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(54) Title: FXR INHIBITOR, BILE ACID SEQUESTRANT AS COMBINATION THERAPY FOR CHOLESTEROL REDUCTION

(57) Abstract: This invention relates to a composition containing both a bile acid sequestrant and a Farnesoid X Receptor antagonist and a method of administering the composition to a subject to modulate lipid metabolism, specifically, to increase cholesterol transport, to decrease Low-Density Lipoprotein Cholesterol (LDL-C), and/or to reduce the risk of atherosclerosis therein. This composition can be used to treat a patient having a cardiovascular disease, atherosclerosis, and/or gallstones.



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DESCRIPTION

FXR INHIBITOR, BILE ACID SEQUESTRANT AS COMBINATION THERAPY FOR CHOLESTEROL REDUCTION

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[0001] The present application claims priority to U.S. provisional patent application 61/439,145 filed on February 3, 2011, which is hereby incorporated by reference in its entirety.

[0002] The present invention relates generally to the field of lipid metabolism.
10 In certain aspects, it concerns methods and compositions for enhancing reverse cholesterol transport, reducing atherosclerosis, and/or lowering cholesterol involving both a Farnesoid X Receptor antagonist and a bile acid sequestrant.

BACKGROUND OF THE INVENTION

15 [0003] It has been shown that a lipid disorder, such as high blood cholesterol and triglycerides, increases the risk for atherosclerosis, and thus for heart disease, stroke, high blood pressure, and other pathological conditions. In particular, atherosclerosis is the leading cause of heart disease. Lowering the levels of cholesterol and triglycerides is a way to reduce the risk for cardiovascular diseases and conditions. While a number of drugs are being used
20 for this purpose, more Americans die from cardiovascular diseases than cancer. Therefore, there remains a need for additional therapeutics to lower cholesterol as a way to prevent and/or treat cardiovascular diseases and conditions.

SUMMARY OF THE INVENTION

[0004] Embodiments overcome a major deficiency in the art by providing
25 methods and pharmaceutical compositions related to the treatment of high cholesterol, atherosclerosis and/or cardiovascular diseases.

[0005] Methods and compositions are based in part on the finding that using a combination of a bile acid sequestrant or an inhibitor of ileal bile acid transport with a Farnesoid X Receptor (FXR) antagonist substantially increases reverse cholesterol transport.
30 Such methods and compositions are contemplated in some embodiments for use on a human subject.

[0006] Thus, embodiments generally include a method for modulating lipid metabolism comprising administering to a subject an effective amount of a bile acid sequestrant and a FXR antagonist. In some embodiments, lipids are modulated by the lowering of their levels. In particular embodiments, the lipid is cholesterol, such as Low Density Lipoprotein-Cholesterol (LDL-C). In some embodiments, the subject is determined to be in need of having lipid levels modulated. In certain embodiments, a subject is evaluated for the level of lipids. In some cases, the bile acid sequestrant and/or the FXR antagonist are administered to the subject after the subject has been evaluated for cholesterol levels. In certain embodiments, the subject has been determined to be at risk for a cardiovascular disease based on the evaluation. In some cases, the LDL-C levels are determined to be at least 100 mg/dl.

[0007] Throughout the application, the term “bile acid sequestrant” refers to a compound that is capable of sequestering bile acids and preventing their reabsorption from the gut, thus disrupting the enterohepatic circulation of bile acids and reducing bile acid levels. The term “FXR antagonist” refers to a compound that blocks or dampens agonist-mediated responses of the farnesoid X receptor, or that otherwise inhibits the function of the farnesoid X receptor. The term “FXR inhibitors” refers to FXR antagonists and methods for reducing the expression of the farnesoid X receptor. In specific embodiments the FXR antagonist directly inhibits binding to the farnesoid X receptor.

[0008] In one embodiment, the administration of a bile acid sequestrant and a FXR inhibitor is useful for increasing reverse cholesterol transport, decreasing LDL-C, and reducing the risk of atherosclerosis in a subject in need thereof.

[0009] In a further embodiment, the bile acid sequestrant and the FXR antagonist are formulated into a pharmaceutical composition when administered to a subject in need thereof.

[0010] In another embodiment, the bile acid sequestrant and FXR antagonist are administered to a human, especially a patient having atherosclerosis, cardiovascular disease, or other conditions associated with atherosclerosis or high blood cholesterol.

[0011] In some further embodiments, a bile acid sequestrant is administered to a patient in need thereof who is already given a FXR antagonist; or a FXR antagonist is administered to a patient in need thereof who is already given a bile acid sequestrant.

[0012] In a further embodiment, a bile acid sequestrant and/or a FXR antagonist are administered to a subject in need thereof intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, 5 topically, intratumorally, intramuscularly, intraperitoneally, subcutaneously, subconjunctival, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, *via* a catheter, and/or *via* a lavage.

[0013] In still further embodiments, there is a pharmaceutical composition 10 comprising a bile acid sequestrant and a FXR antagonist. Alternatively, embodiments concern a composition that is a nutritional composition or one that can be ingested.

[0014] In one embodiment, the pharmaceutical composition of a bile acid sequestrant and a FXR antagonist, further comprises a pharmaceutically acceptable carrier.

[0015] In a further embodiment, the pharmaceutical composition takes the form 15 of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain about 10% to 95% of active ingredient, preferably about 25% to about 70%.

[0016] In still further embodiments, the pharmaceutical composition comprises X amount of bile acid sequestrant and Y amount of FXR antagonist.

