Published in final edited form as:

Atherosclerosis. 2013 March ; 227(1): 58–64. doi:10.1016/j.atherosclerosis.2012.10.064.

Two apolipoprotein E mimetic peptides with similar cholesterol reducing properties exhibit differential atheroprotective effects in LDL-R null mice

Shaila P. Handattu*, Gaurav Nayyar*, David W. Garber, Mayakonda N. Palgunachari, Candyce E. Monroe, Tamara D. Keenum, Vinod K. Mishra, Geeta Datta, and G.M. Anantharamaiah

Department of Medicine and Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, Alabama 35294

Abstract

Objective—We investigated two apoE mimetic peptides with similar long-term plasma cholesterol reducing abilities for their effects on atherosclerotic lesions in Western diet-fed female LDL receptor (LDL-R) null mice.

Methods and Results—Single doses of peptides Ac-hE18A-NH₂ and mR18L were administered retro-orbitally to LDL-R null mice on Western diet and plasma cholesterol was measured at 10 min, 4 hours, and 24 hours post administration. Peptide mR18L and not Ac-hE18A-NH₂ reduced plasma cholesterol levels significantly at 4 hours post administration. However, multiple administrations (100 µg/mouse twice weekly for 8 weeks) resulted in a similar reduction in plasma cholesterol. Only the plasma from the Ac-hE18A-NH₂ group had significantly reduced reactive oxygen species levels at the end of the treatment protocol. Both mR18L and Ac-hE18A-NH₂ showed reduced atherosclerotic lesion areas. However, peptide Ac-hE18A-NH₂ was significantly more effective in inhibiting atherosclerosis. Both peptides reduced total plaque macrophage load compared to the saline treated animals, with peptide Ac-hE18A-NH₂ having a greater reduction. Incubation of HepG2 cells and THP-1 monocyte-derived macrophages with both peptides in the presence of oxidized phospholipid showed that Ac-hE18A-NH₂ promotes the secretion of apoE from the cells whereas mR18L does not.

Conclusions—Despite similar reductions in plasma cholesterol levels, Ac-hE18A-NH₂ was more effective in inhibiting lesions than mR18L, possibly due to its ability to promote the secretion of apoE from hepatocytes and macrophages.

Keywords

Atherosclerosis; cholesterol; peptides; oxidation; inflammation

© 2012 Elsevier Ireland Ltd. All rights reserved.

Corresponding author at: David W. Garber University of Alabama at Birmingham 1720 2nd Ave South BDB room D-654 Birmingham, AL 35294, USA. Tel No +1 205-934-1218, Fax +1 205-975-8079 Dgarber@uab.edu.

*These authors contributed equally to these studies.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Disclosures

G. M. Anantharamaiah is a principal in Bruin Pharma, a startup biotechnology company and owns stock in Lipimetix LLC. M. N. Palgunachari owns stock in Lipimetix LLC.

Introduction

Cardiovascular disease is still the major cause of death in the United States despite changes in lifestyle measures and the use of new pharmacologic approaches [1, 2]. Both lipid accumulation, primarily low density lipoprotein (LDL) cholesterol, as well as inflammatory stimuli lead to the formation and progression of atherosclerotic plaque [3]. Apolipoprotein E (apoE), a protein component of very low density lipoprotein (VLDL) and large high density lipoproteins (HDL), is necessary for the receptor mediated hepatic uptake of apo B-containing remnant lipoproteins [4, 5]. In addition to its role in lipid transport and lipoprotein metabolism, apoE also plays an important role in coagulation, macrophage function, oxidative processes, central nervous system physiology, inflammation, and cell signaling [6]. It has been shown that region 141-150 of apoE, which is a highly positively charged region containing lysine and arginine residues, is the putative receptor binding region required for interaction of apoE with the LDL-receptor (LDL-R) [7].

