Abstract:

Title: APOE MIMETIC PEPTIDE COMPOSITIONS

Disclosure are aqueous compositions comprising a mixture of synthetic apolipoprotein E (ApoE)-mimicking peptides and a polyethylene sorbitan ester, and their use in reducing plasma cholesterol.
APOE MIMETIC PEPTIDE COMPOSITIONS

REFERENCE TO RELATED APPLICATIONS


BACKGROUND


[0003] Synthetic ApoE mimicking peptides that enhance LDL/VLDL binding, increase LDL/VLDL degradation, and lower LDL/VLDL cholesterol in individuals with atherosclerosis are previously described. See e.g., U.S. Patent No. 6,506,880, WO 2009/032702, and Ac-hel8A-NH₂ (AEM-28), Ac-[R]hel8A-NH₂ (AEM-28(R)), and Aha-[R]hE18A-NH₂ (AES-21) in U.S. Provisional Application Serial No. 62/031,585. These peptides demonstrated good efficacy in reducing plasma cholesterol. Improved synthetic ApoE mimicking peptides are disclosed in PCT/US20 15/04 1162, and include Ac-MyrA[R]hE 18A-NH₂ (AEM-28-14) and Ac-Octa[R]hE18A-NH₂ (AEM-28-8).

SUMMARY

[0004] It has now been found that certain synthetic mimetic ApoE peptides which contain longer chain aminoalkyl groups (e.g., Ac-MyrA[R]hE18A-NH₂ (AEM-28-14) and Ac-
Octa[R]hE18A-NH₂ (AEM-28-8)), and which are otherwise effective in reducing plasma cholesterol (see e.g., PCT/US2015/041162), are poorly soluble in aqueous formulations such as in phosphate-buffered saline (PBS). This deficiency diminishes the ability to effectively use these longer-chain peptides in a clinical setting such as for administration via injection. More significantly, it has also been found that certain ApoE peptides (e.g., AEM-28) have low maximum tolerated dose levels (MTDs) and/or low no-observed adverse effect levels (NOAELs). It is reported herein that these problems are overcome by adding a polyoxyethylene sorbitan fatty acid ester to the aqueous formulations comprising these synthetic ApoE mimetic peptides. For example, when AEM-28-14 and AEM-28-8 were formulated in 2% polysorbate 80 (e.g., Tween™ 80) in PBS, complete dissolution occurred and the NOAELs for both peptides were up to 15 mg/kg, with minimal effects observed at 25 mg/kg. See Examples 2 and 3. In the case of AEM-28, the addition of a polyoxyethylene sorbitan fatty acid ester increased the NOAEL dose from ≤ 5 mg/kg to 10 mg/kg. See Example 3.

It was further found that compositions comprising certain longer-chain mimetic ApoE peptides (e.g., AEM-28-14 and AEM-28-8) and a polyoxyethylene sorbitan fatty acid exhibit no adverse effects and elicit superior reductions in plasma cholesterol. For example, AEM-28-14 formulated in 0.8% polysorbate 80 (e.g., Tween™ 80) reduced cholesterol from 450 mg/dL to zero in some animals, thereby essentially removing all circulating cholesterol for at least 6 hours post-dose from the initial dose of 4 mg/kg. See e.g., Figure 1 and Example 4. Similarly, AEM-28-8 formulated in 0.8% polysorbate 80 (e.g., Tween™ 80) at a dose of 4 mg/kg showed a change of total cholesterol to 9% of baseline. See e.g., Figure 1 and Example 4.

Based on these discoveries, new aqueous pharmaceutical formulations of synthetic mimetic ApoE peptides containing polyoxyethylene sorbitan fatty acid esters are disclosed herein.

Further provided are methods of reducing plasma cholesterol, methods of effecting plasma LDL and/or plasma VLDL, and methods of treating lipid disorders, atherosclerosis, or acute coronary syndrome (ACS) using one or more of the disclosed pharmaceutical formulations described herein.

**BRIEF DESCRIPTION OF THE FIGURES**

**FIG. 1** illustrates the change in cholesterol (mg/dL) vs time for AEM-28-8, AEM-28-14, and AEM-28 at concentrations of 0.4 or 4 mg/kg, where a) represents total cholesterol and b) represents percent (%) change in total cholesterol.
FIG. 2 illustrates the change in cholesterol (mg/dL) vs time for AEM-28-8, AEM-28-14, and AEM-28 at concentrations of 1 or 2 mg/kg, where a) represents total cholesterol and b) represents percent (%) change in total cholesterol.

FIG. 3 shows the effect of AEM-28-14 in 2% Tween® 20/PBS in hypercholesterolemic cynomolgus monkeys.

DETAILED DESCRIPTION

A. General Description of the Compositions

Provided herein are aqueous formulations comprising i) a synthetic apolipoprotein E (ApoE)-mimicking peptide and ii) a polyethylene sorbitan ester.

In a first exemplary embodiment, provided herein are aqueous pharmaceutical compositions comprising i) a synthetic apolipoprotein E (ApoE) mimicking peptide of the formula \((\text{CH}_3)(\text{CH}_2)_x\text{C}(0)\text{NH}(\text{CH}_2)_y\text{C}(0)\text{LRRLRRRLR-DWLKAFYDVAEKLKEAF-NH}_2\), wherein \(x\) is an integer from 0 to 6; and \(y\) is an integer from 1 to 20; or a pharmaceutically acceptable salt thereof; and ii) a polyethylene sorbitan ester.

B. Compositions and Definitions

The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

Ac-hE18A-NH\(_2\) as used herein means a synthetic apolipoprotein E (ApoE)-mimicking peptide where "Ac" is acetyl, such that the synthetic apolipoprotein E (ApoE)-mimicking peptide has the structure \(\text{H}_2\text{C}(\text{CO})-\text{LRKLKRLLR-DWLKAFYDVAEKLKEAF-NH}_2\). Ac-hE18A-NH\(_2\) and AEM-28 are used interchangeably.

Ac-MyrA[R]hE18A-NH\(_2\) as used herein means a synthetic apolipoprotein E (ApoE)-mimicking peptide where "Ac" is acetyl and "MyrA" is myristic acid, such that the synthetic apolipoprotein E (ApoE)-mimicking peptide has the structure \(\text{H}_2\text{C}(\text{CO})-\text{NH}(\text{CH}_2)_i\text{C}(\text{CO})-\text{LRRLRRRLR-DWLKAFYDVAEKLKEAF-NH}_2\). Ac-MyrA[R]hE18A-NH\(_2\) and AEM-28-14 are used interchangeably.