[0017] Furthermore, in the embodiments discussed above in the context of 20 methods and/or compositions of the invention, the bile acid sequestrant is colesevelam, cholestyramine, colestilan, colextran, BTG 511, filicol, DMP 504, GT31-104, SK&F 97426A, soystatin, or colestipol or a combination thereof.

[0018] Further, in methods and/or compositions embodiments described above, the bile acid sequestrant is a natural compound, including but not limited to saponins and 25 chitosan derivatives. The term “natural compound” refers to a compound or substance produced by a living, organism found in nature. In some embodiments, the natural compound is isolated or purified. The term “saponin”, in some embodiments, refers to amphipathic glycosides containing one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative. It will further be appreciated by those of ordinary skill in the art that 30 other saponins may also be useful in the embodiments described.

[0019] Further, in methods and/or compositions embodiments described above, ileal bile acid transport inhibitors include but are not limited to SC-435, 264W94, 2164U90, or benzothiepinines.

[0020] In methods and/or compositions embodiments described above, the FXR antagonist is a natural compound, preferably a guggulipid, a stigmaterol, a tuberatulide, lithocholate, oleanolic acid or a combination thereof. Tuberatulides include but are not limited to tuberatulide A, tuberatulide B, 2'-epi-tuberatulide B, Yezoquinolide, (R)-Sargachromenol, and (S)-Sargachromenol.

[0021] Further, in methods and/or compositions embodiments described above, the FXR antagonist is a synthetic compound. In some embodiments, the FXR antagonist is CDRI 80/574, QRX-401, or a substituted-isoxazole derivative. Substituted-isoxazole derivatives include but are not limited to 3-{2-Chloro-4-[3-(2,6-dichlorophenyl)-5-naphthalen-2-ylisoxazol-4-ylmethoxy]benzoylamino}benzoic acid and 3-{4-Biphenyl-4-yl-3-(2,6-dichlorophenyl)-isoxazol-4-ylmethoxy}-2-chlorobenzoylamino}benzoate.

[0022] In specific embodiments, methods and/or compositions include an FXR antagonist that is a guggulipid, its active component guggulsterone, or a derivative thereof, in combination with a bile acid sequestrant that is colesevelam. In certain embodiments, the colesevelam is administered as part of a diet on a regular basis. Guggulipid, its active component guggulsterone, or a derivative thereof may be given before, after, and or concurrently with the colesevelam. Embodiments concerning other FXR antagonists or bile acid sequestrants may also be similarly administered.

[0023] Furthermore, in methods and/or compositions embodiments described above, the FXR inhibitor is a FXR anti-sense and/or a FXR iRNA.

[0024] In another aspect, methods of inhibiting expression of FXR in a cell are provided. The method includes contacting the cell with a complex comprising an FXR anti-sense or FXR iRNA, wherein the iRNA-inducing agent is complementary to at least a portion of the mRNA. In another aspect of the invention, in methods and/or compositions embodiments described above, the FXR antagonist binds FXR.

[0025] Embodiments discussed in the context of methods and/or compositions of the invention may be employed with respect to any other method or composition described

herein. Thus, an embodiment pertaining to one method or composition may be applied to other methods and compositions of the invention as well.

[0026] As used herein the specification, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising", the words
5 "a" or "an" may mean one or more than one.

[0027] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." As used herein "another" may mean at least a second or more.

10 [0028] Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[0029] Throughout this application, the term "effective amount" is used to indicate that the compounds are administered at an amount sufficient to treat a condition in a
15 subject in need thereof. In some embodiments, the condition is, but is not limited to, high cholesterol, atherosclerosis, gallstones, or conditions associated therewith. In some embodiments, an "effective amount" is an amount that can reduce symptoms of the condition in a majority of subject by at least about 20% when compared to untreated subjects.

[0030] An "iRNA," as the term is used herein and in the art, encompasses RNA
20 molecules or vectors whose presence within a cell results in RNA interference and leads to reduced expression of a transcript to which the iRNA-inducing entity is targeted. Suitably, for some embodiments, delivery of an iRNA-inducing agent reduces expression of the target transcript to a level that confers a therapeutic effect. Delivery of an iRNA-inducing agent suitably reduces expression of the target transcript at least about 25%, at least about 50%, at
25 least about 80%, at least about 90%, at least about 95%, at least about 98%, at least about 99% or 100%, relative to expression of the transcript in an appropriate control cell. The percentage reduction in expression can be calculated by the following equation:

30
$$\frac{[(\text{Expression target transcript in sample} - \text{Expression of target transcript in control}) / \text{Expression target transcript in sample}] \times (-100)}{}$$

[0031] Specifically contemplated iRNA inducing agents include short interfering RNA ("siRNA"), short hairpin RNA ("shRNA"), and iRNA-inducing vectors, each of which is defined below. Selection of appropriate target sequences for iRNA may take into account factors such as synthetic considerations, avoidance of targeting unwanted transcripts, and other considerations, as described by Manoharan (2004), which is incorporated herein by reference in its entirety.