Our earlier studies have shown that a synthetic dual-domain apoE mimetic peptide, in which the putative receptor binding region of 141–150 of human apoE was covalently linked to a lipid associating class A amphipathic helical peptide 18A to obtain Ac-hE18A-NH₂, promotes the uptake and degradation of LDL in murine fibroblasts [8], promotes clearance of atherogenic lipoprotein particles in several mouse models [9], and improves endothelial function and increases paraoxonase-1 (PON-1) activity in Watanabe Heritable Hyperlipidemic rabbits (WHHL) [10]. Datta et al. [11] showed that peptide Ac-hE18A-NH₂ promotes the secretion and recycling of apoE from hepatocytes and macrophages, and possesses several anti-inflammatory properties. We showed that multiple administrations of this peptide resulted in a cumulative reduction in plasma cholesterol and inhibited atherosclerosis progression in apoE null mice [12]. Recently we compared the apoA-I mimetic peptide 4F with Ac-hE18A-NH₂, administered at equal dosage, frequency, and route of administration in older female apoE null mice [13]. Although both peptides exhibited similar anti-inflammatory properties, only Ac-hE18A-NH₂ reduced plasma cholesterol. Both peptides reduced atherosclerotic lesions, but mice administered Ac-hE18A-NH₂ exhibited a significantly greater reduction in lesions [13].

In this paper we have extended our investigations to compare the atheroprotective effects of this peptide to the apoE mimetic single domain peptide mR18L (with the sequence Ac-GFRRFLGSWARIYRAFG-NH₂) which has also been shown to reduce plasma cholesterol and inhibit atherosclerosis in apoE null mice [14]. The peptide mR18L was designed by modifying a model cationic class L-peptide [15].

In the present investigations, we have used female LDL-R null mice on Western diet to determine if the dual domain peptide may have additional atheroprotective effects due to its ability to enhance the secretion of apoE from hepatocytes. We have compared the anti-atherogenic abilities of the two apoE mimetic peptides Ac-hE18A-NH₂ and mR18L for reducing plasma cholesterol, enhancing apoE secretion in macrophages and hepatocytes, reducing plasma reactive oxygen species, and ability to reduce lesion formation. The results show that Ac-hE18A-NH₂ is a better atheroprotective peptide than mR18L in LDL-R null mice on Western diet, perhaps due to its ability to promote apoE secretion from hepatocytes and macrophages.

Materials and Methods

2.1. Synthesis of Peptides

Peptides Ac-hE18A-NH₂ with the sequence Ac-LRKLRKRLLRDWLKAIFYDKVAEKLKEAF-NH₂ and mR18L with the sequence Ac-

GFRRFLGSWARIYRAFVG-NH₂ were synthesized by the solid phase peptide synthesis method using fluorenylmethyloxycarbonyl (Fmoc) amino acids and suitable protected amino acids as described previously [11]. The peptides were purified by preparative HPLC, and the purity and identity of the peptides were determined by analytical HPLC and mass spectrometry. Oxidized lipids (oxPAPC) were prepared by 72 hour air oxidation of palmytoylarachadonyl phosphatidyl choline (PAPC) purchased from Avanti Polar Lipids (Birmingham, AL).

2.2. Effect of short-term administration on plasma cholesterol and long-term administration on lesion inhibition in female LDL-R null mice on Western diet

Twelve week old LDL-R null female mice were purchased from the Jackson laboratory (Bar Harbor, ME) and were acclimatized for two weeks. After feeding Western diet (TekLad Adjusted Calories Diet, No. 88137) for two weeks animals were randomized into three groups. To determine short term effects on plasma cholesterol levels, peptides Ac-hE18A-NH₂ and mR18L (100 µg/dose in 100 µl saline/mouse) or equal volume of saline were administered retro-orbitally (6 mice per group) and plasma samples were analyzed at 10 min, 4 h, and 24 h post peptide administration. For determining the effect of these peptides on atherosclerotic plaque formation, peptides were administered retro-orbitally two times a week for eight weeks (n=9 to 11 per group). The control group received 100 µl of saline. Blood was collected from the retro-orbital sinus under anesthesia at time points shown in the figures and cholesterol was measured manually using a commercially available kit (Infinity Cholesterol Reagent, Thermo Scientific). At the time of euthanasia, blood was collected *via* cardiac puncture under ketamine-xylazine anesthesia. All protocols involving mice were approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee. Peptide concentrations were determined as described by us previously [16].

