



Published in final edited form as:

Atherosclerosis. 2015 August ; 241(2): 709–715. doi:10.1016/j.atherosclerosis.2015.06.018.

Apolipoprotein A-I Mimetic Peptide Reverses Impaired Arterial Healing after Injury by Reducing Oxidative Stress

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Abstract

Objective—Endothelial cell (EC) migration is essential for healing of arterial injuries caused by angioplasty, but a high cholesterol diet inhibits endothelial repair. *In vivo* studies suggest that apolipoprotein A-I (apoA-I), the major protein constituent of HDL, is essential for normal healing of arterial injuries. ApoA-I mimetics, including 4F, have been designed to mimic the amphipathic portion of the apoA-I molecule. This study was undertaken to determine if 4F improves endothelial migration and healing.

Methods—A razor scrape assay was used to analyze the effect of 4F on EC migration *in vitro*. Endothelial healing *in vivo* was assessed following electrical injury of carotid arteries in mice. Markers of oxidative stress were also examined.

Results—Lipid oxidation products inhibited EC migration *in vitro*, but preincubation with L-4F preserved EC migration. Endothelial healing of carotid arterial injuries in mice on a high cholesterol diet was delayed compared with mice on a chow diet with 27.8% vs. 48.2% healing, respectively, at 5 days. Administration of D-4F improved endothelial healing in mice on a high cholesterol diet to 43.4%. D-4F administration had no effect on lipid levels but decreased markers of oxidation. *In vivo*, there was a significant inverse correlation between endothelial healing and plasma markers of oxidative stress.

Conclusion—These studies suggested that an apoA-I mimetic can improve endothelial healing of arterial injuries by decreasing oxidative stress.

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Keywords

Endothelium; hypercholesterolemia; apolipoprotein A-I mimetic; D-4F; L-4F; endothelial migration; arterial healing

INTRODUCTION

Cardiovascular disease has a major impact on the length and quality of life. The population is aging and between the years 2010 and 2030, the number of people over the age of 65 is expected to increase by 73% in the United States [1], and the number of cardiovascular interventions (angioplasties, stents, and vascular grafts) performed in 2030 is expected to be 1.73 times the number performed in 2010. The long-term results of vascular interventions are compromised by intimal hyperplasia and continued thrombogenicity of the incompletely endothelialized surface. Until the endothelial cell (EC) surface continuity is restored, the angioplasty site remains thrombogenic relative to the adjacent normal artery. Additionally, the development of intimal hyperplasia is more severe if restoration of the endothelial monolayer is delayed [2, 3]. Many factors that inhibit EC migration *in vitro* or delay arterial healing *in vivo* have been identified. Oxidized LDL and lysophosphatidylcholine (lysoPC), the major lysophospholipid in oxidized LDL, block EC migration *in vitro* [4, 5], and a high cholesterol diet retards endothelial healing of arterial injuries in a mouse model [6]. Also, healing of carotid injuries is delayed in mice deficient in apolipoprotein A-I (apoA-I), the major protein component of HDL, and reconstitution of apoA-I allows normal healing [7]. These findings suggest the importance of apoA-I in EC migration.

ApoA-I has anti-oxidant and anti-inflammatory properties as well as cardiovascular protection and reverse cholesterol transport functions [8–11]. ApoA-I can inhibit LDL oxidation, remove lipid hydroperoxides, decrease monocyte chemotaxis, and protect EC from apoptosis [9, 12–15]; all properties that would aid in endothelial healing of arterial injuries. In fact, apoA-I Milano administration decreases intimal thickening and macrophage content after balloon injury of arteries in cholesterol-fed rabbits [16].

ApoA-I mimetics have been developed to replicate the anti-atherogenic functions of apoA-I. An 18 amino acid peptide, 4F (Ac-DWFKAFYDKVAEKFKAEAF-NH₃) [17], reproduces the helical and amphipathic portion of apoA-I which is key to its function [18]. Phenylalanine residues on the non-polar face increase the hydrophobicity and lipid binding ability [19]. D-4F and L-4F, composed of the D- and L-isomers of the amino acids, respectively, have comparable profiles of action [20], and function similarly to apoA-I. D-4F is stable when administered orally, but L-4F must be delivered parenterally [17]. ApoA-I mimetics can improve reverse cholesterol transport, increase levels of pre-beta HDL (the fraction most important in reverse cholesterol transport), decrease atherosclerotic lesion formation, prevent oxidation of LDL, decrease LDL-induced monocyte chemotactic activity, and increase the anti-inflammatory properties of HDL [12, 13, 17, 21–23].