[0032] A "short, interfering RNA," or "siRNA," comprises an RNA duplex that is about 19 to about 27 base pairs in length and optionally further comprises one or two single-stranded overhangs. siRNA can be chemically synthesized, or can be transcribed *in vitro* from a DNA template, or *in vivo* from an RNA precursor. An siRNA may be formed from two RNA molecules that hybridize, or may alternatively be generated from a single RNA molecule that includes a self-hybridizing portion. It is generally preferred that free 5' ends of siRNA molecules have phosphate groups, and free 3' ends have hydroxyl groups. The duplex portion of an siRNA may, but typically does not, contain one or more unpaired nucleotides. One strand of an siRNA includes a portion that hybridizes with a target transcript. In certain embodiments of the invention, one strand of the siRNA is precisely complementary with a region of the target transcript, meaning that the siRNA hybridizes to the target transcript without a single mismatch. In other embodiments, one or more mismatches between the siRNA and the targeted portion of the target transcript may exist. In most embodiments of the invention in which perfect complementarity is not achieved, it is generally preferred that any mismatches be located at or near the siRNA termini. An siRNA used in accordance with the invention is suitably hybridizable to a target transcript and capable of inducing its degradation.

[0033] The term "short hairpin RNA" refers to an RNA molecule comprising at least two complementary portions hybridized or capable of hybridizing to form a double-stranded (duplex) structure of sufficient length to mediate RNAi (typically about 19-27 base pairs in length), and at least one single-stranded portion, typically between approximately 1 and 10 nucleotides in length, that forms a loop structure. The duplex portion may, but typically does not, contain one or more unpaired nucleotides. Not to be bound by theory, it is thought that shRNAs are precursors of siRNAs and are, in general, similarly capable of inhibiting expression of a target transcript.

[0034] As used herein, "an RNAi-inducing vector" is a vector whose presence within a cell results in transcription of one or more RNAs that self-hybridize or hybridize to each other to form an shRNA or siRNA. The term generally encompasses any construct comprising a polynucleotide operably linked to expression signal(s) so that one or more RNA molecules that hybridize or self-hybridize to form an siRNA or shRNA are transcribed when the vector is present within a cell. Thus, the vector provides a template for intracellular synthesis of the RNA-inducing agent or precursors thereof. An RNAi-inducing vector is considered to be targeted to a transcript if presence of the vector within a cell results in production of one or more RNAs that hybridize to each other or self-hybridize to form an siRNA or shRNA that is targeted to the transcript, *i.e.*, if presence of the vector within a cell results in production of one or more siRNAs or shRNAs targeted to the transcript. Genetic constructs for the delivery of iRNA molecules are described, for example, in U.S. Pat. No. 6,573,099, which is incorporated herein by reference. A further example of the use of shRNA expression plasmids to reduce gene expression *in vivo* in rats has been described by Zhang *et al.* (2003), which is also incorporated herein by reference.

[0035] In particular embodiments, the iRNA-inducing agent is suitably stabilized by chemical modification. For example, iRNAs may be crosslinked to increase half-life in the body. For example, a 3' OH terminus of one of the strands of double-stranded siRNA can be modified, or the two strands can be crosslinked and modified at the 3' OH terminus. The siRNA derivative can contain a single crosslink or multiple crosslinks. Additionally, stability may be enhanced by including nucleotide analogs at one or more free ends in order to reduce digestion, *e.g.*, by exonucleases. The inclusion of deoxynucleotides, *e.g.*, pyrimidines such as deoxythymidines at one or more free ends, may serve this purpose. It will further be appreciated by those of ordinary skill in the art that effective siRNA agents for use in accordance with certain embodiments of the invention may comprise one or more moieties that are not nucleotides or nucleotide analogs. Further suitable chemical modifications are described by, *e.g.*, Manoharan (2004), incorporated herein by reference in its entirety.

[0036] In a further embodiment, micro RNA ("miRNA") may be delivered to cells according to the invention. As used herein, "micro RNA" or "miRNA" refers to single-stranded, non-coding RNA molecules of about 19 to about 27 base pairs which regulate gene expression in a sequence specific manner.

[0037] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0038] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. Embodiments may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0039] FIG. 1 illustrates the effects of colesvelam on reverse cholesterol transport in wild type mice and FXR knockout mice.

DETAILED DESCRIPTION OF THE INVENTION

[0040] The Farnesoid X Receptor (FXR), first encoded as an ‘orphan’ nuclear receptor in 1995, is now recognized as an important modulator of bile and lipid metabolism. FXR, which is also referred to as the “bile acid receptor,” protects cells from bile acid toxicity by up regulating genes that export bile acids from cells such as bile salt export pump (BSEP) and repressing genes involved with bile acid synthesis (Caudel *et al.*, 2005; Lee *et al.*, 2006; Cariou and Staels, 2007).

[0041] FXR knockout mice (FXR^{-/-}) have been generated and compared to wild-type mice. The knockout mice had increased plasma triglyceride and HDL-cholesterol levels (Lambert *et al.*, 2003; Sinal *et al.*, 2000; Hanniman *et al.*, 2005; Zhang *et al.*, 2006)). Bile acids such as chenodeoxycholic acid, which is a potent FXR agonist, lowers triglycerides in humans; bile acid sequestrants, which reduce hepatic bile acid levels, increase triglyceride levels (Davidson, 2006).

[0042] The synthetic FXR agonist GW4064 has been shown to lower triglyceride levels in animal models (Zhang *et al.*, 2006). FXR affects lipid metabolism largely by down regulating SREBP-1c gene expression (Caudel *et al.*, 2005; Lee *et al.*,

2006). This is mediated in part through the FXR-mediated induction of a small heterodimer partner (SHP), an atypical member of the nuclear receptor superfamily because it lacks a DNA-binding domain, which can inactivate the liver X receptor- α (LXR α), a positive regulator of the SREBP-1c gene (Watanabe *et al.*, 2004). In addition, FXR induces apoC-II
5 expression (Kast *et al.*, 2001) and represses apoC-III expression (Claudel *et al.*, 2003), which activate and inhibit lipoprotein lipase respectively. Therefore, induction of the FXR target genes would result in marked reduction in plasma triglycerides by repressing triglyceride synthesis and increasing triglyceride clearance.