2.3. Column cholesterol lipoprotein profiles

Plasma cholesterol lipoprotein profiles were measured using the CLiP method as described by us previously [17]. In brief, 10 µl of plasma were analyzed using a single Superose 6 column (Pharmacia, Piscataway, NJ). Cholesterol reagent was immediately introduced following the column, and the eluent–reagent mixture was allowed to enter a post-column reaction coil. Cholesterol content of the eluent mixture was spectrophotometrically detected at 564 nm and the absolute values were determined by comparing with a control sample of known value. The resulting profiles were decomposed into component peaks and analyzed for relative area using PeakFit (Systat Software Inc., Chicago, IL).

2.4. Plasma Reactive Oxygen Species (ROS) Levels

ROS levels were measured using 2',7'-dichlorodihydrofluorescein diacetate (DCFDA) (Invitrogen, Carlsbad, CA) as previously described [18].

2.5. Lesion Analysis

At the end of eight weeks blood samples were taken under anesthesia by cardiac puncture, and hearts were excised and subjected to histological examination and quantification of lesion area as described earlier [19]. In brief, mice were perfused through the left ventricle with saline (exiting the right atrium), then hearts were excised. After the lower two-thirds of the hearts were removed, the remaining tissue was frozen in freezing medium (OCT Tissue-Tek; Miles Laboratories Ltd, Elkhart, IN) and sectioned in a cryostat at -20°C. Alternate 10-µm sections were saved on slides and observed for the beginning of the aortic root. Sections were then collected for an additional 600 µm, or until the aortic cross section was rounded and the valve cusps were no longer evident. Slides were stained with Oil Red O, and counterstained with hematoxylin. Stained lesion cross-sectional areas were measured in

consecutive slides 80 μm apart by image analysis (SigmaScan Pro; Systat Software Inc., Chicago, IL), and the average lesion area was determined for each aortic sinus over the 400- μm length (five slides) providing the greatest mean lesion area. *En face* preparations of the entire aorta from the aortic arch to the iliac bifurcation [13] were done in the same mice. The aortae were excised, cleaned, and opened longitudinally with extremely fine Vannas scissors, then pinned flat on a black wax surface. The aorta was then stained with Oil Red O and lesions were quantified by video capture under a stereo dissecting microscope. Lesion and total areas were determined using SigmaScan (Systat) and lesion area was expressed as a percentage of total area.

For total macrophage area, slides were immunostained with rat anti-mouse CD68 (macrosialin) antibody (BD Pharminogen) and a fluorescent secondary antibody (Goat anti rat Alexaflour-555; Invitrogen, Carlsbad, CA). Cross sectional areas were determined by image analysis as described above.

2.6. Cell Culture

HepG2 cells and THP-1 monocytes were obtained from American Tissue Culture Collection (ATCC), USA. HepG2 cells were grown in MEM containing 10% FBS and antibiotics. THP-1 monocytes were grown in RPMI media (ATCC, USA) containing 10% FBS and antibiotics and differentiated into macrophages by the addition of phorbol myristate acetate (PMA).

2.7. ApoE levels and secretion

Plasma apoE levels were measured in samples taken at euthanasia (6 randomly chosen samples from each group) using mouse apoE ELISA (Mybiosource, San Diego CA).

To measure the ability of peptides to overcome the oxidized lipid-mediated inhibition of apoE secretion, HepG2 cells and THP-1 monocyte derived macrophages were incubated overnight with and without peptide (5 $\mu\text{g}/\text{ml}$) following pretreatment with or without different amounts (10 and 60 $\mu\text{g}/\text{ml}$) of oxPAPC for 6 hours. Amounts of apoE secreted into the media were determined by human apoE ELISA (Mabtech Inc., Cincinnati OH).

2.8. Statistics

Groups were compared by one-way analysis of variance (ANOVA) when the data were normally distributed or one-way ANOVA on ranks when normality failed. Post-hoc analyses were done using two-tailed Student's t-test. Groups were considered significantly different when $p < 0.05$. Data are expressed as mean $\pm$ SEM.

Results

Short-term effect of peptides on plasma cholesterol levels

Garber *et al.* showed that peptide Ac-hE18A-NH₂ did not reduce plasma cholesterol levels in LDL-receptor null mice on normal chow or Western diet [9]. To compare the acute effects of peptides Ac-hE18A-NH₂ and mR18L on plasma cholesterol levels, the peptides (100 $\mu\text{g}/\text{mouse}$) were administered intravenously to LDL-R null mice on Western diet. Blood was drawn before administration, 10 min, 4 hrs and 24 hrs post-administration for plasma cholesterol analysis. As shown in **Figure 1**, mice administered with mR18L showed a decrease in plasma cholesterol that was significantly greater than both the control and Ac-hE18A-NH₂ groups at the 4 hr time point. However, this reduction was not maintained for 24 hrs. Mice administered with either saline or Ac-hE18A-NH₂ had similar changes in plasma cholesterol levels. These results are consistent with earlier studies [9].