This study was undertaken to determine if an apoA-I mimetic can promote EC migration. Using a razor scrape assay, the effect of L-4F on endothelial migration *in vitro* was assessed.

The ability of D-4F to promote endothelial healing of a carotid injury in chow-fed mice and reverse the detrimental effect of a high cholesterol diet was also studied.

MATERIALS AND METHODS

Bovine aortic EC culture and migration study

Bovine aortic EC (BAEC) were isolated from adult bovine aortas, cultured, and used between passage 4 and 10, as previously described [24]. To assess early EC migration during a time period not influenced by cell proliferation, a razor scrape migration assay was used. In the appropriate study groups, cells were pretreated for 3 hours with medium, 0.43 $\mu\text{mol/L}$ of L-4F [17], or 0.43 $\mu\text{mol/L}$ of a scrambled peptide containing the same amino acids as in L-4F (Ac-DWFAKDYFKKAFVEEFAK-NH₃) (GenScript, Piscataway, NJ). At the end of 3 hours, EC in one region were removed with a razor blade, as previously described [24]. LysoPC (1-palmitol-2-hydroxy-*sn*-glycerol-3-phosphocholine; 12.5 $\mu\text{mol/L}$, Avanti Polar Lipids, Alabaster, AL) was added to appropriate wells. After 24 hours, EC were fixed and an observer, blinded to experimental conditions, counted the number of migrating EC using NIH Image 1.63 software (<http://rsb.info.nih.gov/ij/>).

Measurement of reactive oxygen species (ROS)

Intracellular ROS were monitored by oxidation of dichloro-dihydro-fluorescein diacetate (DCF-DA) assay, as previously described [25]. EC were grown to 70% confluence and loaded with DCF-DA (10 $\mu\text{mol/L}$, Life Technologies, NY) for 20 min. EC were washed, incubated for 3 h with L-4F (0.43 $\mu\text{mol/L}$) or scrambled peptide (0.43 $\mu\text{mol/L}$), washed, then lysoPC was added for 15 min, as indicated. DCF fluorescence was detected at 480 nm with a Leica DM IRB inverted fluorescence microscope (Heidelberg, Germany) using an FITC filter. A minimum of 3 randomly selected locations were captured in each well for analysis.

Immunoblot analysis for NAD(P)H oxidase activation and externalization

NAD(P)H oxidase activation was assessed by evaluating p47^{phox} phosphorylation. Confluent BAEC were incubated in serum-free DMEM containing 0.1% gelatin. Cells were incubated for 3 hours with either medium or L-4F (0.43 $\mu\text{mol/L}$). Following pretreatment, the cells were washed, and lysoPC (12.5 $\mu\text{mol/L}$) was added as appropriate. After a 15 minute incubation period, cells were lysed, proteins (50 $\mu\text{g/lane}$) resolved by SDS-PAGE and detected by immunoblot analysis using an antibody specific for phospho-p47^{phox} (Ser³⁴⁵) (Assay Biotech, Sunnyvale, CA). The membranes were reprobed for actin (Chemicon, Temecula, CA) to confirm equal loading of the lanes.

Translocation of NAD(P)H oxidase subunits, p47^{phox} and p67^{phox}, to the cell membrane was evaluated. EC were incubated with L-4F (0.43 $\mu\text{mol/L}$) or the scrambled peptide (0.43 $\mu\text{mol/L}$) for 3 h, washed, then lysoPC (12.5 $\mu\text{mol/L}$) was added for 15 min as indicated. EC were lysed and cell membranes disrupted by passage through a 20- to 25-gauge needle. The cytosolic fraction was separated from the membrane fraction by centrifuged at 15,000 g for 20 min at 4°C. The supernatant containing the cytosolic fraction was removed for analysis. To disrupt the nuclear membranes, the pellet was resuspended in buffer (50 mM HEPES, 150 mM NaCl, 1 mM EDTA, 200 μM Na₃VO₄, 100 mM NaF, 1% Triton X-100, pH 7.4)

containing protease inhibitors (Complete, Roche, Indianapolis, IN) and centrifuged at 15,000 g for 20 min at 4°C. The supernatant containing the nuclear proteins was discarded. The pellet containing the membrane fraction was resuspended for analysis. The presence of p47^{phox} and p67^{phox} in the cytosolic and membrane fractions was assessed by Western blot using anti-p47^{phox} antibody (1:1000, Cell Signaling Technology) and anti-p67^{phox} antibody (1:1000, Cell Signaling Technology, MA).