[0043] FXR upregulation appears to have a negative effect HDL metabolism
10 and on reverse cholesterol transport (RCT), the process by which cholesterol in peripheral tissues is effluxed to HDL for transport to the liver. Activation of FXR results in decreased expression of apoA-I (Claudel *et al.*, 2002), the major apoprotein in HDL, and hepatic lipase (Sirvent *et al.*, 2004), which is involved in the catabolism of HDL. FXR inhibits LXR α activity, at least in part by induction of SHP expression (Watanabe *et al.*, 2004). LXR α
15 inhibition would decrease expression of ABCA-1 (Kalaany and Mangelsdorf, 2006), which is essential for the efflux of free cholesterol into nascent HDL from macrophages (Zannis *et al.*, 2006). Since HDL-C is a primary source of cholesterol for bile acid synthesis, FXR appears to downregulate reverse cholesterol transport by repressing the expression of hepatic SR-BI (Malerod *et al.*, 2005), a receptor that mediates selective uptake of cholesteryl esters from
20 HDL. FXR also downregulates ApoA-I and ABCA-1 which have all been shown to be necessary for RCT (Zhang *et al.*, 2003; Wang *et al.*, 2007; Zhang *et al.*, 2005).

[0044] Colesevelam, a bile acid sequestrant, is likely to decrease FXR activity by reducing bile acid levels in hepatocytes by interrupting enterohepatic recirculation. Downregulation of FXR may potentially lead to increased synthesis of apoA-I, ABCA1 and
25 hepatic lipase, which should significantly increase HDL levels. However, FXR inhibition also increases the expression of SR-BI, phospholipid transfer protein (PLTP), and CETP (probably mediated by reduced inhibition of LXR), which would decrease HDL levels. The net effect of colesevelam on plasma HDL levels in humans is a modest increase, but there could be a marked increase in RCT. In a cholesterol-fed rabbit model of atherosclerosis, we
30 were able to show that colesevelam increased expression of LXR and its target genes and markedly reduced atherosclerosis compared to controls (Szapary *et al.*, 2003). However, colesevelam markedly reduced plasma cholesterol levels in these animals and, therefore, it

was difficult to assess the effects of LXR activation of atherosclerosis independent of the cholesterol-lowering effects.

[0045] It has also been shown that FXR antagonists such as the natural compound guggulipid adversely affect LCL-C (Szapary *et al.*, 2003).

5 [0046] Pharmaceutical compositions described herein comprise an effective amount of a bile acid sequestrant and/or a FXR antagonist and/or additional agent dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, such
10 as, for example, a human, as appropriate. The preparation of a pharmaceutical composition that contains at least a bile acid sequestrant and/or a FXR antagonist or additional active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference. Moreover, for animal (*e.g.*, human) administration, it
15 will be understood that preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biological Standards.

[0047] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (*e.g.*, antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts,
20 preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329, incorporated herein by reference). Except insofar as any conventional carrier is incompatible
25 with the active ingredient, its use in the pharmaceutical compositions is contemplated.

[0048] Embodiments may comprise different types of carriers depending on whether it is to be administered in solid, liquid or aerosol form, and whether it need to be sterile for such routes of administration as injection. The present invention can be administered intravenously, intradermally, transdermally, intrathecally, intraarterially,
30 intraperitoneally, intranasally, intravaginally, intrarectally, topically, intramuscularly, subcutaneously, mucosally, orally, topically, locally, inhalation (*e.g.*, aerosol inhalation),

injection, infusion, continuous infusion, localized perfusion bathing target cells directly, via a catheter, via a lavage, in cremes, in lipid compositions (*e.g.*, liposomes), or by other method or any combination of the forgoing as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference).

[0049] Compounds may be formulated into a composition in a free base, neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts, *e.g.*, those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric or mandelic acid. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine or procaine. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as formulated for parenteral administrations such as injectable solutions, or aerosols for delivery to the lungs, or formulated for alimentary administrations such as drug release capsules and the like.

[0050] Methods and compositions that are suitable for administration may be provided in a pharmaceutically acceptable carrier with or without an inert diluent. The carrier should be assimilable and includes liquid, semi-solid, *i.e.*, pastes, or solid carriers. Except insofar as any conventional media, agent, diluent or carrier is detrimental to the recipient or to the therapeutic effectiveness of a the composition contained therein, its use in administrable composition for use in practicing the methods of the present invention is appropriate. Examples of carriers or diluents include fats, oils, water, saline solutions, lipids, liposomes, resins, binders, fillers and the like, or combinations thereof. The composition may also comprise various antioxidants to retard oxidation of one or more component. Additionally, the prevention of the action of microorganisms can be brought about by preservatives such as various antibacterial and antifungal agents, including but not limited to parabens (*e.g.*, methylparabens, propylparabens), chlorobutanol, phenol, sorbic acid, thimerosal or combinations thereof.

[0051] In some embodiments, the composition is combined with the carrier in any convenient and practical manner, *i.e.*, by solution, suspension, emulsification, admixture,

encapsulation, absorption and the like. Such procedures are routine for those skilled in the art.