Ac-Octa[R]hE18A-NH\(_2\) as used herein means a synthetic apolipoprotein E (ApoE)-mimicking peptide where "Ac" is acetyl and "Octa" is octanoic acid, such that the synthetic apolipoprotein E (ApoE)-mimicking peptide has the structure \(\text{H}_2\text{C}(\text{CO})-\text{NH}(\text{CH}_2)_j\text{C}(\text{CO})-\text{LRRLRRRLR-DWLKAFYDVAEKLKEAF-NH}_2\). Ac-Octa[R]hE18A-NH\(_2\) and AEM-28-8 are used interchangeably.

The disclosed Apo E mimicking peptides disclosed herein comprise amino acids that contain basic groups (e.g., -NH\(_2\)) and acid groups (e.g., -COOH). The basic groups can be protonated when the Apo E mimicking peptides are dissolved in an acidic aqueous
solution; and the acid group can be deprotonated when the Apo E mimicking peptides are dissolved in basic solution. "Pharmaceutically acceptable salt thereof" refers to Apo E mimicking peptides that have been obtained from such solutions which contain acids or bases that are suitable for pharmaceutical use, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric, sulfuric, acetic, benzenesulfonic, benzoic, citric, ethanesulfonic, fumaric, gluconic, glycolic, isethionic, lactic, lactobionic, maleic, malic, methanesulfonic, succinic, p- toluenesulfonic, and tartaric acids. Suitable pharmaceutically acceptable basic salts include e.g., ammonium salts, alkali metal salts (such as sodium and potassium salts) and alkaline earth metal salts (such as magnesium and calcium salts). Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Company, Easton, PA, 1990, p 1445, the disclosure of which is hereby incorporated by reference.

[0018] As used herein, polyethylene sorbitan fatty acid esters are amphipathic, nonionic surfactants composed of fatty acid esters of polyoxyethylene sorbitan. Typically "fatty acid ester of polyoxyethylene sorbitan" refers to a mixture of fatty acid esters of polyoxyethylene sorbitan, such as a mixture of fatty acid esters of polyoxyethylene comprising primarily sorbitan polyoxyethylene (20) sorbitan monolaurate or sorbitan polyoxyethylene (20) sorbitan oleic acid (e.g., at least 50% w/w, 60% w/w, 70% w/w, 80% w/w or 90% w/w).

Examples include, but are not limited to, polysorbate 20 (comprising polyoxyethylene (20) sorbitan monolaurate) such as e.g., Tween™ 20 comprised of e.g., a polyethylene sorbitan ester with a calculated molecular weight of 1,225 daltons, assuming 20 ethylene oxide units, 1 sorbitol, and 1 lauric acid as the primary fatty acid; polysorbate 40 (comprising polyoxyethylene (20) sorbitan monopalmitate) such as e.g., Tween™ 40; polysorbate 60 (comprising polyoxyethylene (20) sorbitan monostearate) such as e.g., Tween™ 60; and polysorbate 80 (comprising polyoxyethylene (20) sorbitan monooleate) such as e.g., Tween™ 80 comprised of e.g., polyethylene sorbitan ester with a calculated molecular weight of 1,310 daltons, assuming 20 ethylene oxide units, 1 sorbitol, and 1 oleic acid as the primary fatty acid. Examples include Tween™ 80.

[0019] No observed adverse effect level (NOAEL) refers to the highest tested dose of a peptide described in the compositons herein at which there is no biologically or statistically significant (e.g., alteration of morphology, functional capacity, growth, development or life span) increase in the frequency or severity of any adverse effects in the exposed subject when compared to a control subject or group.

[0020] The maximum tolerated dose (MTD) is the highest dose of a drug (e.g., a composition as described herein) or treatment that does not cause unacceptable side effects.
"Dose" or "dosage" as used herein refers to a specific quantity of a therapeutic agent, such as an Apo E mimetic, that is taken at specific times.

As used herein, "average particle size" means the average size of particles relative to the total amount the same particles in solution.

As used herein, "effective amount" is meant to mean a sufficient amount of the composition or Apo E mimetic to provide the desired effect. For example, an effective amount of an Apo E mimetic can be an amount that provides a therapeutic affect and provides sustained therapeutic effects after withdrawal of the treatment. An effective amount of an Apo E mimetic is an amount that is able to cause a benefit illustrated by a decrease in atherosclerosis, a decrease in artery wall stiffness, a decrease in isolated systolic hypertension, a decrease in arterial inflammation, an increase in anti-oxidant capability of the HDL fraction and/or an improvement in myocardial function, as well as an amount that allows for a sustained therapeutic effect after withdrawal of the Apo E mimetic. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of disease (or underlying genetic defect) that is being treated, the particular compound used, its mode of administration, and the like. Thus, it is not possible to specify an exact "effective amount." However, an appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine experimentation.

As used herein, "sample" is meant to mean an animal; a tissue or organ from an animal; a cell (either within a subject, taken directly from a subject, or a cell maintained in culture or from a cultured cell line); a cell lysate (or lysate fraction) or cell extract; or a solution containing one or more molecules derived from a cell or cellular material (e.g. a polypeptide or nucleic acid), which is assayed as described herein. A sample may also be any body fluid or excretion (for example, but not limited to, blood, urine, stool, saliva, tears, bile) that contains cells or cell components.

As used herein, "subject" refers to the target of administration, e.g. an animal. Thus the subject of the disclosed methods can be a vertebrate, such as a mammal. For example, the subject can be a human. The term does not denote a particular age or sex. Subject can be used interchangeably with "individual" or "patient".

As used herein, "modulate" is meant to mean to alter, by increasing or decreasing.

As used herein, the terms "treatment," "treat," and "treating" refer to reversing, alleviating, delaying the onset of, or inhibiting the progress of a disease or disorder, or one or more symptoms thereof, as described herein. In some embodiments, treatment may be administered after one or more symptoms have developed, i.e., therapeutic treatment. In
other embodiments, treatment may be administered in the absence of symptoms. For example, treatment may be administered to a susceptible individual prior to the onset of symptoms (e.g., in light of a history of symptoms and/or in light of genetic or other susceptibility factors), i.e., to reduce the likelihood of developing. Susceptible individual being one who is at risk of developing one or more of the conditions recited herein.

[0028] As used herein, "lipoprotein" or "lipoproteins" is meant to mean a biochemical assembly that contains both proteins and lipids. The lipids or their derivatives may be covalently or non-covalently bound to the proteins. Many enzymes, transporters, structural proteins, antigens, adhesins, and toxins are lipoproteins. Examples include the high density and low density lipoproteins of the blood, the transmembrane proteins of the mitochondrion and the chloroplast, and bacterial lipoproteins.

[0029] As used herein, "high-density lipoprotein" (HDL) is meant to mean a class of lipoproteins, varying somewhat in their size (8-11 nm in diameter), that can transport cholesterol. HDL cholesterol is cholesterol that is associated with HDLs. About one-fourth to one-third of blood cholesterol is carried by high-density lipoprotein (HDL). HDL cholesterol is known as "good" cholesterol, because high levels of HDL seem to protect against heart attack. Low levels of HDL (less than 40 mg/dL in men and less than 50 mg/dL in women) also increase the risk of heart disease. Medical experts think that HDL tends to carry cholesterol away from the arteries and back to the liver, where it is passed from the body. Some experts believe that that HDL removes excess cholesterol from arterial plaque, thus slowing its buildup.