Mouse model

Six-week-old male C57Bl/6 mice were randomly divided into four study groups: 1) chow diet, 2) high cholesterol (HC) diet (Harlan Teklad, Madison, WI) containing 21% milk fat and 0.2% cholesterol by weight, 3) chow diet plus D-4F, and 4) HC diet plus D-4F. D-4F (21.7 µmol/L, GenScript) was administered in the drinking water starting two weeks prior to carotid injury and continued until the end of study. The Institutional Animal Care and Use Committee approved the study protocol. The animal protocols and care complied with the American Association of Laboratory Care Guidelines and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, 1996).

At eight weeks of age, a perivascular electrical injury of the right common carotid artery was performed as previously described [6]. Electrocautery was applied to the artery using bipolar forceps with 4 mm wide tips. The injury was produced by applying 2 watts of power to the artery for 3 seconds.

Mouse carotid artery harvest and analysis for EC migration

At 120 hours after carotid artery injury, when the injury is approximately 50% healed in mice on a chow diet, the mice were anesthetized. Blood was drawn from the inferior vena cava and Evans Blue (5% in PBS, 100 µl) was injected intravenously to stain arteries not having an intact EC surface [6]. Arteries were perfusion-fixed, removed, pinned flat, imaged, and analyzed as previously described [6].

Plasma assays

Plasma levels of total cholesterol, HDL, LDL/VLDL, lysoPC, and thiobarbituric acid reactive substances (TBARS) were assessed at the conclusion of the study. Plasma from blood collected at the time of carotid removal, with added butylated hydroxytoluene (BHT), was stored under N₂ at -80° C until analyzed for total cholesterol, HDL cholesterol, and LDL/VLDL cholesterol by a cholesterol oxidase method (Infinity Cholesterol Reagent; Thermo Fisher Scientific, Inc., Waltham, MA), as previously described [26]. Plasma lysoPC concentration was determined by an enzymatic method (Azwell LPC Assay Kit; Cosmo Bio USA Inc., Carlsbad, CA), and lipid peroxidation was measured as TBARS by a fluorometric assay, as previously described [6, 26].

Urine assays

Urine was collected during the first 24 hours after injury and for the 24 hours prior to carotid artery removal using a metabolic cage (Fisher Scientific, Pittsburgh, PA), and 8-isoprostanes measured as an additional marker of systemic oxidative stress, as previously described [27].

Urine 8-isoprostane levels were normalized to creatinine to correct for variation in urine concentration.

Tissue lipid analysis

The injured right and uninjured left common carotid arteries were isolated from surrounding tissue and immersed in a cold solution of 0.15 mol/L NaCl, 0.1 mmol/L BHT, and 1 mmol/L EDTA. Samples were stored under N₂ at -80° C until analyzed. After combining carotid tissue from four mice to achieve a measurable level of cholesterol, tissue was minced, lipids extracted, and cholesterol was measured by HPLC, as previously described [26].

Immunostaining for oxidized LDL

Oxidized LDL in the carotid arteries, as a marker of oxidative stress, was assessed by immunohistochemistry as previously described [6]. OxLDL was identified by use of an antibody to hypochlorite-oxidized LDL (1:2000, Calbiochem, Gibbstown, NJ). Tissue stained for oxLDL was scored on a scale of 0 to 4 by three observers blinded to the treatment group, giving a qualitative measure of oxLDL content.

Statistical analysis

GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA) was utilized for statistical analysis. The results were represented as the mean±standard deviation (SD) or mean ±standard error (SE) of the mean, as indicated. Student t-test and ANOVA followed by Tukey's posthoc multiple comparison test were used for the analysis. $P<0.05$ was considered statistically significant.