[0052] In a specific embodiment, the composition is combined or mixed thoroughly with a semi-solid or solid carrier. The mixing can be carried out in any convenient manner such as grinding. Stabilizing agents can be also added in the mixing process in order to protect the composition from loss of therapeutic activity, *i.e.*, denaturation in the stomach. Examples of stabilizers for use in the composition include buffers, amino acids such as glycine and lysine, carbohydrates such as dextrose, mannose, galactose, fructose, lactose, sucrose, maltose, sorbitol, mannitol, *etc.*

[0053] In further embodiments, methods may concern the use of a pharmaceutical lipid vehicle compositions that include a bile acid sequestrant and a FXR antagonist, one or more lipids, and an aqueous solvent. As used herein, the term "lipid" will be defined to include any of a broad range of substances that is characteristically insoluble in water and extractable with an organic solvent. This broad class of compounds is well known to those of skill in the art, and as the term "lipid" is used herein, it is not limited to any particular structure. Examples include compounds which contain long-chain aliphatic hydrocarbons and their derivatives. A lipid may be naturally occurring or synthetic (*i.e.*, designed or produced by man). However, a lipid is usually a biological substance. Biological lipids are well known in the art, and include for example, neutral fats, phospholipids, phosphoglycerides, steroids, terpenes, lysolipids, glycosphingolipids, glycolipids, sulphatides, lipids with ether and ester-linked fatty acids and polymerizable lipids, and combinations thereof. Of course, compounds other than those specifically described herein that are understood by one of skill in the art as lipids are also encompassed by the compositions and methods described herein.

[0054] One of ordinary skill in the art would be familiar with the range of techniques that can be employed for dispersing a composition in a lipid vehicle. For example, the composition comprising a bile acid sequestrant and a FXR antagonist may be dispersed in a solution containing a lipid, dissolved with a lipid, emulsified with a lipid, mixed with a lipid, combined with a lipid, covalently bonded to a lipid, contained as a suspension in a lipid, contained or complexed with a micelle or liposome, or otherwise associated with a lipid or lipid structure by any means known to those of ordinary skill in the art. The dispersion may or may not result in the formation of liposomes.

[0055] The actual dosage amount of a composition that is administered to an animal patient can be determined by physical and physiological factors such as body weight, severity of condition, the type of disease being treated, previous or concurrent therapeutic interventions, idiopathy of the patient and on the route of administration. Depending upon the dosage and the route of administration, the number of administrations of a preferred dosage and/or an effective amount may vary according to the response of the subject. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject.

[0056] In certain embodiments, pharmaceutical compositions may comprise, for example, at least about 0.1% of an active compound. In other embodiments, the an active compound may comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, about 2%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, and any range derivable therein. Naturally, the amount of active compound(s) in each therapeutically useful composition may be prepared in such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

[0057] In other non-limiting examples, a dose may also comprise from about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 mg/kg/body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg/body weight to about 100 mg/kg/body weight, about 5

microgram/kg/body weight to about 500 milligram/kg/body weight, *etc.*, can be administered, based on the numbers described above.

[0058] In certain embodiments, one of more agents is administered as part of a diet. In certain embodiments, a subject is administered or intakes about, at least about, or at most about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 15.5, 16.0, 16.5, 17.0, 17.5, 18.0, 18.5, 19.0, 19.5, 20.0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, 265, 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 325, 330, 335, 340, 345, 350, 355, 360, 365, 370, 375, 380, 385, 390, 395, 400, 410, 420, 425, 430, 440, 441, 450, 460, 470, 475, 480, 490, 500, 510, 520, 525, 530, 540, 550, 560, 570, 575, 580, 590, 600, 610, 620, 625, 630, 640, 650, 660, 670, 675, 680, 690, 700, 710, 720, 725, 730, 740, 750, 760, 770, 775, 780, 790, 800, 810, 820, 825, 830, 840, 850, 860, 870, 875, 880, 890, 900, 910, 920, 925, 930, 940, 950, 960, 970, 975, 980, 990, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 6000, 7000, 8000, 9000, 10000 milligrams (mg) or micrograms (mcg) or $\mu\text{g/kg}$ or micrograms/kg/day or micrograms/kg/hour or mg/kg or milligrams/kg/day, or milligrams/kg/hour, and any range derivable therein. In certain embodiments, the agent is about, at most about, or at least about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7,

9.8, 9.9, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 15.5, 16.0, 16.5, 17.0, 17.5, 18.0, 18.5, 19.0, 19.5, 20.0 percent of a patient's diet (by weight of ingested food), and any range derivable therein. In further embodiments, the combined amount of the antagonist and sequestrant totals an amount recited above or anywhere else in the disclosure.

5 **[0059]** In certain embodiments, one or more agents is a nutritional or dietary supplement. In further embodiments, it is provided in powder, pill, or liquid format.