Effect of multiple administrations of peptides on plasma lipids

Although, reduction in plasma cholesterol levels upon a single administration of peptide Ac-hE18A-NH₂ was similar to the control group, multiple administrations (100 µg/mouse, twice weekly) resulted in a reduction in plasma cholesterol levels when measured 24 hours after the most recent treatment. Plasma cholesterol levels at week 2, 4 and 6 and at the termination of the experiment (**Figure 2A**) show upon multiple administrations of the peptides that both mR18L and Ac-hE18A-NH₂ reduced plasma cholesterol to a similar extent as compared to the animals administered with saline. Also, lipoprotein profile analysis at week 4 suggested that the cholesterol reduction occurred in all lipoprotein fractions, with the greatest reduction in the VLDL-like lipoprotein fraction (**Figure 2B**). Representative cholesterol profiles are shown in **Supplemental Figure 1**. Plasma apoE levels measured at the time of euthanasia in 6 randomly chosen samples from each group were not significantly different despite the reduction in plasma cholesterol in the peptide groups (control, 180.22 ± 11.41 ; mR18L, 158.77 ± 16.26 ; Ac-hE18A-NH₂, 181.06 ± 18.79 µg/mL). When normalized to plasma cholesterol, there was a non-significant trend for higher apoE levels per unit of cholesterol in the peptide groups, with Ac-hE18A-NH₂ having the highest apoE levels per unit cholesterol (**Supplemental Figure 2**).

Effect of chronic administration of peptides on plasma ROS levels

Plasma samples taken at euthanasia were analyzed for ROS levels. As shown in **Figure 3**, plasma ROS levels of mR18L treated mice did not change significantly from that of the control group after 8 weeks of administration, whereas Ac-hE18A-NH₂ treated mice had significantly lower plasma ROS levels compared to both control and mR18L treated mice.

Effect of Ac-hE18A-NH₂ and mR18L on atherosclerotic lesions and macrophage content

After 8 weeks of peptide administration, aortic sinus sections and aortic *en face* preparations were analyzed for atherosclerotic lesions using Oil Red O staining. Compared with the control group, both Ac-hE18A-NH₂ and mR18L groups had significant inhibition of atherosclerotic lesion formation as seen in aortic sinus sections (**Figure 4A**) and *en face* preparations (**Figure 4C**). However, this reduction was significantly greater in animals treated with Ac-hE18A-NH₂ as compared to the animals treated with peptide mR18L.

Treatment with peptides Ac-hE18A-NH₂ and mR18L significantly reduced total macrophage content of the aortic sinus lesions compared with the saline treated animals (**Figure 4B**). However, Ac-hE18A-NH₂ treated animals had significantly less area covered with macrophages as compared to the animals treated with peptide mR18L.

Effects of peptides on apoE secretion from cultured hepatocytes and macrophages

Secretion of apoE released from hepatocytes and macrophages plays an important role in lipoprotein metabolism. We studied the inhibitory effect of oxidized lipids on apoE secretion from HepG2 cells and THP-1 monocyte-derived macrophages and the ability of peptides Ac-hE18A-NH₂ and mR18L to overcome these effects. Incubation of HepG2 cells with peptide Ac-hE18A-NH₂ resulted in a 2.5 fold increase in apoE secretion into the media. We also saw a dose dependent reduction in the secretion of apoE after treating the cells with oxPAPC (**Figure 5A**). However, incubation of cells with peptide Ac-hE18A-NH₂ after pre-incubation with oxPAPC not only mitigated the oxPAPC-induced reduction in apoE secretion, it further promoted apoE secretion beyond the levels of the control cells treated with the peptide (**Figure 5A**). Peptide mR18L did not cause an increase in apoE secretion from cultured hepatocytes. However, it was able to mitigate the reduction associated with oxPAPC incubation, although not to the levels of control cells treated with this peptide (**Figure 5B**).

