Oral Administration of an Apo A-I Mimetic Peptide Synthesized From D-Amino Acids Dramatically Reduces Atherosclerosis in Mice Independent of Plasma Cholesterol

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Abstract—When apolipoprotein A-I mimetic peptides synthesized from either D- or L-amino acids were given orally to LDL receptor-null mice, only the peptide synthesized from D-amino acids was stable in the circulation and enhanced the ability of HDL to protect LDL against oxidation. The peptide synthesized from L-amino acids was rapidly degraded and excreted in the urine. When a peptide synthesized from D-amino acids (D-4F) was administered orally to LDL receptor-null mice on a Western diet, lesions decreased by 79%. When added to the drinking water of apoE-null mice, D-4F decreased lesions by approximately 75% at the lowest dose tested (0.05 mg/mL). The marked reduction in lesions occurred independent of changes in total plasma or HDL-cholesterol. **(*Circulation*. 2002;105:290-292.)**

Key Words: atherosclerosis ■ HDL ■ apo A-I ■ LDL oxidation

Infusion[1] or transgenic expression[2] of apo A-I, the major apolipoprotein of HDL, protects against atherosclerosis in animals. Proposed mechanisms by which apo A-I protects include reverse cholesterol transport[3] and removal of low levels of oxidized lipids, "seeding molecules" required to oxidize LDL.[4–6] Apo A-I and class A amphipathic helical peptide analogs of apo A-I remove these "seeding molecules" and prevent LDL oxidation.[4,5] Intraperitoneal administration of an apo A-I mimetic peptide enhanced the ability of HDL to protect LDL against oxidation and protected mice from diet-induced atherosclerosis without changing plasma cholesterol levels.[7] The major limitation for the use of apo A-I or apo A-I mimetic peptides as pharmacological agents has been the need for parenteral administration. Mammalian enzymes such as proteases recognize peptides and proteins synthesized from L-amino acids but rarely recognize those synthesized from D-amino acids. We report here that orally administered apo A-I mimetic peptides synthesized from D-amino acids dramatically inhibit atherosclerosis in mice independent of changes in total plasma or HDL-cholesterol.

Methods

Mice

Female LDL receptor-null or apoE-null mice on a C57BL/6J background were from Jackson Laboratory, Bar Harbor, Maine. LDL receptor-null mice were maintained on chow diet (Ralston Purina) until they were 4-weeks old when they were switched to a Western diet (Teklad, Madison, WI, diet No. 88137) for 6 weeks. ApoE-null mice were maintained on chow diet throughout the study. LDL receptor-null mice received the test peptide or a vehicle control by gastric gavage twice daily for the periods indicated. At 4-weeks old, the test peptide was added to the drinking water of some of the apoE-null mice and the apoE-null mice were continued on the chow diet. The lyophilized peptide was easily dissolved in a measured quantity of drinking water resulting in a clear solution and was measured and replaced with fresh solution every other day.

Mice were bled under anesthesia from the retroorbital venous plexus with Animal Research Committee approval. Atherosclerotic lesions were measured as described.[8]

Lipoproteins

LDL and HDL were isolated as described.[4] Blood was obtained from normal humans with institutional review board approval and informed consent.

Cocultures, Cellular Oxidation of LDL, Monocyte Isolation, and Monocyte Chemotaxis Assay

Human blood monocytes and aortic endothelial and smooth muscle cells were isolated and cultured, and cellular oxidation of LDL in the presence and absence of HDL was determined as described.[4]

Synthesis and Preparation of Apo A-I Mimetic Peptides

Apo A-I mimetic peptides were synthesized as described[9] except that in some instances each amino acid in the peptide was the D-stereoisomer. The peptides are based on the sequence Ac-D-W-L-K-A-F-Y-D-K-V-A-E-K-L-K-E-A-F-NH_2 (Ac-18A-NH_2 or 2F). 2F or an analog of 2F with the primary amino acid sequence Ac-D-W-F-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH_2 (4F)[9] was used. Peptides synthesized from L-amino acids are designated with an L (eg, L-4F) and peptides synthesized from D-amino acids are designated with a D (eg, D-4F). Some peptides were radiolabeled

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using IODO-BEAD reagent (Pierce), according to manufacturer's recommendations. Liposomes made of L-α-1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphocholine (Avanti Polar Lipids) with and without D-4F were made according to manufacturer's recommendations. Extraction and detection of intact peptides from mouse plasma was performed as described[10] using reverse phase HPLC.

Other Methods

Protein[4] and cholesterol[11] content of lipoproteins was determined as described.[11] Differences among groups were determined by ANOVA (Excel), with significance defined as $P<0.05$.