[0060] In some embodiments, the compositions of the present invention are formulated to be administered via an alimentary route. Alimentary routes include all possible routes of administration in which the composition is in direct contact with the alimentary
10 tract. Specifically, the pharmaceutical compositions disclosed herein may be administered orally, buccally, rectally, or sublingually. As such, these compositions may be formulated with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard- or soft- shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

15 **[0061]** In certain embodiments, the active compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and the like (Mathiowitz *et al.*, 1997; Hwang *et al.*, 1998; U.S. Pat. Nos. 5,641,515; 5,580,579 and 5,792, 451, each specifically incorporated herein by reference in its entirety). The tablets, troches, pills, capsules and the like may also contain the
20 following: a binder, such as, for example, gum tragacanth, acacia, cornstarch, gelatin or combinations thereof; an excipient, such as, for example, dicalcium phosphate, mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate or combinations thereof; a disintegrating agent, such as, for example, corn starch, potato starch, alginic acid or combinations thereof; a lubricant, such as, for example, magnesium stearate; a
25 sweetening agent, such as, for example, sucrose, lactose, saccharin or combinations thereof; a flavoring agent, such as, for example peppermint, oil of wintergreen, cherry flavoring, orange flavoring, *etc.* When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets,
30 pills, or capsules may be coated with shellac, sugar, or both. When the dosage form is a capsule, it may contain, in addition to materials of the above type, carriers such as a liquid carrier. Gelatin capsules, tablets, or pills may be enterically coated. Enteric coatings prevent

denaturation of the composition in the stomach or upper bowel where the pH is acidic. See, *e.g.*, U.S. Pat. No. 5,629,001. Upon reaching the small intestines, the basic pH therein dissolves the coating and permits the composition to be released and absorbed by specialized cells, *e.g.*, epithelial enterocytes and Peyer's patch M cells. A syrup or elixir may contain the active compound sucrose as a sweetening agent methyl and propylparabens as preservatives, a dye and flavoring, such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations.

[0062] For oral administration the compositions of the present invention may alternatively be incorporated with one or more excipients in the form of a mouthwash, dentifrice, buccal tablet, oral spray, or sublingual orally- administered formulation. For example, a mouthwash may be prepared incorporating the active ingredient in the required amount in an appropriate solvent, such as a sodium borate solution (Dobell's Solution). Alternatively, the active ingredient may be incorporated into an oral solution such as one containing sodium borate, glycerin and potassium bicarbonate, or dispersed in a dentifrice, or added in a therapeutically- effective amount to a composition that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants. Alternatively the compositions may be fashioned into a tablet or solution form that may be placed under the tongue or otherwise dissolved in the mouth.

[0063] Additional formulations which are suitable for other modes of alimentary administration include suppositories. Suppositories are solid dosage forms of various weights and shapes, usually medicated, for insertion into the rectum. After insertion, suppositories soften, melt or dissolve in the cavity fluids. In general, for suppositories, traditional carriers may include, for example, polyalkylene glycols, triglycerides or combinations thereof. In certain embodiments, suppositories may be formed from mixtures containing, for example, the active ingredient in the range of about 0.5% to about 10%, and preferably about 1% to about 2%.

[0064] In further embodiments, the composition of the present invention may be administered via a parenteral route. As used herein, the term "parenteral" includes routes that bypass the alimentary tract. Specifically, the pharmaceutical compositions disclosed herein may be administered for example, but not limited to intravenously, intradermally,

intramuscularly, intraarterially, intrathecally, subcutaneous, or intraperitoneally U.S. Pat. Nos. 6,537,514, 6,613,308, 5,466,468, 5,543,158; 5,641,515; and 5,399,363 (each specifically incorporated herein by reference in its entirety).

[0065] Solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (U.S. Patent 5,466,468, specifically incorporated herein by reference in its entirety). In all cases the form must be sterile and must be fluid to the extent that easy injectability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*i.e.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0066] For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous, and intraperitoneal administration. In this connection, sterile aqueous media that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in isotonic NaCl solution and either added hypodermoclysis fluid or injected at the proposed site of

infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human
5 administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

[0067] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally,
10 dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired
15 ingredient from a previously sterile-filtered solution thereof. A powdered composition is combined with a liquid carrier such as, *e.g.*, water or a saline solution, with or without a stabilizing agent.

[0068] In other embodiments, the active compound, *i.e.* the bile acid sequestrant and/or the FXR antagonist, may be formulated for administration via various miscellaneous
20 routes, for example, topical (*i.e.*, transdermal) administration, mucosal administration (intranasal, vaginal, *etc.*) and/or inhalation.

[0069] Pharmaceutical compositions for topical administration may include the active compound formulated for a medicated application such as an ointment, paste, cream or powder. Ointments include all oleaginous, adsorption, emulsion and water-solubly based
25 compositions for topical application, while creams and lotions are those compositions that include an emulsion base only. Topically administered medications may contain a penetration enhancer to facilitate adsorption of the active ingredients through the skin. Suitable penetration enhancers include glycerin, alcohols, alkyl methyl sulfoxides, pyrrolidones and luarocapram. Possible bases for compositions for topical application
30 include polyethylene glycol, lanolin, cold cream and petrolatum as well as any other suitable absorption, emulsion or water-soluble ointment base. Topical preparations may also include emulsifiers, gelling agents, and antimicrobial preservatives as necessary to preserve the active

ingredient and provide for a homogenous mixture. Transdermal administration of the present invention may also comprise the use of a "patch". For example, the patch may supply one or more active substances at a predetermined rate and in a continuous manner over a fixed period of time.

5 **[0070]** In certain embodiments, the pharmaceutical compositions may be delivered by eye drops, intranasal sprays, inhalation, and/or other aerosol delivery vehicles. Methods for delivering compositions directly to the lungs via nasal aerosol sprays has been described *e.g.*, in U.S. Pat. Nos. 5,756,353 and 5,804,212 (each specifically incorporated herein by reference in its entirety). Likewise, the delivery of drugs using intranasal
10 microparticle resins (Takenaga *et al.*, 1998) and lysophosphatidyl-glycerol compounds (U.S. Pat. No. 5,725,871, specifically incorporated herein by reference in its entirety) are also well-known in the pharmaceutical arts. Likewise, transmucosal drug delivery in the form of a polytetrafluoroethylene support matrix is described in U.S. Pat. No. 5,780,045 (specifically incorporated herein by reference in its entirety).