RESULTS

LysoPC-induced inhibition of EC migration is attenuated by pretreatment with L-4F

The ability of an apoA-I mimetic peptide, L-4F, to alter EC migration *in vitro* was assessed in BAEC. Treatment with L-4F led to an insignificant increase in EC migration relative to control (n=3, $P=NS$, Fig. 1A & 1B)). Treatment with the scrambled peptide did not alter EC migration relative to control (n=3, $P=NS$, Fig. 1A & 1B). Next, the ability of L-4F to preserve migration of EC exposed to lysoPC was assessed. EC were pretreated with PBS, L-4F, or scrambled peptide for 3 hours, washed, then incubated with lysoPC. LysoPC inhibited EC migration by 72% (n=3, $P<0.001$; Fig. 1A & 1B). After pretreatment with L-4F, lysoPC only inhibited migration to 32% of control, a significant improvement over cells incubated with lysoPC without L-4F pretreatment (n=3, $P<0.001$ compared to lysoPC, Fig. 1A). Pretreatment with scrambled peptide did not change the inhibitory effect of lysoPC on EC migration. These results suggested that 4F has a specific action in countering the anti-migratory effect of lysoPC.

L-4F binds lipids, especially oxidized lipids [28], but in the studies above, L-4F was removed and cells washed prior to the addition of lysoPC. No L-4F was present in the medium to bind the lysoPC. To assess whether L-4F could further improve EC migration by directly binding lysoPC, the experiment was repeated but without removal of L-4F or washing of EC. Under these conditions, L-4F improved EC migration in the presence of

lysoPC even further, with only a 4% decrease in migration from the medium alone (n=3, $P<0.001$ compared to lysoPC, Fig. 1B). Pretreatment with the scrambled peptide prior to incubation with lysoPC did not improve EC migration compared with cells incubated with lysoPC alone. The results suggested that L-4F partially preserved EC migration by a direct effect on EC in the presence of lysoPC, and also binds lysoPC to further preserve migration.

L-4F reduces intracellular ROS

Intracellular ROS was assessed by monitoring oxidation of DCF. LysoPC increased ROS in EC (Supplemental Fig. 1A). When EC were pretreated with L-4F, but not the scrambled peptide, lysoPC did not increase ROS in EC (Supplemental Fig. 1A).

L-4F does not inhibit lysoPC induced activation and externalization of NADP(H) oxidase

To explore the ability of L-4F to block lysoPC-induced NAD(P)H oxidase activation, p47^{phox} phosphorylation was assessed. Incubation of BAEC with lysoPC resulted in phosphorylation of p47^{phox} (n=3, Supplemental Fig. 1B). Pretreatment with L-4F did not alter the lysoPC-induced p47^{phox} phosphorylation. (n=3, Supplemental Fig. 1B).

Externalization of NADH(P)H oxidase subunits p47^{phox} and p67^{phox} was assessed. The p47^{phox} and p67^{phox} subunits translocated from the cytoplasm to the membrane following BAEC incubation with lysoPC (n=3, Supplemental Fig. 1C). Pretreatment with L-4F did not alter the lysoPC-induced translocation of p47^{phox} and p67^{phox}. L-4F did not block the lysoPC-induced inhibition of EC migration by preventing the activation and externalization of NAD(P)H oxidase subunits.

Mouse study groups

Ninety-seven 8-week old C57Bl/6 mice were used to assess the effect of an apoA-I mimetic on endothelial healing of an arterial injury. Two weeks prior to carotid injury, the mice were assigned to a diet and treatment group and maintained on the assigned diet and medication until 120 hours after the injury. Of the mice that underwent the carotid injury, no mice died during the procedure or thrombosed the injured artery. Table 1 details the demographics for the mice that underwent carotid injury. Mice fed the chow and HC diets were not significantly different with respect to age, but the hypercholesterolemic mice weighed more at the time of injury as compared with the mice on a chow diet. Mice treated with D-4F were not significantly different with respect to age or weight at the time of injury as compared with mice on the corresponding diet not receiving D-4F.

Plasma cholesterol

Plasma cholesterol levels were determined from blood samples obtained 120 hours after carotid injury. Mice receiving a HC diet had significantly higher plasma total cholesterol levels than chow-fed mice ($P<0.001$, Table 1). Similarly, a HC diet was associated with a significant increase in plasma HDL and LDL/VLDL levels ($P<0.001$, Table 1). Treatment with D-4F did not significantly alter plasma total cholesterol, HDL, or LDL/VLDL levels compared with mice on the corresponding diet not receiving D-4F treatment (Table 1).