[0030] As used herein, "very Low Density Lipoproteins" (VLDL) is meant to mean a lipoprotein subclass. It is assembled in the liver from cholesterol and apolipoproteins. It is converted in the bloodstream to low density lipoprotein (LDL). VLDL particles have a diameter of 30-80 nm. VLDL transports endogenous products where chylomicrons transport exogenous (dietary) products.

[0031] As used herein, "low-density lipoprotein" or "LDL" is meant to mean a lipoprotein that varies in size (approx. 22 nm) and can contain a changing number of triglycerides and cholesteryl esters they actually have a mass and size distribution. Each native LDL particle contains a single apolipoproteinB-100 molecule (Apo B-100, a protein with 4536 amino acid amino acid residues) and a phospholipid coat that circles the triglycerides and cholesteryl esters, keeping them soluble in the aqueous environment. LDL is commonly referred to as bad cholesterol. LDL cholesterol is cholesterol that is associated with LDLS. When too much LDL cholesterol circulates in the blood, it can slowly build up in
the inner walls of the arteries that feed the heart and brain. Together with other substances, it can form plaque, a thick, hard deposit that can narrow the arteries and make them less flexible. This condition is known as atherosclerosis. If a clot forms and blocks a narrowed artery, then heart attack or stroke can result.

[0032] The phrase "lipid disorder" is meant to mean when a subject has an excess of lipids or increased inflammatory lipids in their blood. Lipids include, but are not limited to lipids such as ox-LDL (i.e., oxidized PAPC (1-palmitoyl 2-arachidonyl phophatidyl choline). Oxidation of PAPC or PLPC, the lipid components of LDL, produce oxidized lipids. Having a lipid disorder can make you more likely to develop inflammatory disease such as atherosclerosis and heart disease. Lipid disorders can be caused by genetic predispositions or diet. Lipid disorders include e.g., coronary artery disease, rheumatoid arthritis, diabetes, Alzheimer's disease, peripheral arterial disease (PAD), cerebral vascular disease, diabetes-derived cardiovascular diseases, macular degeneration, congestive heart failure, and systemic lupus.

[0033] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed method and compositions belong.

[0034] As described above, in a first embodiment, provided herein are aqueous pharmaceutical compositions comprising i) a synthetic apolipoprotein E (ApoE) mimicking peptide of the formula \((\text{CH}_3)(\text{CH}_2)_x\text{C}(0)\text{NH}(\text{CH}_2)_y\text{C}(0)\text{-LRRLRRRLLR-DWLKAFYDKVAEKLKEAF-NH}_2\) wherein \(x\) is an integer from 0 to 6; and \(y\) is an integer from 1 to 20; or a pharmaceutically acceptable salt thereof; and ii) a polyoxyethylene sorbitan fatty acid ester.

[0035] In a second embodiment, \(x\) in the peptides described herein is an integer from 0 to 3, wherein the remaining features are as described in the first embodiment. Alternatively, \(x\) is 0, wherein the remaining features are as described in the first embodiment.

[0036] In a third embodiment, \(y\) in the peptides described herein is an integer from 3 to 18, wherein the remaining features are as described in the first or second embodiment. In one alternative, \(y\) is an integer from 4 to 16, wherein the remaining features are as described in the first or second embodiment. In another alternative, \(y\) is an integer from 6 to 13, wherein the remaining features are as described in the first or second embodiment.

[0037] In a fourth embodiment, the ApoE mimicking peptide in the aqueous compositions described herein is of the formula \(\text{H}_3\text{C}(0)\text{-NH(\text{CH}_2)_6(0)-LRRLRRRLLRDWLKAFYDKVAEKLKEAF-NH}_2\), \(\text{H}_3\text{C}(0)\text{-NH(\text{CH}_2)_7(0)-LRRLRRRLLR-}\)
DWLKAFYDKVAEKLKEAF-NH$_2$, or H$_2$C(CO)-NH(CH$_2$)$_3$(CO)-LRRLRRLLR-DWLKAFYDKVAEKLKEAF-NH$_2$, or a pharmaceutically acceptable salt thereof, wherein the remaining features are as described in the first, second, or third embodiment. In one alternative, the ApoE mimicking peptide is of the formula H$_2$C(CO)-NH(CH$_2$)$_3$(CO)-LRRLRRRLR-DWLKAFYDKVAEKLKEAF-NH$_2$, or a pharmaceutically acceptable salt thereof, wherein the remaining features are as described in the first, second, or third embodiment. In yet another alternative, the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles, wherein the remaining features are as described in the first, second, or third embodiment.

[0038] In a fifth embodiment, the aqueous pharmaceutical composition comprises sterile water for injection (WFI), saline, or phosphate-buffered saline (PBS), or a combination thereof, wherein the remaining features are as described in the first, second, third, or fourth embodiment.

[0039] In a sixth embodiment, the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles, wherein the remaining features are as described in the first, second, third, fourth, or fifth embodiment.

[0040] In a seventh embodiment, the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles having an average particle size of less than 20 nm, wherein the remaining features are as described in the first, second, third, fourth, fifth, or sixth embodiment. Alternatively, the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles having an average particle size ranging from 2 nm to 17 nm, wherein the remaining features are as described in the first, second, third, fourth, fifth, or sixth embodiment. In another alternative, the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles having an average particle size ranging from 5 nm to 16 nm, wherein the remaining features are as described in the first, second, third, fourth, fifth, or sixth embodiment. In another alternative, the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles having an average particle size ranging from 5 nm to 15 nm, wherein the remaining features are as described in the first, second, third, fourth, fifth, or sixth embodiment. In yet another alternative, the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles having an average particle size ranging from 8 nm to 16 nm, wherein the remaining features are as described in the first, second, third, fourth, fifth, or sixth embodiment. In yet another alternative, the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form
particles having an average particle size ranging from 5 nm to 12 nm, wherein the remaining features are as described in the first, second, third, fourth, fifth, or sixth embodiment. In yet another alternative, the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles having an average particle size ranging from 6 nm to 10 nm, wherein the remaining features are as described in the first, second, third, fourth, fifth, or sixth embodiment. In yet another alternative, the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles having an average particle size ranging from 7 nm to 9 nm, wherein the remaining features are as described in the first, second, third, fourth, fifth, or sixth embodiment.

[0041] In an eighth embodiment, the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles that are micelles, wherein the remaining features are as described in the first, second, third, fourth, fifth, sixth, or seventh embodiment.

[0042] In a ninth embodiment, the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles that are micelles, which are homogenously distributed throughout the aqueous formulation, wherein the remaining features are as described in the first, second, third, fourth, fifth, sixth, seventh, or eighth embodiment.