Similarly, in THP-1 monocyte-derived macrophages, treatment with Ac-hE18A-NH₂ not only promoted apoE secretion, overnight incubation of cells with this peptide after pretreatment with oxPAPC for 6 hours increased apoE secretion above the levels of control cells treated with peptide alone (**Figure 5C**). Treatment with mR18L promoted secretion of apoE from THP-1 cells in the absence of oxPAPC and slightly above control levels in the presence of low-dose oxPAPC, but apoE secretion was reduced from control levels in the presence of high-dose oxPAPC (**Figure 5D**).

3 Discussion

Earlier we reported that a single administration of peptide Ac-hE18A-NH₂ does not significantly reduce plasma cholesterol levels in LDL-R null mice on either normal chow or Western diet [9]. In agreement with the earlier observations, a single administration of Ac-hE18A-NH₂ did not reduce plasma cholesterol levels in these animals (**Figure 1**). It was shown then that radiolabelled Ac-hE18A-NH₂ did not associate with lipoproteins in these mice [9]; we speculate that mR18L does associate acutely, thus leading to the initial reduction in cholesterol. Preliminary data (not shown) suggests that this is the case, in that plasma from LDL-R null mice on Western diet mixed with Ac-hE18A-NH₂ did not show a change in mobility using agarose gel electrophoresis, while plasma mixed with mR18L did. However, plasma cholesterol was significantly reduced in animals treated with mR18L at 4 hours post injection. In contrast, multiple administrations of both peptides resulted in a similar reduction of plasma cholesterol levels. Reduction was seen in all lipoprotein classes, but was greatest in VLDL (**Figure 2**). Plasma from Ac-hE18A-NH₂ (and not from mR18L) mice showed reduced plasma ROS, suggesting that Ac-hE18A-NH₂ has greater anti-oxidative properties (**Figure 3**).

At the end of the experimental protocol, both peptides reduced lesion and macrophage area in the aortic sinus, although Ac-hE18A-NH₂ did so to a greater extent compared to peptide mR18L (**Figure 4**). Lesion area was also reduced in *en face* total aorta preparations, with Ac-hE18A-NH₂ again having a significantly greater reduction.

Despite the significant reduction in plasma cholesterol in the peptide groups relative to the controls (**Figure 2**), apoE levels in total plasma were not reduced. This may have reflected increased apoE secretion, as suggested by the trend to increased apoE relative to plasma cholesterol shown in **Supplemental Figure 2**. However, such increased apoE secretion may be obscured by clearance of plasma apoE through pathways other than the LDL-R, such as heparan sulfate proteoglycans and/or LDL-R related protein (LRP) [20]. Datta et al. have shown previously that peptide Ac-hE18A-NH₂ can promote the secretion of apoE from hepatocytes [11]. Unlike apoE null mice, LDL-R null mice express endogenous apoE. Therefore, we wanted to determine if peptides Ac-hE18A-NH₂ and mR18L enhance secretion of apoE from HepG2 cells and THP-1 monocyte-derived macrophages in the presence or absence of oxidized lipid. As shown in **Figure 5**, oxidized lipids inhibit the secretion of apoE from hepatocytes and macrophages in a dose-dependent manner. Ac-hE18A-NH₂ was able to not only rescue the cells from oxidized lipid-mediated inhibition of apoE secretion, it further promoted apoE secretion from hepatocytes as compared to the control. In contrast, mR18L was only able to mitigate the inhibitory effect of oxidized lipids on apoE secretion in hepatocytes to a certain extent (**Figures 5A and B**). In macrophages, oxidized lipid again suppressed apoE secretion. Ac-hE18A-NH₂ significantly increased secretion in the presence of oxidized lipids, well above control levels (**Figure 5C**). Peptide mR18L significantly increased secretion in the absence of oxidized lipids, but in the presence of lower levels of oxPAPC did so only slightly, and at higher levels of oxPAPC did not significantly increase secretion (**Figure 5D**).

The results of enhanced secretion of apoE in hepatocytes and macrophages by Ac-hE18A-NH₂, reduction of levels of plasma ROS, and previous observations that peptide Ac-hE18A-NH₂ enhances paraoxonase-1 (PON-1) activity [10] all support the idea that Ac-hE18A-NH₂ exhibits a greater anti-atherogenic effect than the peptide mR18L, perhaps due to its ability to enhance the secretion of apoE under increased oxidative stress such as occurs with the Western diet. Future experiments have to be performed to determine if the peptide Ac-hE18A-NH₂ can also cause regression of existing lesions.