15 **[0071]** The term aerosol refers to a colloidal system of finely divided solid of liquid particles dispersed in a liquefied or pressurized gas propellant. The typical aerosol of the present invention for inhalation will consist of a suspension of active ingredients in liquid propellant or a mixture of liquid propellant and a suitable solvent. Suitable propellants include hydrocarbons and hydrocarbon ethers. Suitable containers will vary according to the
20 pressure requirements of the propellant. Administration of the aerosol will vary according to subject's age, weight and the severity and response of the symptoms.

[0072] Embodiments also concern kits, such as therapeutic kits. For example, a kit may comprise one or more pharmaceutical composition as described herein and optionally instructions for their use. Kits may also comprise one or more devices for accomplishing
25 administration of such compositions. For example, a subject kit may comprise a pharmaceutical composition and catheter for accomplishing direct intraarterial injection of the composition into a cancerous tumor. In other embodiments, a subject kit may comprise pre-filled ampoules of a protein isoform specific antibody construct, optionally formulated as a pharmaceutical, or lyophilized, for use with a delivery device.

30 **[0073]** Kits may comprise a container with a label. Suitable containers include, for example, bottles, vials, and test tubes. The containers may be formed from a variety of

materials such as glass or plastic. The container may hold a composition which includes an antibody that is effective for therapeutic or non-therapeutic applications, such as described above. The label on the container may indicate that the composition is used for a specific therapy or non-therapeutic application, and may also indicate directions for either *in vivo* or *in vitro* use, such as those described above. The kit of the invention will typically comprise the container described above and one or more other containers comprising materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

Examples

[0074] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1

[0075] A novel assay was previously developed to assess the effects of various therapeutic approaches on RCT in murine models (Zhang *et al.*, 2003; Wang *et al.*, 2007; zhang *et al.*, 2005). This assay involves injecting ³H-cholesterol-labeled macrophages (*i.e.*, the murine macrophage cell line J774) intraperitoneally into mice. The radiolabeled cholesterol in the macrophages is effluxed, presumably to nascent HDL particles. Cholesterol efflux from the macrophages is detected by the appearance of the radiolabeled cholesterol in the plasma, liver, and feces. This technique was utilized to show that an LXR agonist increases the rate of RCT from macrophages to feces *in vivo*.

[0076] The effect of colesvelam on RCT utilizing this novel assay in different mouse models was evaluated to determine the mechanism(s) by which bile acid sequestrants may affect RCT. The effects of colesvelam on RCT in wild mice and FXR knockout mice were evaluated. In addition, since the presence of CETP appears to affect the rate of RCT, the effects of colesvelam in transgenic mice with human apoB-100 and CETP (apoB/CETP

double transgenic mice) were also evaluated. These studies determined the effect of colesevelam on RCT and the potential role of FXR in mediating these effects.

[0077] As shown in FIG. 1, wild type mice and FXR knockout mice were fed with control diet or diet with colesevelam for 2 weeks prior to intraperitoneal injection with J774 cells loaded with acetylated LDL and ³H-cholesterol. The feces was collected at 48 hours and radioactivity in the feces were determined. The change in radioactivity in the feces reflects an increase in reverse cholesterol transport (RCT).

[0078] Colesevelam increased the radioactivity in the feces, which represents the H3 labeled cholesterol in the macrophages effluxed into HDL, taken up by the hepatocyte or intestinal cells and excreted into the lumen. In the wild type mice, colesevelam increased uptake in the feces of H3 labeled cholesterol by 1.8 fold, which is similar to the effects of ezetimibe (a cholesterol absorption inhibitor or by decreasing cholesterol absorption in the animal model). ApoB/CETP transgenic mice displayed a 4.5 fold increase in the radioactivity in the feces with colesevelam treated animals compared to controls. This probably reflects increased RCT in these animals due to the presence of CETP, with more cholesterol in bile delivered to the intestine which is then inhibited from reabsorption by colesevelam.

[0079] Surprisingly, in contrast to our initial hypothesis that the effect of colesevelam on RCT would be markedly reduced in FXR knockout mice, we instead observed the opposite treatment of FXR knockout mice led to a 60 fold increase in radioactivity in the feces. Therefore, the combination of a bile acid sequestrant with antagonism to FXR appears to markedly increase RCT. This level of increase (60 fold) in RCT has never been shown before in other approaches to increase RCT, including transgenic apoA1 or LXR agonists (Zhang *et al.*, 2003).

[0080] These results indicate that FXR antagonism will result in increased bile acid synthesis, which requires a net flux of cholesterol into the liver to synthesize the bile. The increased quantity of bile is excreted from the liver into the intestine, and in the FXR knockout model, the bile would be markedly reabsorbed. However, due to the bile acid sequestrants, the bile is not reabsorbed, and instead remains in the feces for excretion. Therefore the combination of FXR antagonism and bile acid binding promotes significant removal of cholesterol/bile acids from the body, potentially enhancing both RCT and LDL-C clearance through the upregulation of the LDL receptors.