[0043] In a tenth embodiment, the molar ratio of ApoE mimicking peptide to polyoxyethylene sorbitan fatty acid ester in the aqueous compositions described herein is less than 0.200, wherein the remaining features are as described in the first, second, third, fourth, fifth, sixth, seventh, eighth, or ninth embodiment. Alternatively, the molar ratio of ApoE mimicking peptide to polyoxyethylene sorbitan fatty acid ester in the aqueous compositions described herein is less than or equal to 0.170, wherein the remaining features are as described in the first, second, third, fourth, fifth, sixth, seventh, eighth, or ninth embodiment. Alternatively, the molar ratio of ApoE mimicking peptide to polyoxyethylene sorbitan fatty acid ester in the aqueous compositions described herein is less than 0.165, wherein the remaining features are as described in the first, second, third, fourth, fifth, sixth, seventh, eighth, or ninth embodiment. Alternatively, the molar ratio of ApoE mimicking peptide to polyoxyethylene sorbitan fatty acid ester in the aqueous compositions described herein ranges from 0.010 to 0.170, wherein the remaining features are as described in the first, second, third, fourth, fifth, sixth, seventh, eighth, or ninth embodiment. In another alternative, the molar ratio of ApoE mimicking peptide to polyoxyethylene sorbitan fatty acid ester in the aqueous compositions described herein ranges from 0.020 to 0.170, wherein the remaining features are as described in the first, second, third, fourth, fifth, sixth, seventh, eighth, or ninth embodiment. In another alternative, the molar ratio of ApoE mimicking
peptide to polyoxyethylene sorbitan fatty acid ester in the aqueous compositions described herein ranges from 0.030 to 0.170, wherein the remaining features are as described in the first, second, third, fourth, fifth, sixth, seventh, eighth, or ninth embodiment. In another alternative, the molar ratio of ApoE mimicking peptide to polyoxyethylene sorbitan fatty acid ester in the aqueous compositions described herein ranges from 0.050 to 0.170, wherein the remaining features are as described in the first, second, third, fourth, fifth, sixth, seventh, eighth, or ninth embodiment. In another alternative, the molar ratio of ApoE mimicking peptide to polyoxyethylene sorbitan fatty acid ester in the aqueous compositions described herein ranges from 0.019 to 0.050, wherein the remaining features are as described in the first, second, third, fourth, fifth, sixth, seventh, eighth, or ninth embodiment. In another alternative, the molar ratio of ApoE mimicking peptide to polyoxyethylene sorbitan fatty acid ester in the aqueous compositions described herein ranges from 0.014 to 0.019, wherein the remaining features are as described in the first, second, third, fourth, fifth, sixth, seventh, eighth, or ninth embodiment.

[0044] In an eleventh embodiment, the polyoxyethylene sorbitan fatty acid ester is selected from polysorbate 20, polysorbate 40, polysorbate 60, and polysorbate 80, wherein the remaining features are as described in the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, or tenth embodiment. In one alternative, the polyoxyethylene sorbitan fatty acid ester is polysorbate 20 or polysorbate 80, wherein the remaining features are as described in the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, or tenth embodiment. In another alternative, the polyoxyethylene sorbitan fatty acid ester is polysorbate 80, wherein the remaining features are as described in the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, or tenth embodiment.

[0045] In a twelfth embodiment, the ApoE mimicking peptide, or pharmaceutically acceptable salt thereof, is present at a concentration ranging from 0.1 mg/mL to 10 mg/mL, wherein the remaining features are as described in the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, or eleventh embodiment. Alternatively, the ApoE mimicking
peptide, or pharmaceutically acceptable salt thereof, is present at a concentration ranging from 0.8 mg/mL to 5.5 mg/mL, wherein the remaining features are as described in the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, or eleventh embodiment. In another alternative, the ApoE mimicking peptide, or pharmaceutically acceptable salt thereof, is present at a concentration of 1 mg/mL, 2.5 mg/mL, or 5 mg/mL, wherein the remaining features are as described in the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, or eleventh embodiment.

[0046] Other examples of compositions, including independent components of the peptides and/or phospholipids described herein, are provided in the EXEMPLIFICATION. In certain embodiment, the compositions described herein encompasses all of the disclosed peptides and phospholipids, and combinations and variations thereof, as further set forth in the EXEMPLIFICATION.

C. Uses and Administration

[0047] Disclosed herein are methods for affecting plasma LDL, plasma VLDL, or both, comprising administering a composition comprising i) a synthetic apolipoprotein E(ApoE)-mimicking peptide of the formula (CH₃)(CH₂)ₓC(0)NH(CH₂)ₙC(0)-LRRRLRRLLL-R-DWLKAFYDKVAEKLKEAF-NHₓ² wherein x is an integer from 0 to 6; and y is an integer from 1 to 20; or a pharmaceutically acceptable salt thereof; and ii) a phospholipid as described herein. In one aspect, the plasma LDL, plasma VLDL, or both, are affected. In another aspect, binding of LDL to a cell of the subject is enhanced. In another aspect, degradation of LDL by a cell of the subject is increased. In another aspect, LDL cholesterol in the subject is lowered. In another aspect, binding of VLDL to a cell of the subject is enhanced. In another aspect, degradation of VLDL by a cell of the subject is increased. In another aspect, VLDL cholesterol in the subject is lowered. In another aspect, total plasma concentration of cholesterol in the subject is lowered. In one aspect, in the methods of affecting plasma LDL, plasma VLDL, or both, the disclosed synthetic apolipoprotein E (ApoE)-mimicking peptides are administered in an amount of about 0.01 mg/kg to about 20 mg/kg, e.g., 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 mg/kg, or any range in between.

[0048] Also disclosed herein are methods of treating atherosclerosis comprising administering to a subject in need thereof i) a synthetic apolipoprotein E(ApoE)-mimicking peptide having the formula (CH₃)(CH₂)ₓC(0)NH(CH₂)ₙC(0)-LRRRLRRLLL-R-DWLKAFYDKVAEKLKEAF-NHₓ² wherein x is an integer from 0 to 6; and y is an integer from 1 to 20; or a pharmaceutically acceptable salt thereof; and ii) a phospholipid as
described herein. In one aspect in the methods of treating atherosclerosis, the disclosed synthetic apolipoprotein E (ApoE)-mimicking peptides are administered in an amount of about 0.01 mg/kg to about 20 mg/kg, e.g., 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 mg/kg, or any range in between.