Conclusions

A single administration of Ac-hE18A-NH₂ does not reduce plasma cholesterol levels in Western diet-fed LDL-R null mice. However, multiple administrations reduce plasma cholesterol levels to the same extent as peptide mR18L. Ac-hE18A-NH₂ is more effective than mR18L in reducing atherosclerotic aortic lesions in Western diet-fed female LDL-R null mice, perhaps due to its ability to enhance apoE secretion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by grants R01 HL090803 and P01 HL34343 from NIH and by a gift from the Julius H. Caplan Charity Foundation, Inc. in memory of Miles D. Garber Jr., M.D.

References

1. Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet*. 1994; 344:1383–1389. [PubMed: 7968073]
2. Shepherd J, Cobbe SM, Ford I, et al. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *N Engl J Med*. 1995; 333:1301–1307. [PubMed: 7566020]
3. National Cholesterol Education Program. Second report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). *Circulation*. 1994; 89:1333–1445. [PubMed: 8124825]
4. Bradley WA, Hwang SL, Karlin JB, et al. Low-density lipoprotein receptor binding determinants switch from apolipoprotein E to apolipoprotein B during conversion of hypertriglyceridemic very-low-density lipoprotein to low density lipoproteins. *J Biol Chem*. 1984; 259:14728–14735. [PubMed: 6501314]
5. Gianturco SH, Gotto AM Jr, Huang SLC, et al. Apolipoprotein E mediates uptake of Sf 100–400 hypertriglyceridemic very low density lipoproteins by the low density lipoprotein receptor pathway in normal human fibroblasts. *J. Biol. Chem*. 1983; 258:4526–4533.
6. Davignon J. Apolipoprotein E and atherosclerosis: beyond liver effect. *Arterioscler Thromb Vasc Biol*. 2005; 25:267–269. [PubMed: 15681305]
7. Zaiou M, Arnold KS, Newhouse YM, et al. Apolipoprotein E-low density lipoprotein receptor interaction: Influences of basic residue and amphipathic α -helix organization in the ligand. *J. Lipid Res*. 2000; 41:1087–1095. [PubMed: 10884290]
8. Datta G, Chaddha M, Garber DW, et al. The receptor binding domain of apolipoprotein E, linked to a model class A amphipathic helix, enhances internalization and degradation of LDL by fibroblasts. *Biochemistry*. 2000; 39:213–220. [PubMed: 10625496]
9. Garber DW, Handattu S, Aslan I, Datta G, Chaddha M, Anantharamaiah GM. Effect of an arginine-rich amphipathic helical peptide on plasma cholesterol in dyslipidemic mice. *Atherosclerosis*. 2003; 168:2229–2237.