Reendothelialization after arterial injury

After the electrical injury, chow-fed mice reendothelialized $48.2 \pm 1.9\%$ (Fig. 2) of the injured area by 120 hours. Reendothelialization was significantly decreased in the high cholesterol group with coverage of only $27.8 \pm 1.4\%$ at 120 hours ($P < 0.0001$, Fig. 2). Treatment with D-4F improved endothelial healing to $56.6 \pm 4.3\%$ in chow-fed mice (Fig. 2), but the results did not reach statistical significance ($P = 0.1$). Treatment with D-4F significantly increased reendothelialization from $27.8 \pm 1.4\%$ in HC-fed mice to $43.4 \pm 3.4\%$ in HC-fed mice treated with D-4F ($P = 0.002$, Fig. 2). Thus, D-4F significantly improved endothelial healing in the hypercholesterolemic mice.

Tissue cholesterol and oxLDL

Tissue cholesterol levels were higher in the injured right carotid artery than the uninjured left carotid artery. They either approached or reached statistical significance when comparing the injured artery with the corresponding uninjured artery within the same treatment group (Table 2). D-4F treatment did not significantly change the tissue cholesterol levels compared to mice in the same dietary group without D-4F treatment (Table 2). In fact, neither uninjured arterial cholesterol levels nor injured carotid tissue cholesterol levels were significantly different across the treatment groups.

Tissue oxLDL scores were a marker of local oxidative stress. OxLDL score was not significantly different between the injured and uninjured carotid artery in chow-fed mice or chow-fed mice treated with D-4F (Table 2). Mice on the HC and the HC+D-4F diet had a significantly higher tissue oxLDL score in the injured artery compared with the uninjured artery, but D-4F did not lower oxLDL scores compared with mice not treated with D-4F (Table 2).

Markers of systemic oxidative stress

Plasma TBARS and lysoPC levels were determined from blood samples obtained 120 hours after injury as markers of systemic oxidative stress. Mice on the HC diet had significantly higher TBARS levels compared with chow-fed mice ($P < 0.001$, Table 3). Treatment with D-4F did not affect the TBARS levels in normocholesterolemic mice but significantly reduced TBARS levels in hypercholesterolemic mice ($P < 0.001$, Table 3). Plasma lysoPC levels were also significantly higher in hypercholesterolemic mice compared to normocholesterolemic mice ($P < 0.001$, Table 3). D-4F treatment significantly reduced plasma lysoPC levels in chow-fed ($P < 0.001$, Table 3) and in HC mice ($P < 0.001$, Table 3).

To assess the immediate and longer impact of the injury and diet on systemic oxidative stress, urine was collected for 8-isoprostane determination for the first 24 hours after injury (Day 1) and for the 24 hours prior to sacrifice (Day 5). On Day 1 and 5, hypercholesterolemic mice had significantly higher levels of 8-isoprostane compared to chow-fed mice ($P < 0.001$, Table 3). Treatment with D-4F did not affect the 8-isoprostane levels of chow-fed mice on Day 1, but D-4F was associated with a significant decrease in 8-isoprostane levels in hypercholesterolemic mice on Day 1 and in both dietary groups on Day 5 ($P < 0.001$, Table 3). Overall, administration of D-4F to mice on a HC diet resulted in a decrease in markers of systemic oxidative stress. Considering plasma TBARS and lysoPC

levels, and urinary 8-isoprostane levels as measures of systemic oxidative stress, mice on a HC diet had increased oxidative stress compared with those on a chow diet. D-4F reduced the level of oxidative stress. Interestingly, for all study groups, there was a significant inverse correlation between endothelial healing and plasma TBARS ($r=-0.695$, $P<0.001$), plasma lysoPC ($r=-0.597$, $P=0.002$), urine 8-isoprostane on Day 1 ($r=-0.582$, $P<0.01$), and urine 8-isoprostane levels on Day 5 ($r=-0.590$, $P<0.01$).

DISCUSSION

Our studies demonstrate the ability of 4F to ameliorate the inhibitory effects of lysoPC on EC migration *in vitro* and improve endothelial healing of arterial injuries in hypercholesterolemic mice. L-4F does not significantly stimulate EC migration under control conditions, but the inhibitory effect of lysoPC is significantly decreased when EC are pretreated with L-4F. This effect is seen when the L-4F is removed prior to the addition of lysoPC suggesting that L-4F has an effect directly on EC, perhaps by altering membrane mechanical properties that are important in migration [29, 30]. When L-4F is also present during incubation with lysoPC, inhibition of migration is completely prevented suggesting that L-4F can also bind lysoPC and prevent its inhibitory effect.

