecithin:cholesterol acyltransferase (LCAT). In turn, LCAT aids the esterification of free cholesterol during the maturation of nascent HDL into larger HDL particles that are rich with phospholipids and cholesterol taken up from the tissues. As a part of this mechanism, ApoA-I is clearly an indispensable component of HDL and its atheroprotective capacities.

Peptide analogs, which are much smaller than apoA-I, can be synthesized to mimic the properties of this protein.

Previous studies have shown peptide mimetics of ApoA-I to: strongly associate with phospholipids to form lipid/protein complexes [9,10], promote cholesterol efflux [11,12], activate an enzyme needed for HDL metabolism in the circulation [13], and remove oxidized fatty acids from LDL [1,2]. One 18-amino acid peptide mimetic of ApoA-I known as 18A has been extensively studied. This mimetic contains the amino acid sequence: D-W-L-K-A-F-Y-D-K-V-A-E-K-L-K-E-A-F [14] and is abbreviated as 2F for its two phenylalanine residues [15]. Following its synthesis, acetylation of the N-terminal and amidation of the C-terminal stabilized the peptide's structure [16]. When the non-polar amino acid residues of the peptide were systematically replaced with 1 to 5 phenylalanine molecules (F), an abrupt increase in hydrophobicity and the ability to associate with phospholipids was observed between peptides 4F (2 phenylalanine replacements) and 5F (3 phenylalanine replacements). However, these peptides were less effective in activating LCAT. In a human artery wall co-culture model, peptides 4F, 5F, and 6F were comparable in their ability to inhibit LDL-induced monocyte chemotactic activity [15]. Taken together, these results suggest that a specific balance between peptide-peptide and peptide-lipid interactions is required in order to achieve optimal biological activity of such amphipathic peptides [15].

ApoA-I and ApoA-I mimetic peptides are limited in their use as pharmacological agents because proteases in the stomach prevent their effective oral administration; as a result, these peptides must be administered parenterally. While mammalian proteases are specific for peptides and proteins synthesized from L-amino acids, they do not recognize peptides or proteins synthesized from D-amino acids. When LDL-receptor null mice (i.e. a mouse model of

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human hypercholestermia) were given either L-4F or D-4F via gastric tube, only D-4F remained intact in the circulation after four hours [17]. In subsequent experiments, L-4F or D-4F dissolved in saline was administered *via* gastric tube to LDL-receptor null mice; control mice received saline alone. When the mice were bled after six hours, there was no difference in the HDL of the mice that had received L-4F or saline alone, as shown in (Fig. 1B and C). In contrast, the HDL of the mice that had received D-4F was highly anti-inflammatory, as indicated by the reduced ability of HDL to inhibit LDL-induced monocyte chemotactic activity [17]. Interestingly, LDL-receptor null mice that had been placed on a high-fat, high-cholesterol diet for 6 weeks and were administered D-4F twice daily by gastric tube showed no significant difference in total plasma cholesterol or HDL cholesterol levels compared to control mice that received

only saline. However, atherosclerotic lesions in the mice that received D-4F were dramatically reduced [17]. These results suggest that raw values of lipoprotein *quantity* are not the only indicators of atherosclerotic risk; in fact, the *quality* of the lipoproteins present in the circulation is also of significance.

ApoE is another important apolipoprotein of HDL; in mice, apoE is responsible for clearance of lipoproteins by the liver. ApoE null mice develop hyperlipidemia and atherosclerosis that resembles human atherosclerosis, even when placed on a low-fat chow diet. Furthermore, these mice produce pro-inflammatory HDL. When D-4F was added to the drinking water of apoE null mice, however, their HDL became anti-inflammatory such that their LDL-induced monocyte chemotactic activity was notably reduced [17]. As