10. Gupta H, White CR, Handattu S, et al. Apolipoprotein E mimetic peptide dramatically lowers plasma cholesterol and restores endothelial function in Watanabe heritable hyperlipidemic rabbits. *Circulation*. 2005; 111:3112–3118. [PubMed: 15939819]
11. Datta G, White CR, Dashti N, et al. Anti-inflammatory and recycling properties of an apolipoprotein mimetic peptide, Ac-hE18A-NH(2). *Atherosclerosis*. 2010; 208:134–141. [PubMed: 19656510]
12. Nayyar G, Handattu SP, Monroe CE, et al. Two adjacent domains (141-150 and 151- 160) of apoE covalently linked to a class A amphipathic helical peptide exhibit opposite atherogenic effects. *Atherosclerosis*. 2010; 208:449–457. [PubMed: 21030022]
13. Nayyar, G.; Garber, DW.; Palgunachari, MN., et al. Apolipoprotein E mimetic is more effective than apolipoprotein A-I mimetic in reducing lesion formation in older female apo E null mice.. *Atherosclerosis*. (<http://dx.doi.org/10.1016/j.atherosclerosis.2012.05.040>)
14. Handattu SP, Datta G, Epand RM, et al. Oral administration of L-mR18L, a single domain cationic amphipathic helical peptide, inhibits lesion formation in ApoE null mice. *J Lipid Res*. 2010; 51:3491–3499. [PubMed: 20841495]
15. Segrest JP, De Loof H, Dohlman JG, Brouillette CG, Anantharamaiah GM. Amphipathic helix motif: classes and properties. *Proteins*. 1990; 8:103–117. [PubMed: 2235991]
16. Palgunachari MN, Mishra VK, Lund-Katz S, et al. Only the two end helices of eight tandem amphipathic helical domains of human apo A-I have significant lipid affinity: implications for HDL assembly. *Arterioscler Thromb Vasc Biol*. 1996; 16:328–338. [PubMed: 8620350]
17. Garber DW, Kulkarni KR, Anantharamaiah GM. A sensitive and convenient method for lipoprotein profile analysis of individual mouse plasma samples. *J Lipid Res*. 2000; 41:1020–1026. [PubMed: 10828095]
18. Nayyar G, Mishra VK, Handattu SP, et al. Sidedness of interfacial arginine residues and anti-atherogenicity of apolipoprotein A-I mimetic peptides. *J Lipid Res*. 2012; 53:849–858. [PubMed: 22377531]
19. Garber DW, Datta G, Chaddha M, et al. A new synthetic class A amphipathic peptide analogue protects mice from diet-induced atherosclerosis. *J Lipid Res*. 2001; 42:545–552. [PubMed: 11290826]
20. Mahley RW, Ji ZS. Remnant lipoprotein metabolism: key pathways involving cell- surface heparan sulfate proteoglycans and apolipoprotein E. *J. Lipid. Res*. 1999; 40:1–16. [PubMed: 9869645]

Highlights

ApoE mimetic peptides Ac-hE18A-NH₂ and mR18L were studied in LDL-R null mice.

Chronic treatment of both peptides reduced cholesterol levels to the same extent.

Ac-hE18A-NH₂ and not mR18L reduced plasma reactive oxygen species.

While both peptides reduced atherosclerosis, Ac-hE18A-NH₂ was more effective.

Ac-hE18A-NH₂ increased *in vitro* apoE secretion in the presence of ox-lipid.

Figure 1. Acute administration of mR18L and not Ac-hE18A-NH₂ lowers plasma cholesterol levels in western diet fed female LDL-R knockout mice

LDL-R knockout mice were fed with Western diet for two weeks and were administered either Ac-hE18A-NH₂ (▲) or mR18L (■) (both peptides at 100 µg/mouse in 100 µL, retro-orbitally). Saline administered mice (●) served as controls. mR18L administered mice showed a significant decrease in plasma cholesterol levels 4 hours post administration compared to the saline ($\dagger p < 0.001$) and Ac-hE18A-NH₂ ($* p < 0.01$) groups. Plasma cholesterol levels of Ac-hE18A-NH₂ administered mice were not different from saline administered mice at any time point. N=4 in saline group and N=6 in mR18L and Ac-hE18A-NH₂ groups. In this and subsequent figures, Ac-hE18A-NH₂ is abbreviated as hE18A.

Figure 2. Chronic administration of the peptide Ac-hE18A-NH₂ and mR18L decreases plasma cholesterol

16 week old western diet fed female LDL-R knockout mice were administered with saline (● and black bar), mR18L (■ and gray bar) or Ac-hE18A-NH₂ (▲ and dark gray bar), retro-orbitally. 100 µg of each peptide in 100 µl saline was administered 2 times a week for 8 weeks. Plasma samples were drawn every two weeks, 24 hours after the most recent treatment. **A)** After 2 weeks of treatment plasma from mice treated with mR18L ([†]p<0.01 vs saline) and Ac-hE18A-NH₂ (*p<0.05 vs saline) showed significantly lower cholesterol levels compared to the saline treated group. Reduction in plasma cholesterol levels was maintained at week 4 ([†]p<0.01 vs saline), week 6 ([†]p<0.01 vs saline), and week 8 ([#]p<0.001 vs saline) for both the peptides. **B)** At the end of four weeks of peptide treatment, lipoprotein cholesterol profile analysis were performed on four plasma samples from each group, and showed that most of the reduction was in the VLDL-like particles ([†]p<0.01 vs saline) and LDL (*p<0.01 vs saline) cholesterol fractions. However there was a small but significant reduction in HDL cholesterol in animals treated with mR18L ([§]p<0.05 vs saline) and Ac-hE18A-NH₂ (*p<0.01 vs saline).