Also disclosed are methods of treating a subject with a lipid disorder comprising administering to a subject in need thereof: i) a synthetic apolipoprotein E(ApoE)-mimicking peptide having the formula \((\text{CH}_3)\text{(CH}_2)\_x\text{C}(0)\text{NH(\text{CH}}_2\text{)}\_y\text{C}(0)\text{-LRRLRRLLL}-\text{DLWKAFYDKVAEKLKEAF-NH}_2\), wherein \(x\) is an integer from 0 to 6; and \(y\) is an integer from 1 to 20; or a pharmaceutically acceptable salt thereof; and ii) a phospholipid as described herein. In one aspect, the lipid disorder is selected from coronary artery disease, rheumatoid arthritis, systemic lupus, diabetes, Alzheimer's disease, peripheral artery disease (PAD), diabetes-derived cardiovascular diseases, macular degeneration, and congestive heart failure. In one aspect in the methods of treating a lipid disorder, the disclosed synthetic apolipoprotein E (ApoE)-mimicking peptides are administered in an amount of about 0.01 mg/kg to about 20 mg/kg, e.g., 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 mg/kg, or any range in between.

Also disclosed are methods of treating acute coronary syndrome (ACS) comprising administering to a subject in need thereof: i) a synthetic apolipoprotein E(ApoE)-mimicking peptide having the formula \((\text{CH}_3)\text{(CH}_2)\_x\text{C}(0)\text{NH(\text{CH}}_2\text{)}\_y\text{C}(0)\text{-LRRLRRLLL}-\text{DLWKAFYDKVAEKLKEAF-NH}_2\), wherein \(x\) is an integer from 0 to 6; and \(y\) is an integer from 1 to 20; or a pharmaceutically acceptable salt thereof; and ii) a phospholipid as described herein. In one aspect in the methods of treating ACS, the disclosed synthetic apolipoprotein E (ApoE)-mimicking peptides are administered in an amount of about 0.01 mg/kg to about 20 mg/kg, e.g., 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 mg/kg, or any range in between.

The dose or dosage of the compositions described herein can vary depending on many factors, such as but not limited to, age, condition, sex and extent of the disease in the patient, route of administration, length of treatment cycle, or whether other drugs are included in the regimen, and can be determined by one of skill in the art. Effective dosages can be determined empirically, and making such determinations is within the skill in the art. The dosage ranges for the administration of the compositions are those large enough to produce the desired effect in which the disease is treated. For example, the dosage can be an amount effective to provide therapeutic effects and provide or allow for sustained therapeutic effects even after the treatment is withdrawn. The therapeutic effects
can be, but are not limited to, a reduction in atherosclerotic lesions, decrease in arterial stiffness, decrease in isolated systolic hypertension, increase in vasoresponsiveness or improvement in cardiac function. The therapeutic effects can be measured by markers of arterial inflammation such as, but not limited to, C-reactive protein. The therapeutic effects can be measured by atherosclerosis imaging techniques, including MRI, intravascular ultrasound, ultrafast imaging CT scans, B-mode ultrasonography, virtual histology intravascular ultrasound, optical coherence tomography, or other known methods. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. The dosage can be adjusted by the individual physician in the event of any counter-indications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products.

In the methods described herein, any suitable route of administration can be used for the disclosed compositions. Suitable routes of administration can, for example, include topical, enteral, local, systemic, or parenteral. For example, administration can be epicutaneous, inhalational, enema, conjunctival, eye drops, ear drops, alveolar, nasal, intranasal, enteral, oral, intraoral, transoral, intestinal, rectal, intrarectal, transrectal, injection, infusion, intravenous, intraarterial, intramuscular, intracerebral, intraventricular, intracerebroventricular, intracardiac, subcutaneous, intramuscular, intradermal, intrathecal, intraperitoneal, intravesical, intracavernosal, intramedullar, intraocular, intracranial, transdermal, transmucosal, transnasal, inhalational, intracisternal, epidural, peridural, intravitreal, etc. The disclosed compositions can be used in and with any other therapy.

In one aspect, the disclosed compositions are aqueous formulations that exists in e.g., H₂O (e.g., water for injection (WFI)), PBS (e.g., sterile PBS), and the like.

The foregoing formulations and administration methods are intended to be illustrative and not limiting. It will be appreciated that, using the teaching provided herein, other suitable formulations and modes of administration can be readily devised.

**Examples**

**General Peptide Synthesis**

Peptides described herein were prepared via standard solid-phase synthetic procedures. Ac-hE18A-NH₂, Ac-[R]hE18A-NH₂, and Ac-Aha[R]hE 18A-NH₂ were
prepared according to the procedures described in U.S. Patent No. 6,506,880 and U.S. Provisional No. 62/031,585, and following standard solid-phase synthetic procedures.

**Example 1: General Peptide Synthesis**

[0058] Peptides described herein were prepared via standard solid-phase synthetic procedures. Ac-hE18A-NH₂ was prepared according to the procedures described in U.S. Patent No. 6,506,880 and U.S. Provisional No. 62/031,585, and following standard solid-phase synthetic procedures.

**Example 2: Composition Development and Preparation**

[0059] AEM-28 was found to readily dissolve in 0.9% saline, while AEM-28-08 and AEM-28-14 formed suspensions with rapid settling. To overcome this obstacle, the polyethylene sorbitan ester Tween was added. The addition of Tween to AEM-28-08 and AEM-28-14 was found to not only dissolve the peptides, but also formed small, uniform micelles, thereby producing smaller, more consistent API containing particles. Table 1 shows results from this study.

[0060] **Table 1**

<table>
<thead>
<tr>
<th>Ref</th>
<th>API</th>
<th>Conc. A (mg/mL)</th>
<th>polyethylene sorbitan ester (weight basis in vehicle)</th>
<th>Molar Ratio API/polyethylene sorbitan ester</th>
<th>Vehicle</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AEM-28-14</td>
<td>2.5</td>
<td>0 (none)</td>
<td>infinity</td>
<td>0.9% Saline (USP)</td>
<td>Clear with some particles</td>
</tr>
<tr>
<td>2</td>
<td>AEM-28-14</td>
<td>2.5</td>
<td>0.1% Tween® 20</td>
<td>0.841</td>
<td>0.9% Saline (USP)</td>
<td>Some Precipitate</td>
</tr>
<tr>
<td>3</td>
<td>AEM-28-14</td>
<td>2.5</td>
<td>0.5% Tween® 20</td>
<td>0.168</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>4</td>
<td>AEM-28-14</td>
<td>2.5</td>
<td>2.0% Tween® 20</td>
<td>0.042</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>5</td>
<td>AEM-28-14</td>
<td>2.5</td>
<td>5.0% Tween® 20</td>
<td>0.017</td>
<td>Clear</td>
<td>Clear</td>
</tr>
<tr>
<td>6</td>
<td>AEM-28-14</td>
<td>10.0</td>
<td>5.0% Tween® 20</td>
<td>0.067</td>
<td>Gel-like precipitate</td>
<td>Cloudy suspension</td>
</tr>
<tr>
<td>7</td>
<td>AEM-28-14</td>
<td>100.0</td>
<td>5.0% Tween® 20</td>
<td>0.673</td>
<td>PBS 1x</td>
<td>Clear with some particles</td>
</tr>
<tr>
<td>8</td>
<td>AEM-28-14</td>
<td>2.5</td>
<td>0 (none)</td>
<td>infinity</td>
<td>PBS 1x</td>
<td>Clear with some particles</td>
</tr>
<tr>
<td>9</td>
<td>AEM-28-14</td>
<td>2.5</td>
<td>5.0% Tween® 20</td>
<td>0.017</td>
<td>PBS 1x</td>
<td>Clear with some particles</td>
</tr>
</tbody>
</table>

AAdjusted for peptide content, as applicable

[0061] Table 1A below shows the correlation between the molar ratio of AEM-28-14/polyethylene sorbitan ester and particle size. No visible particles were seen.
Example 3: Peptide Tolerability

Formulations for Session 1 and Session 2 were prepared as outlined in Table 2 below.