Results

In vitro, both L-2F and D-2F were equally able to block LDL oxidation and LDL-induced monocyte chemotactic activity (data not shown). In vivo, after oral administration, only D-4F remained intact in the circulation (Figure 1A) and significantly enhanced HDL protective capacity (Figure 1B) and decreased LDL-induced monocyte chemotactic activity (Figure 1C). Two hours after oral administration of radiolabeled peptides the urine from mice given L-4F had approximately 15 times more radioactivity than was the case for mice given D-4F (data not shown). Twice-daily administration of D-4F by gavage reduced atherosclerotic lesions in LDL receptor-null mice on a Western diet by 79% (Figure 1D). Total plasma cholesterol was not significantly different: 761±69 mg/dL, 677±52 mg/dL, and 699±31 for the D-4F, Liposome, and Saline groups, respectively. HDL-cholesterol levels were also not significantly different: 73±9 mg/dL, 66±9, and 67±6 for the D-4F, Liposome, and Saline groups, respectively.

When apoE-null mice were given D-4F in their drinking water there was a substantial increase in the protective capacity of their HDL (Figure 2A), a decrease in the ability of their LDL to induce monocyte chemotactic activity (Figure 2B), and there was an approximately 75% reduction in lesions at the lowest dose tested (Figure 2C). There was no significant difference in body, heart, or liver weights or in the amount of water consumed (2.5 mL/day/mouse) between the apoE-null mice receiving or not receiving D-4F (data not shown). Furthermore, the plasma total cholesterol was not significantly different: 556±110 mg/dL for mice not receiving peptide, 638±85, 612±63, 558±81, 529±17, 534±12, and 579±5 mg/dL for mice receiving D-4F at 0.05, 0.1, 0.2, 0.4, 1.0, and 2.0 mg/mL, respectively. HDL-cholesterol was slightly but not significantly increased in the mice receiving the highest doses of D-4F compared with mice that did not receive D-4F (34±6 mg/dL for mice without peptide, 29±6, 22±8, 30±6, 27±5, 39±5, and 43±6 mg/dL for mice receiving D-4F at 0.05, 0.1, 0.2, 0.4, 1.0, and 2.0 mg/mL, respectively).

Discussion

The marked reduction in atherosclerotic lesions in the present study occurred independent of changes in total plasma or HDL-cholesterol. The studies presented here together with our earlier studies[4–7] suggest that the ability of HDL to protect LDL against oxidation may be more important than HDL-cholesterol levels in determining lesion development.