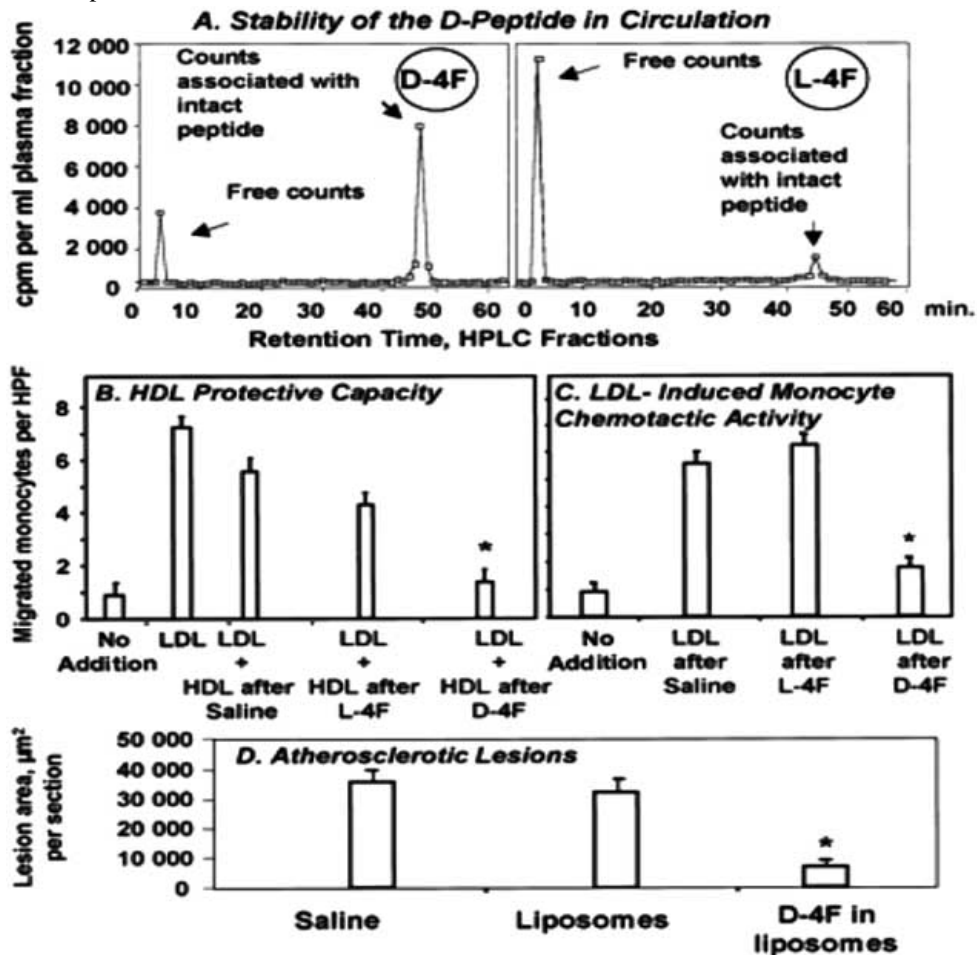


Fig. (1). Studies in LDL receptor-null mice. **A**, Radiolabeled L-4F and D-4F were administered by oral gavage. After 4 hours, blood was collected, plasma separated, delipidated, and analyzed by reverse phase HPLC. **B** and **C**, L-4F and D-4F were administered by oral gavage. Blood was collected after 6 hours, and plasma HDL and LDL were isolated by fast protein liquid chromatography. * $P < 0.0005$. **B**, Human LDL was added to cocultures alone (LDL) or together with mouse HDL taken from mice that received saline (LDL+HDL after Saline) or L-4F (LDL+HDL after L-4F) or D-4F (LDL+HDL after D-4F), and monocyte chemotactic activity was determined. **C**, Mouse LDL was isolated from mice receiving saline (LDL after Saline) or L-4F (LDL after L-4F) or D-4F (LDL after D-4F) and was added to cocultures and monocyte chemotactic activity determined. **D**, Mice were placed on a Western diet and were given by oral gavage 100 μL of saline alone (Saline), or 100 μL of liposomes without D-4F (Liposomes), or 2.5 mg D-4F in 100 μL liposomes (D-4F in liposomes), twice daily for 6 weeks. The mice were bled, and subsequently euthanized, aortic root fixed, sectioned, and the extent of lesions determined. * $P < 0.05$.

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in previous experiments, the total plasma cholesterol and HDL cholesterol levels of these mice were not reduced, but atherosclerotic lesions were remarkably decreased. Once again, these results demonstrate that administration of D-4F induced a change in the quality of HDL, despite no change in its quantity.

The conversion of pro-inflammatory HDL to anti-inflammatory HDL in apoE null mice has significant implications for therapeutic applications in humans. Previous studies reported that patients who had coronary atherosclerosis but none of the risk factors for the disease (i.e. the patients did not smoke; were not diabetic; and had plasma total cholesterol, triglycerides, LDL, and HDL levels comparable to that of healthy control individuals) had dysfunctional, pro-inflammatory HDL [18]. In contrast, the HDL of the healthy control individuals was anti-inflammatory, like the HDL of the LDL-receptor null mice and apoE null mice that received D-4F [18]. Since atherogenesis has been shown to be strongly linked to the presence of pro-inflammatory HDL, it is hypothesized that apoA-I mimetic peptides like D-4F will come to play an important role in the treatment of atherosclerosis through their ability to alter the inflammatory properties of HDL [17].

Van Lenten *et al.* found that in mice infected with the influenza virus, HDL lost its anti-inflammatory properties following infection [19]. Such changes in the properties of HDL have serious ramifications for increasing the likelihood of an atherosclerotic clinical event. When monocyte/macrophage migration to atherosclerotic plaques increases, plaques become more vulnerable to rupture and cause thrombosis. Van Lenten *et al.* tested the effects of D-4F on the properties of HDL by inducing respiratory influenza A infection in mice that had been fed a high-fat, high-cholesterol diet [20]. After infection, experimental mice were given daily intraperitoneal injections of D-4F dissolved in saline, while control mice received saline injections alone. The mice that received D-4F had anti-inflammatory HDL, while the HDL of the control mice became highly pro-inflammatory [20]. These results further support the potentially therapeutic benefits for treatment of atherosclerosis with D-4F by improving the quality of patients' HDL (i.e. converting pro-inflammatory HDL to anti-inflammatory HDL).

Finally, apoA-I mimetic peptides like D-4F may also prove to be helpful in treating other symptoms of atherosclerosis, such as abnormal vasorelaxation. Pritchard and colleagues found that mice fed a high-fat diet, as well as mouse models of sickle cell disease, showed dramatic improvements in vasoreactivity following injection with L-4F [21].

In sum, apoA-I mimetic peptides synthesized from D-amino acids show promise as a new class of oral pharmacological agents for the treatment of atherosclerosis. Furthermore, by increasing the anti-inflammatory properties of HDL and thus reducing atherosclerotic lesions – without changing total plasma cholesterol or HDL levels – D-4F has shown the value of monitoring the *quality* of HDL as well as its *quantity*. With further research, apoA-I mimetic peptides like D-4F may prove to be yet another part of achieving greater cardiovascular health.

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