The dose formulations for Groups 1-5 were formulated with Tween® 80 in PBS IX. The dose formulation for Group 6 was formulated with Tween® 20 in PBS IX. All formulations for Sessions 1 and 2 were clear colorless solutions at the time of intravenous dose administration. Following dosing of each session, the residual dose formulations were stored -20 ± 5°C until final disposition.

A total of thirty nine naïve female CD-I mice were received from Charles River Laboratories, Kingston, NY. Following an acclimation period, the animals were assigned to
the study based on acceptable health as determined by a staff veterinarian. Animals were placed into 6 groups (3 groups per session) for intravenous dose administration. Animal fasting was not required for this study. The final study design is presented in Table 3.

Table 3

<table>
<thead>
<tr>
<th>Session</th>
<th>Group</th>
<th>Subgroup</th>
<th>No. of Females</th>
<th>API</th>
<th>Dose Level&lt;sup&gt;a&lt;/sup&gt; (mg/kg)</th>
<th>Dose Conc.&lt;sup&gt;a&lt;/sup&gt; (mg/mL)</th>
<th>Dose Volume (mL/kg)</th>
<th>Dose Vehicle</th>
<th>Dose Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>A</td>
<td>2</td>
<td>AEM-28</td>
<td>5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
<td>5</td>
<td>10</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>A</td>
<td>2</td>
<td>AEM-28-08</td>
<td>5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
<td>5</td>
<td>10</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>2</td>
<td></td>
<td>15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5</td>
<td>6</td>
<td>25</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>2</td>
<td></td>
<td>25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>A</td>
<td>2</td>
<td>AEM-28-14</td>
<td>5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
<td>5</td>
<td>10</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
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<td></td>
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<td>15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5</td>
<td>6</td>
<td>25</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>2</td>
<td></td>
<td>25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5</td>
<td>10</td>
<td></td>
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</tr>
<tr>
<td>4</td>
<td>1</td>
<td>A</td>
<td>2</td>
<td>AEM-28-08</td>
<td>20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>IV</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>2</td>
<td></td>
<td>30&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>5</td>
<td>1</td>
<td>A</td>
<td>2</td>
<td>AEM-28-14</td>
<td>20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>2</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>2</td>
<td></td>
<td>30&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>A</td>
<td>2</td>
<td>AEM-28-14</td>
<td>20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B</td>
<td>2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>2</td>
<td></td>
<td>30&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Adjusted for peptide content, as applicable
<sup>b</sup>Subgroup dosing staggered by a minimum of 1 hour. Higher doses to be administered if no significant adverse clinical signs are observed for either animal dosed at the lower dose level within the group.
<sup>c</sup>Dosing between animals within the subgroup staggered by a minimum of 1 hour.

Each animal in Sessions 1 and 2 received a single intravenous dose of prepared test article at the target dose levels and dose volumes indicated in the table above. Animals were monitored continuously for any adverse clinical signs for the first hour following dose administration, at which point tolerability and dose escalation were assessed. In addition, clinical observations were performed for all animals at 2, 3, 4, 5, 6, 7 and 24 hours post-dose. A summary of clinical observations are as follows.
- Previously, data in mice with AEM-28 in saline/PBS generated a no observed adverse effect level (NOAEL) dose of \( \leq 5 \) mg/kg and an MTD of 10 mg/kg. However, with AEM-28 formulated in 2% Tween\(^\circ\) 80/PBS, a higher NOAEL dose was observed at 10 mg/kg.

- AEM-28-08 and AEM-28-14 in 5% Tween\(^\circ\) 80/PBS generated a NOAEL up to 15 mg/kg, with minimal effects observed at 25 mg/kg.

- AEM-28-08 in 10% Tween\(^\circ\) 80/PBS was well tolerated at 20 and 25 mg/kg.

- AEM-28-14 in 10% Tween\(^\circ\) 80/PBS was well tolerated up to 30 mg/kg with minimal effects.

- AEM-28-14 in 2% Tween\(^\circ\) 20/PBS generated a NOAEL at 25 mg/kg.

These results show that AEM-28-08 and AEM-28-14 were well-tolerated in animal models. Surprisingly, it was also found that replacement of saline/PBS with Tween/PBS for AEM-28 generated a greater NOAEL dose. Because AEM-28-08 and AEM-28-14 did not dissolve in water to the same extent as AEM-28.

**Example 4: Peptide Efficacy**

All formulations for Sessions 1 & 2 were clear colorless solutions at the time of intravenous dose administration. Following dosing of Sessions 1 & 2, the residual dose formulations were stored -20+5°C until final disposition. A total of twenty-two naïve female Apo-E (-/-) mice were received from Jackson Laboratories. Following an acclimation period, the animals were assigned to the study based on acceptable health as determined by a staff veterinarian. All animals were fasted overnight before dosing and food was returned after the 6-hour post dose blood collections. The final study design is presented in Table 4 below.

**Table 4**

<table>
<thead>
<tr>
<th>Session</th>
<th>Group</th>
<th>No. of Females</th>
<th>API</th>
<th>Dose Level(^A) (mg/kg)</th>
<th>Dose Conc.(^A) (mg/mL)</th>
<th>Dose Volume (mL/kg)</th>
<th>Dose Vehicle</th>
<th>Dose Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>4</td>
<td>AEM-28</td>
<td>4</td>
<td>0.4</td>
<td>10</td>
<td>0.8% Tween(^\circ) 80 in PBS IX</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4</td>
<td>AEM-28-08</td>
<td>0.4</td>
<td>0.04</td>
<td>10</td>
<td>0.08% Tween(^\circ) 80 in PBS IX</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4</td>
<td>AEM-28-08</td>
<td>4</td>
<td>0.4</td>
<td>10</td>
<td>0.8% Tween(^\circ) 80 in PBS IX</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
<td>AEM-28-14</td>
<td>0.4</td>
<td>0.04</td>
<td>10</td>
<td>0.08% Tween(^\circ) 80 in PBS IX</td>
<td>IV</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4</td>
<td>AEM-28-14</td>
<td>4</td>
<td>0.4</td>
<td>10</td>
<td>0.8% Tween(^\circ) 80 in PBS IX</td>
<td>IV</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>4</td>
<td>AEM-28-14</td>
<td>1</td>
<td>0.1</td>
<td>10</td>
<td>0.2% Tween(^\circ) 80 in PBS IX</td>
<td>IV</td>
</tr>
<tr>
<td>Tween® 80 in PBS IX</td>
<td>Tween® 80 in PBS IX</td>
<td>Tween® 80 in PBS IX</td>
<td>Tween® 80 in PBS IX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------------</td>
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<td>---------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>0.4%</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>0.2%</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.2</td>
<td>0.4%</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.1</td>
<td>0.04%</td>
<td>IV</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.2</td>
<td>0.08%</td>
<td>IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AAdjusted for peptide content, as applicable