Figure 3. Chronic administration of the peptide Ac-hE18A-NH₂ and not mR18L decreases plasma reactive oxygen species (ROS) levels

At the end of 8 weeks of treatment, plasma ROS levels were significantly reduced in the Ac-hE18A-NH₂ group (dark gray bar) compared with either control (black bar; †p<0.001) or mR18L group (light gray bar; †p<0.001).

Figure 4. Peptide Ac-hE18A-NH₂ is more effective in reducing atherosclerotic lesion progression than the peptide mR18L

A) Quantification of aortic sinus lesions. At the end of the experiment, animals were assessed for aortic sinus atherosclerotic lesions. Oil Red O stained areas in the aortic sinus were measured in all three groups. After 8 weeks of treatment, mice treated with saline (n=9) had significantly more atherosclerotic lesions as compared to animals administered with the peptides mR18L (n=9, *p<0.01 vs saline) and Ac-hE18A-NH₂ (n=10, †p<0.001 vs saline). Peptide Ac-hE18A-NH₂ was better than peptide mR18L in inhibiting atherosclerotic lesion progression (§p<0.05 vs mR18L). Data are expressed as mean ± S.E.M. **B) Quantification of aortic sinus lesional macrophage load.** At the end of the experiment, lesions were assessed for total macrophage load using anti-mouse CD68 antibody. Animals treated with saline (n=9) had significantly greater macrophage area in the aortic sinus as compared to the animals treated with peptides mR18L (n=9, *p<0.05 vs saline) and Ac-hE18A-NH₂ (n=10, †p<0.001 vs saline). Peptide Ac-hE18A-NH₂ group had significantly less macrophage area in the aortic sinus as compared to the mR18L group (§p<0.05 vs mR18L). **C) Quantification of *en face* lesions.** *En face* lesion analysis showed that mice treated with saline (n=9) had significantly greater aortic atherosclerotic lesion area as compared to the peptides mR18L (n=10, †p<0.001 vs saline) and Ac-hE18A-NH₂ (n=11, †p<0.001 vs saline).

Figure 5. A) Peptide Ac-hE18A-NH₂ promotes apoE secretion from HepG2 cells and abolishes oxidized lipids mediated inhibition of apoE secretion

HepG2 cells were incubated with either Ac-hE18A-NH₂ alone for 18 hours or with Ac-hE18A-NH₂ for 18 hours following pretreatment with either 10 or 60 μ g/ml of oxPAPC (oxPAPC 10 and oxPAPC 60 respectively) for 6 hours or with 10 or 60 μ g/ml of oxPAPC alone. Cells that were treated with vehicle served as control. Data represent mean $\pm$ SEM of four wells for each group. Data were adjusted to a percentage of controls (signified by the dashed line). Like letters indicate the groups that are NOT significantly different from each other. B) Peptide mR18L does not promote apoE secretion from HepG2 cells but partially overcomes the inhibitory effect of oxidized lipids on apoE secretion. Peptide mR18L was incubated with HepG2 cells with and without oxPAPC as in Figure 5A. Data represent mean $\pm$ SEM of six wells for each group. Data were adjusted to a percentage of controls (signified by the dashed line). Like letters indicate the groups that are NOT significantly different from each other. C) Effect of Ac-hE18A-NH₂ on apoE secretion in THP-1 monocyte derived macrophages. THP-1 monocyte derived macrophages were incubated with either Ac-hE18A-NH₂ alone for 18 hours or with Ac-hE18A-NH₂ for 18 hours following pretreatment with either 10 or 60 μ g/ml of OxPAPC for 6 hours or with 10 or 60 μ g/ml of OxPAPC alone. Cells that were treated with vehicle served as control. Data represent mean $\pm$ SEM of

six wells for each group. Data were adjusted to a percentage of controls (signified by the dashed line). Like letters indicate the groups that are NOT significantly different from each other. D) Effects of mR18L on apoE secretion in THP-1 monocyte derived macrophages. Peptide mR18L was incubated with THP-1 cells with and without oxPAPC as in Figure 5C. Data represent mean $\pm$ SEM of six wells for each group. Data were adjusted to a percentage of controls (signified by the dashed line). Like letters indicate the groups that are NOT significantly different from each other.