One of the mechanisms by which oxidized LDL and lysoPC inhibit EC migration is through an increase in EC production of superoxide by NAD(P)H oxidase [25]. L-4F is reported to decrease NAD(P)H oxidase activity and p47^{phox} subunits in aortic tissue of rats with chronic kidney disease [31]. In our studies, L-4F decreased ROS, but L-4F did not acutely alter NAD(P)H oxidase activity as evidenced by no change in p47^{phox} phosphorylation and p47^{phox} and p67^{phox} externalization. Thus, the efficacy of L-4F in preserving EC migration *in vitro* does not appear to be related to acute inhibition of NAD(P)H oxidase activity, but L-4F might be acting as a scavenger of ROS or increasing the activity of superoxide dismutase[32].

This study shows that a high cholesterol diet impairs endothelial healing of an arterial injury in mice, and that administration of an apoA-I mimetic improves healing. In mice on a chow diet, the improved healing seen with D-4F administration approaches but does not reach statistical significance ($P=0.1$). In hypercholesterolemic mice, D-4F blocks the inhibition and increases endothelial healing to a similar level as seen in chow-fed mice. The mechanism by which D-4F augments endothelial healing is unclear, but does not appear to be due to changes in the cholesterol or HDL levels because no significant alteration in these plasma lipid levels was found in our studies. Although one study suggests that D-4F administration increases HDL levels [33], others report no change in plasma cholesterol or HDL levels following D-4F administration [17, 18]. D-4F can increase the pre-beta fraction of HDL [22, 23], the fraction active in reverse cholesterol transport [34], but this fraction has not been reported to speed endothelial healing. HDL is postulated to stimulate EC migration through a receptor-mediated pathway [35], but 4F does not significantly increase migration under control conditions *in vitro*, arguing against a ligand-receptor mechanism. Instead, the beneficial effects of L-4F on EC migration *in vitro* are seen only in reducing the adverse effects of lysoPC. Thus, the most plausible explanation for the benefit *in vivo* is that

D-4F ameliorates an inhibitory effect that accompanies hypercholesterolemia and arterial injury, both of which cause an oxidative stress.

Increased levels of lipid oxidation products accompany hypercholesterolemia. ApoA-I mimetics decrease the oxidation of LDL [12, 13], scavenge lipid hydroperoxides from LDL [36], and increase paraoxonase activity [22]. These properties can minimize the oxidative stress and the increased lysoPC levels seen with hypercholesterolemia. Furthermore, 4F has been shown to bind oxidized lipids with an affinity that is 4–6 orders of magnitude greater than that of apoA-I [28], and our *in vitro* studies suggest that 4F can bind lysoPC. Our study shows that D-4F decreases plasma lysoPC levels, either through decreased production or binding of lysoPC, reducing a potent anti-migratory agent. In fact, the plasma lysoPC level inversely correlates with endothelial healing. In addition, D-4F reduces measures of oxidative stress, including plasma TBARS and urinary 8-isoprostane levels, which inversely correlate with endothelial healing. Our results parallel those of previous work demonstrating that antioxidant administration decreases measures of oxidative stress and improves endothelial healing of arterial injuries in hypercholesterolemic mice [6].

ApoA-I mimetics have anti-inflammatory properties [37]. In our study, D-4F administration does not change the cellular infiltration or the percentage of macrophages in the injured artery as assessed by immunohistochemistry in our studies (data not shown). Similarly, D-4F administration does not produce a significant difference in tissue staining for oxLDL in the injured carotid arteries. This could be due to subtle differences between groups that are not detected by the relatively insensitive assessments used, or the magnitude of the injury may overwhelm the anti-inflammatory action of D-4F.

CONCLUSION

Hypercholesterolemia is accompanied by increased oxidative stress and lipid oxidation products that contribute to delayed reendothelialization of arterial injuries [6]. The current studies show that D-4F, an apoA-I mimetic, improves endothelial healing of arterial injuries, and suggest that it works through minimizing the oxidative stress and rise in lipid oxidation products induced by hypercholesterolemia. Understanding the mechanisms of delayed healing of arterial injuries allows the development of mechanism-based therapies, such as apoA-I mimetics, to promote endothelial healing. Administration of apoA-I mimetics has the potential to speed the healing of arterial injuries at angioplasty sites to minimize thrombogenicity and restenosis at these sites. Identifying treatments that can improve endothelial cell migration is one step toward improving the long-term patency of vascular interventions. Improved patency is clinically relevant, especially in patients with multiple comorbidities. Prolonged patency will decrease the need for re-intervention, as well as the potential morbidity and mortality associated with re-intervention. Improved long-term outcomes following intervention will improve patients' quality of life.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This project was supported by Grant Numbers RO1 HL064357, and F32 HL090205 from the National Heart, Lung, and Blood Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Heart, Lung, and Blood Institute or the National Institutes of Health. The National Heart, Lung, and Blood Institute had no role in the study design, data collection, analysis and interpretation of the data, writing of the manuscript, or in the decision to submit this article for publication.