[0072] The cholesterol reduction results for Sessions 1 and 2 in the mouse ApoE knockout models are shown in FIG. 1 and FIG. 2, respectively. Error bars are standard error of the mean.

[0073] As shown in Figure 1, both AEM-28-08 and AEM-28-14 elicit significant reductions in cholesterol levels with AEM-28-14 essentially removing all circulating cholesterol for at least 6 hours post-dose. See e.g., FIG. 1A for total cholesterol levels and Figure 1B where AEM-28-8 and AEM-28-14 each elicit a dramatic reduction in % change of total cholesterol at 4 mg/kg (to 9% and 2% of baseline). This reduction is superior to AEM-28, which exhibited a % change of total cholesterol to 38% of baseline. See e.g., FIG. IB.

[0074] Perhaps most surprising was that no adverse events were observed even with the dramatic reduction in cholesterol levels, particularly in instances where some animals had no detectable cholesterol levels after 6-hours post-treatment with AEM-28-14. For example, AEM-28-14 at 4 mg/kg reduced cholesterol from 450 mg/dl to zero in some animals.

[0075] Even at lower dosages such as 1 mg/kg and 2 mg/kg, approximately 50% of total cholesterol was reduced at 6 hours, with cholesterol levels returning to 100% or greater at 24 hours. See FIG. 2. Dosing at 2 mg/kg showed slightly greater reduction in cholesterol for AEM-28-08 at 1 hour versus AEM-28-14 (70% vs. 60% as in FIG. 2B). Cholesterol levels with AEM-28-14 at 2 mg/kg also remained level through 6 hours with approximately a 60-70% reduction, and cholesterol levels were still below baseline by approximately 20% at 24 hours.

[0076] No substantial difference was observed in cholesterol reduction between the use of Tween® 80 or Tween® 20, although better solubility of the peptides was observed with Tween® 20.
Example 4: Effects in Hypercholesterolemic Cynomolgus Monkeys

The effect of AEM-28-14 in 2% Tween® 20/PBS in Hypercholesterolemic Cynomolgus Monkeys was evaluated. 3 cynomolgus monkeys (2 male and 1 female) were fed "Western diet" to raise cholesterol (0.29 mg cholesterol/kcal, mean cholesterol >310 mg/dl). All animals were surgically instrumented for infusion and were anesthetized before dosing. AEM-28-14 in 2% Tween® 20/PBS was infused through 10-minute slow push IV at 1, 3, and 5 mg/kg. Blood samples were taken for 7 days and the animals were allowed 7 additional days to recover before the next dose. The animals remained on cholesterol enriched Western diet throughout the blood sampling and recovery period.

The animals tolerated the infusions and the LDL fraction results are shown in FIG. 3. As shown, a decrease in LDL fraction resulted for all doses with 5 mg/kg being the most effective.
Listing of Claims:

1. An aqueous pharmaceutical composition comprising i) a synthetic apolipoprotein E(ApoE) mimicking peptide of the formula $(\mathrm{CH}_3)(\mathrm{CH}_2)x\mathrm{C}(0)\mathrm{NH}(\mathrm{CH}_2)y\mathrm{C}(0)-\mathrm{LRRLRRLLR-DWLKAFYDKVAEKLKEAF-NH}_2$, wherein $x$ is an integer from 0 to 6; and $y$ is an integer from 1 to 20; or a pharmaceutically acceptable salt thereof; and ii) a polyoxyethylene sorbitan fatty acid ester.

2. The aqueous pharmaceutical composition of Claim 1, wherein $x$ is an integer from 0 to 3.

3. The aqueous pharmaceutical composition of Claim 1 or 2, wherein $x$ is 0.

4. The aqueous pharmaceutical composition of any one of Claims 1 to 3, wherein $y$ is an integer from 3 to 18.

5. The aqueous pharmaceutical composition of any one of Claims 1 to 4, wherein $y$ is an integer from 4 to 16.

6. The aqueous pharmaceutical composition of any one of Claims 1 to 5, wherein $y$ is an integer from 6 to 13.

7. The aqueous pharmaceutical composition of any one of Claims 1 to 6, wherein the ApoE mimicking peptide is of the formula $\mathrm{H}_3\mathrm{C}(0)-\mathrm{NH}(\mathrm{CH}_2)x(0)-\mathrm{LRRLRRRLRRL-DWLKAFYDKVAEKLKEAF-NH}_2$, $\mathrm{H}_3\mathrm{C}(0)-\mathrm{NH}(\mathrm{CH}_2)y(0)-\mathrm{LRRLRRRLRRL-DWLKAFYDKVAEKLKEAF-NH}_2$, or $\mathrm{H}_3\mathrm{C}(0)-\mathrm{NH}(\mathrm{CH}_2)i(0)-\mathrm{LRRLRRRLRRL-DWLKAFYDKVAEKLKEAF-NH}_2$, or a pharmaceutically acceptable salt thereof.

8. The aqueous pharmaceutical composition of any one of Claims 1 to 7, wherein the ApoE mimicking peptide is of the formula $\mathrm{H}_3\mathrm{C}(0)-\mathrm{NH}(\mathrm{CH}_2)x(0)-\mathrm{LRRLRRRLRRL-DWLKAFYDKVAEKLKEAF-NH}_2$, or a pharmaceutically acceptable salt thereof.
9. The aqueous pharmaceutical composition of any one of Claims 1 to 8, wherein the ApoE mimicking peptide is of the formula \( \text{H}_3\text{C}(-\text{CO})-\text{NH}(-\text{CH}_2)_{i_3}\text{CO}(-\text{LRRLRRRLLRDWLKAFYDKVAEKLKEAF})\text{-NH}_2 \), or a pharmaceutically acceptable salt thereof.

10. The aqueous pharmaceutical composition of any one of Claims 1 to 9, wherein the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles.

11. The aqueous pharmaceutical composition of any one of Claims 1 to 10, wherein the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles having an average particle size of less than 20 nm.