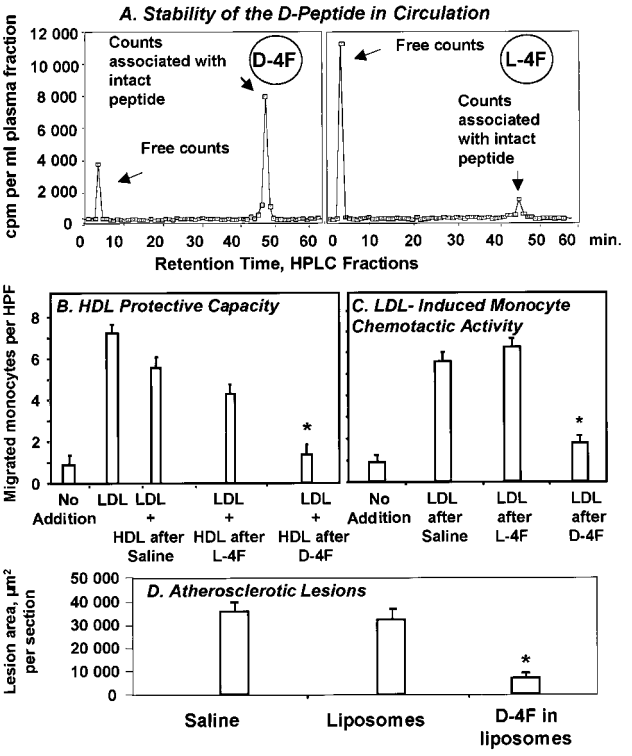

Figure 1. Studies in LDL receptor-null mice (LDL R−/−). A, Radiolabeled L-4F and D-4F were administered by oral gavage (100 μL of saline containing 100 μg of unlabeled peptide plus ^{125}I-peptide with specific activity of 11×10^6 cpm per μg peptide per mouse, n=3). After 4 hours, blood was collected, plasma separated, delipidated, and analyzed by reverse phase HPLC. B and C, L-4F and D-4F (100 μg in 100 μL of saline) were administered by oral gavage (n=5 mice for each). Blood was collected after 6 hours, and plasma HDL and LDL were isolated by fast protein liquid chromatography. Values are mean±SEM for 4 wells in 2 independent experiments. *$P<0.05$. B, Human LDL at 200-μg protein/mL was added to cocultures alone (LDL) or together with mouse HDL at 100-μg cholesterol/mL 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 at 100-μg cholesterol/mL 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) (n=4 mice), or 100 μL of liposomes without D-4F (Liposomes) (n=5 mice), or 2.5 mg D-4F in 100 μL liposomes (D-4F in liposomes) (n=6 mice), twice daily for 6 weeks. The mice were bled, and subsequently euthanized, aortic root fixed, sectioned, and the extent of lesions determined. Values are mean±SEM; *$P<0.05$.

We recently reported data on 27 patients with coronary atherosclerosis who did not smoke, were not diabetic, did not take hypolipidemic medications, and whose total plasma cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol were indistinguishable from 31 age- and sex-matched normal controls.[12] The 27 coronary artery disease patients had dysfunctional HDL[12] similar to the LDL receptor-null and apoE-null mice without D-4F (Figures 1B and 2A).

The use of apo A-I and apo A-I mimetic peptides as pharmacological agents has been limited by the need for parenteral administration. The studies presented here suggest

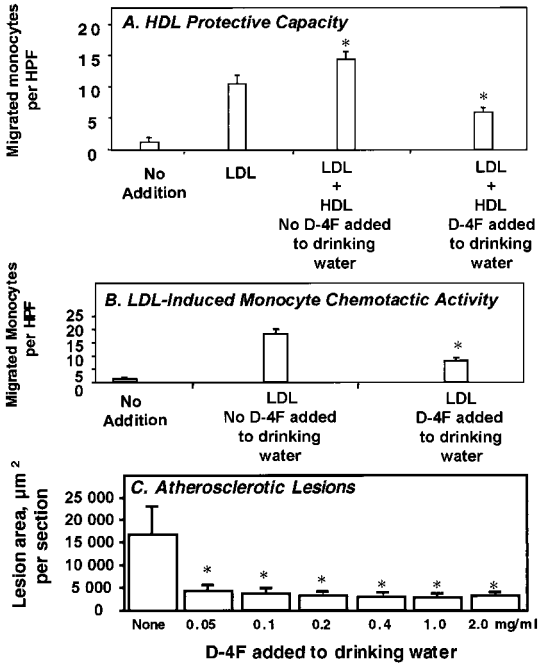

Figure 2. Studies in apoE-null mice. A and B, At 4-weeks old, D-4F was added to the drinking water of some of the mice to give a concentration of 0.05 mg/mL of D-4F (D-4F added to drinking water) (n=8 mice) and no peptide was added to the drinking water of another group of mice (No D-4F added to drinking water) (n=13 mice). The mice consumed approximately 2.5 mL of water per day. All were continued on the chow diet for 5 weeks at which time the mice were bled, and HDL and LDL were isolated and tested in the cocultures. Values are mean±SEM for 4 wells in 2 independent experiments. *$P<0.05$. A, Human LDL at 200-μg protein/mL was added to cocultures alone (LDL) or together with mouse HDL at 50-μg cholesterol/mL taken from mice that did not receive D-4F (LDL+HDL No D-4F added to drinking water) or from mice that received D-4F at 0.05 mg/mL in their drinking water (LDL+HDL D-4F added to drinking water) and monocyte chemotactic activity was determined. B, Mouse LDL was isolated from mice that did not receive D-4F in their drinking water (LDL No D4F added to drinking water) or from mice that received 0.05 mg/mL of D-4F in their drinking water (LDL D-4F added to drinking water) and was added at 50-μg cholesterol/mL to cocultures and monocyte chemotactic activity determined. C, At 4-weeks old, D-4F was added to the drinking water to give concentrations of 0.05 mg/mL (n=8 mice), 0.1 mg/mL (n=8 mice), 0.2 mg/mL (n=8 mice), 0.4 mg/mL (n=8 mice), 1.0 mg/mL (n=4 mice), or 2.0 mg/mL (n=4 mice), and no D-4F was added to the drinking water of control mice (None) (n=13 mice). The mice consumed approximately 2.5 mL of water per day. All were continued on the chow diet for 5 weeks at which time the mice were bled and subsequently euthanized, aortic root fixed, sectioned, and lesions determined. Values are mean±SEM. *$P<0.05$ compared with control mice.

that orally administered apo A-I mimetic peptides synthesized from D-amino acids may be useful for the prevention and treatment of atherosclerosis and other chronic inflammatory illnesses that are caused by oxidized lipids.

Acknowledgments

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