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HIGHLIGHTS

- Hypercholesterolemia leads to increased oxidative stress and lipid oxidation
- Increased oxidative stress impairs arterial healing after injury
- L-4F improves endothelial migration *in vitro* in presence of oxidized lipids
- D4-F improves endothelial healing after carotid injury in hypercholesterolemic mice
- ApoA-I mimetics may reduce thrombogenicity and restenosis of angioplasty sites

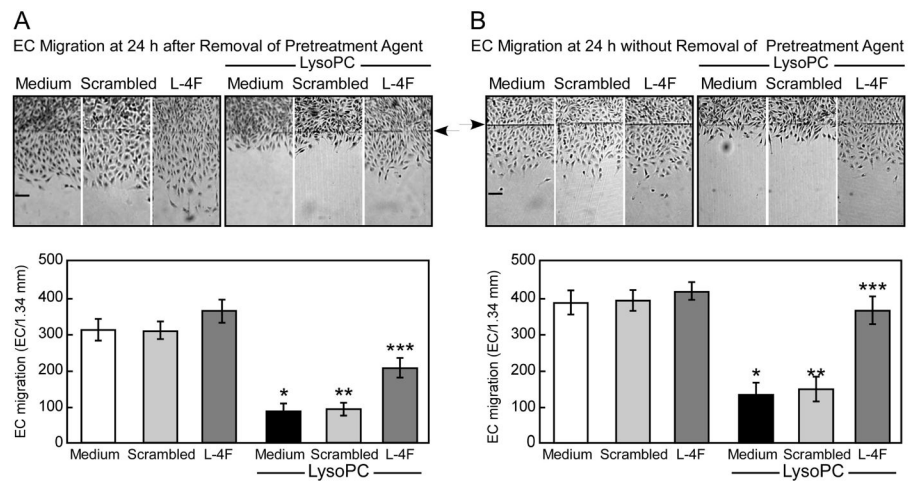


Fig. 1.

The anti-migratory effect of lysophosphatidylcholine (lysoPC) is attenuated in endothelial cells (EC) pretreated with the apolipoprotein A-I mimetic peptide L-4F. Confluent bovine aortic EC (BAEC) were incubated with medium, L-4F (0.43 $\mu\text{mol/L}$), or scrambled peptide (0.43 $\mu\text{mol/L}$) for 3 hours prior to initiation of the migration assay. Migration was initiated, and lysoPC (12.5 $\mu\text{mol/L}$) was added as indicated during the migration assay. Migration was quantified after 24 hours. Arrow indicates starting line of migration. Original magnification, $\times 40$. Bar, 100 μm . The bottom panel represents migration results by mean \pm SD ($n=3$, * $P<0.001$ compared with medium control, ** $P<0.001$ compared with medium control and scrambled peptide control, *** $P<0.01$ compared with medium+lysoPC and with L-4F control). (A) EC were washed 3 times with PBS prior to the addition of lysoPC. (B) Pretreatment agents were not removed during incubation with lysoPC.

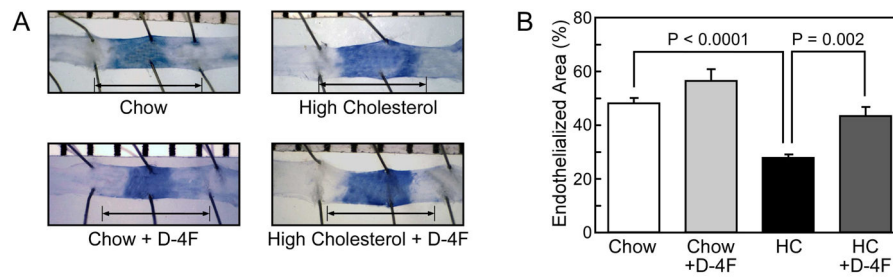


Fig. 2.