12. The aqueous pharmaceutical composition of any one of Claims 1 to 11, wherein the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles having an average particle size ranging from 2 nm to 17 nm.

13. The aqueous pharmaceutical composition of any one of Claims 1 to 12, wherein the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles having an average particle size ranging from 5 nm to 15 nm.

14. The aqueous pharmaceutical composition of any one of Claims 1 to 13, wherein the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles having an average particle size ranging from 5 nm to 12 nm.

15. The aqueous pharmaceutical composition of any one of Claims 1 to 14, wherein the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles having an average particle size ranging from 6 nm to 10 nm.

16. The aqueous pharmaceutical composition of any one of Claims 1 to 15, wherein the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles that are micelles.

17. The aqueous pharmaceutical composition of any one of Claims 1 to 16, wherein the ApoE mimicking peptide and polyoxyethylene sorbitan fatty acid ester form particles that are micelles homogenously distributed throughout the aqueous formulation.
18. The aqueous pharmaceutical composition of any one of Claims 1 to 17, wherein the molar ratio of ApoE mimicking peptide to polyoxyethylene sorbitan fatty acid ester is less then 0.200.

19. The aqueous pharmaceutical composition of any one of Claims 1 to 18, wherein the molar ratio of ApoE mimicking peptide to polyoxyethylene sorbitan fatty acid ester ranges from 0.010 to 0.170.

20. The aqueous pharmaceutical composition of any one of Claims 1 to 19, wherein the molar ratio of ApoE mimicking peptide to polyoxyethylene sorbitan fatty acid ester ranges from 0.010 to 0.050.

21. The aqueous pharmaceutical composition of any one of Claims 1 to 20, wherein the molar ratio of ApoE mimicking peptide to polyoxyethylene sorbitan fatty acid ester ranges from 0.013 to 0.020.

22. The aqueous pharmaceutical composition of any one of Claims 1 to 21, wherein the molar ratio of ApoE mimicking peptide to polyoxyethylene sorbitan fatty acid ester ranges from 0.014 to 0.019.

23. The aqueous pharmaceutical composition of any one of Claims 1 to 18, wherein the molar ratio of ApoE mimicking peptide to polyoxyethylene sorbitan fatty acid ester is less then or equal to 0.170.

24. The aqueous pharmaceutical composition of any one of Claims 1 to 18 and 23, wherein the molar ratio of ApoE mimicking peptide to polyoxyethylene sorbitan fatty acid ester ranges from 0.020 to 0.170.

25. The aqueous pharmaceutical composition of any one of Claims 1 to 18, 23, and 24, wherein the molar ratio of ApoE mimicking peptide to polyoxyethylene sorbitan fatty acid ester ranges from 0.030 to 0.170.
26. The aqueous pharmaceutical composition of any one of Claims 1 to 18 and 23 to 25, wherein the molar ratio of ApoE mimicking peptide to polyoxyethylene sorbitan fatty acid ester ranges from 0.050 to 0.170.

27. The aqueous pharmaceutical composition of any one of Claims 1 to 26, wherein the polyoxyethylene sorbitan fatty acid ester is selected from polysorbate 20, polysorbate 40, polysorbate 60, and polysorbate 80.

28. The aqueous pharmaceutical composition of any one of Claims 1 to 27, wherein the polyoxyethylene sorbitan fatty acid ester is polysorbate 20 or polysorbate 80.

29. The aqueous pharmaceutical composition of any one of Claims 1 to 28, wherein the polyoxyethylene sorbitan fatty acid ester is polysorbate 80.

30. The aqueous pharmaceutical composition of any one of Claims 1 to 29, wherein the ApoE mimicking peptide, or pharmaceutically acceptable salt thereof, is present at a concentration ranging from 0.1 mg/mL to 10 mg/mL.

31. The aqueous pharmaceutical composition of any one of Claims 1 to 30, wherein the ApoE mimicking peptide, or pharmaceutically acceptable salt thereof, is present at a concentration ranging from 0.8 mg/mL to 5.5 mg/mL.

32. The aqueous pharmaceutical composition of any one of Claims 1 to 31, wherein the ApoE mimicking peptide, or pharmaceutically acceptable salt thereof, is present at a concentration of 1 mg/mL, 2.5 mg/mL, or 5 mg/mL.

33. A method of reducing plasma cholesterol in a subject in need thereof comprising administering to the subject an effective amount of the aqueous pharmaceutical composition of any one of Claims 1 to 32.

34. A method of treating a subject with a condition selected from atherosclerosis, acute coronary syndrome (ACS), coronary artery disease, rheumatoid arthritis, diabetes, Alzheimer's disease, peripheral arterial disease (PAD), cerebral vascular disease, diabetes derived cardiovascular diseases, macular degeneration, congestive heart failure, and systemic
lupus, comprising administering to a subject an effective amount of the aqueous pharmaceutical composition of any one of Claims 1 to 32.

35. The method of Claim 34, wherein the condition is atherosclerosis.
FIG. 2A

Total Cholesterol (mg/dL)

Pre-Dose 1 Hour 6 Hour 24 Hour

Time (hours)

1) AEM-28-08 in Tween80 at 1 mg/kg
2) AEM-28-08 in Tween80 at 2 mg/kg
3) AEM-28-14 in Tween80 at 1 mg/kg
4) AEM-28-14 in Tween80 at 2 mg/kg
5) AEM-28-14 in Tween20 at 1 mg/kg
6) AEM-28-14 in Tween20 at 2 mg/kg

FIG. 2B

% Change in Total Cholesterol

1 Hour 6 Hour 24 Hour

Time (hours)

1) AEM-28-08 in Tween80 at 1 mg/kg
2) AEM-28-08 in Tween80 at 2 mg/kg
3) AEM-28-14 in Tween80 at 1 mg/kg
4) AEM-28-14 in Tween80 at 2 mg/kg
5) AEM-28-14 in Tween20 at 1 mg/kg
6) AEM-28-14 in Tween20 at 2 mg/kg
FIG. 3

AEM-28-14 in Monkeys: LDL Fraction of Baseline - Means ± SEM

* = points significantly different from baseline (p < 0.001)
+ = point significantly different from baseline (p=0.005)

LDL Fraction of Baseline

Time (days)

1 mg/kg
3 mg/kg
5 mg/kg
**INTERNATIONAL SEARCH REPORT**

International application No
PCT/US2017/012678

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**A. CLASSIFICATION OF SUBJECT MATTER**
INV. A61K38/17  A61K47/26  C07K14/775  A61P3/06

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According to International Patent Classification (IPC) or to both national classification and IPC

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**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
A61K  C07K  A61P

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal , WPI Data

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**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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Further documents are listed in the continuation of Box C. See patent family annex.

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**Date of the actual completion of the international search**
18 May 2017

**Date of mailing of the international search report**
24/05/2017

**Name and mailing address of the ISA/Authorized officer**
Fayos, Cecile

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