Endothelial cell migration is preserved in hypercholesterolemic C57Bl/6 mice treated with D-4F, an apolipoprotein A-I mimetic peptide. (A) Representative carotid arteries 120 hours after injury for mice in the chow, chow plus D-4F, high cholesterol (HC), and the HC plus D-4F groups are shown. The area stained by Evans Blue is the area that remained deendothelialized. The arrow identifies the length of the original injury. (B) Reendothelialization reported as a percentage of the original injury. Results are expressed as the mean \pm standard error for mice in each group: chow (n=6), chow plus D-4F (n=6), HC (n=6), and HC plus D-4F (n=6).

Mouse demographics and plasma cholesterol data, presented as mean \pm standard error

Table 1

Group	N	Age (days)	Weight (g)	Total Cholesterol (mmol/L)	HDL (mmol/L)	LDL/VLDL (mmol/L)
Chow	24	56 \pm 0.4	23.7 \pm 0.3 ^a	2.54 \pm 0.03 ^b	1.61 \pm 0.04 ^b	0.67 \pm 0.03 ^b
Chow+D-4F	24	57 \pm 0.3	24.3 \pm 0.3	2.66 \pm 0.03 ^b	1.60 \pm 0.02 ^b	0.67 \pm 0.03 ^b
High Cholesterol	24	57 \pm 0.3	25.8 \pm 0.4	3.89 \pm 0.07	2.15 \pm 0.05	1.30 \pm 0.05
HC+D-4F	25	58 \pm 0.4	25.7 \pm 0.6	3.84 \pm 0.04	2.27 \pm 0.04	1.23 \pm 0.04

HC=High cholesterol

Significant differences ($P < 0.05$) are designated as:

^a Between Chow and High Cholesterol

^b Between all chow groups and all high cholesterol groups

Table 2

Mouse tissue cholesterol and oxidized LDL, presented as mean \pm standard error

Group	Tissue Cholesterol ($\mu\text{g}/\text{mg}$)				Tissue oxLDL				
	N	RCCA	LCCA	N	RCCA	LCCA	N	RCCA	LCCA
Chow	3	0.22 \pm 0.02	0.15 \pm 0.01 ^a	6	1.76 \pm 0.3 ^b	1.39 \pm 0.9			
Chow+D-4F	3	0.25 \pm 0.03	0.17 \pm 0.02 ^a	5	2.87 \pm 0.3	2.20 \pm 0.2			
High Cholesterol	3	0.23 \pm 0.03	0.17 \pm 0.02 ^a	6	3.15 \pm 0.2	2.34 \pm 0.1 ^a			
HC+D-4F	3	0.37 \pm 0.08	0.21 \pm 0.03	7	2.97 \pm 0.2	2.32 \pm 0.1 ^a			

RCCA=Right common carotid artery

LCCA=Left common carotid artery

HC=High cholesterol

^aBetween injured RCCA and LCCA within same study group ($P<0.05$)

^bBetween injured RCCA in the Chow and Chow+D-4F study groups ($P<0.05$)

Table 3

Mouse plasma and urine markers of oxidative stress, presented as mean \pm standard error

Group	LysoPC ($\mu\text{mol/L}$) (n=24)	TBARS ($\mu\text{mol MDA/L}$) (n=12)	8-Iso/Cr - Day 1 (pg/mg) (n=5)	8-Iso/Cr - Day 5 (pg/mg) (n=5)
Chow	599.2 \pm 13.9 ^{a,b}	0.367 \pm 0.01 ^a	1359.2 \pm 59.4 ^a	946.0 \pm 30.5 ^{a,b}
Chow+D-4F	488.9 \pm 12.8	0.369 \pm 0.01	1126.9 \pm 54.7	514.7 \pm 51.9
High Cholesterol	827.0 \pm 9.9 ^c	0.623 \pm 0.01 ^c	2546.0 \pm 190.3 ^c	1151.3 \pm 57.6 ^c
HC+D-4F	722.9 \pm 7.7	0.477 \pm 0.01	1267.3 \pm 182.7	593.8 \pm 42.0

HC=High cholesterol

LysoPC=Lysophosphatidylcholine

TBARS=Thiobarbituric acid reactive substances

8-Iso=8-Isoprostane

Cr=Creatinine

Significant differences ($P < 0.05$) are designated as:

^aBetween Chow and High Cholesterol

^bBetween Chow and Chow+D-4F

^cBetween High Cholesterol and HC+D-4F