EXAMPLE 2**Experimental Groups:**

[0081] The apoB/CETP transgenic murine model will be used to examine the combined effect of colessevelam and FXR antagonists on macrophage RCT. In this murine model, colessevelam treatment was shown to increase the amount of radioactivity in the feces by 4.5 fold, which is 2.5 times more than in wild type mice. Upon observation of increased RCT in animals treated with colessevelam and Guggulipid, the assay will be repeated in the absence of colessevelam to confirm that co-treatment is necessary for the enhancement of RCT. Thus, the treatment groups will be:

1. Control diet with or without colessevelam in FXR^{-/-} mice
2. Colessevelam diet with or without Guggulipid (guggulsterone) in apoB/CETPtg mice
3. Control diet with or without stigmasterol in apoB/CETPtg mice

[0082] Guggulipid is a natural product of the gum resin of the tree *Commiphora mukul* that has been used to lower plasma lipids in humans (Uriza and Moore, 2003) and mice (Urizar *et al.*, 2002). Its active component is guggulsterone and has been shown to be an antagonist of bile acid activation of FXR (Urizar *et al.*, 2002; Wu *et al.*, 2002). However it may also activate other steroid receptors such as mineralcorticoid receptor (Burris *et al.*, 2005).

Drug treatment:

[0083] Each group will have 5 animals and each group will be studied twice (total number per group is 10). At 10 weeks of age, the mice will be switched to the control or drug-containing diets for approximately 2 weeks. The control diet is a powdered cereal based diet (Harlan Teklad 2916). Colessevelam will be mixed into the diet to a final concentration of 2% and guggulsterone at 0.05% (100 mg/kg body weight) (Urizar *et al.*, 2002). After 15 days on diet, the mice will be subjected to the in vivo RCT assay described below.

In vivo RCT assay:

[0084] J774 cells will be grown in RPMI media that is radiolabeled by incubation with 5 μ Ci 3 H-cholesterol and loaded with cholesterol by incubation with 50 μ g/ml acetylated LDL for 48 hours, followed by a 16 hour equilibration period in DMEM with 0.2% BSA. The cells will be extensively washed and total radioactivity in the cells will be determined by scintillation counting. Between 8-10 x 10⁶ cholesterol-loaded cells in 1 ml will be injected into the peritoneal cavity of the mice. The mice will be housed individually and feces will be collected 48 hours post-injection. Blood (50 μ l) is obtained at 6, 24 and 48 hours after injection. All samples will be stored at -20°C until analyzed. At 48 hours, the mice will be perfused transcardially with cold PBS for 2 minutes. The liver will be removed and flash frozen in liquid nitrogen or stored at 4°C in RNALater. The intestine will also removed, cells will be scraped from the intestinal lumen and stored as described for the liver.

[0085] The lipids in the feces and liver will be extracted and amount of radioactivity expressed as a % of the initial total radioactivity injected into the mice. Aliquots of the plasma will be counted in a scintillation counter and counts expressed as % total injected radioactivity per ml plasma.

Analysis of plasma:

[0086] Plasma levels of cholesterol and triglycerides will be measured enzymatically using kits from Roche Diagnostics. Since FXR agonists also influence glucose homeostasis, plasma glucose levels are also measured.

Real Time PCR:

[0087] Total RNA will be isolated from the liver and intestine by the Trizol method. Real time PCR will be used to measure hepatic transcript levels for FXR target genes (CYP7A1, BSEP, SHP, SR-BI) and genes involved in HDL metabolism (ABCA-1, SR-BI). The intestinal transcripts for the bile acid transporters and FGF-15 will be measured.

Statistical analysis:

[0088] Results will expressed as mean $\pm$ SEM. Data is analyzed using StatView 5.0.1 software by ANOVA analysis. The significance level will be set at $p < 0.05$.

Expected Results:

[0089] Colesevelam enhances the removal of cholesterol from lipid laden macrophages in FXR KO mice as well as in conjunction with the FXR antagonists guggulipid and stigmasterol. In addition, due to FXR antagonism of guggulipid and/or stigmasterol the cholesterol lowering effect of colesevelam is synergistically enhanced with combination as compared to monotherapy.

* * *

[0090] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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CLAIMS

1. A method for modulating lipid metabolism in a subject comprising administering to the subject an effective amount of at least two agents: (a) a bile acid sequestrant; and (b) an
5 FXR antagonist that is a guggulipid, a stigmasterol, a tuberatolide, lithocholate, oleanolic acid, CDRI 80/574, QRX-401, or a substituted-isoxazole derivative, or a combination thereof.
2. The method of claim 1, wherein said subject is a patient diagnosed as having a cardiovascular disease.
10
3. The method of claim 2, wherein the subject is a patient diagnosed as having atherosclerosis.
4. The method of claim 1 wherein the subject is a patient with gallstones.
15
5. The method of claim 1, wherein the two agents are administered to the subject intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, intramuscularly, intraperitoneally, subcutaneously, subconjunctival, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, *via* a catheter, and/or *via* a lavage.
20
6. The method of claim 1, wherein the subject ingests the bile acid sequestrant and the FXR antagonist in one or more compositions.
25
7. The method of claim 6, wherein the one or more compositions takes the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain about 10% to about 95% of active ingredient.
30
8. The method of claim 1, wherein the subject is administered the bile acid sequestrant and the FXR antagonist at the same time.

9. The method of claim 1, wherein the subject is administered the bile acid sequestrant prior to being administered the FXR antagonist.
10. The method of claim 1, wherein the bile acid sequestrant is colestevlam.
- 5 11. The method of claim 1, wherein the bile acid sequestrant is cholestyramine.
12. The method of claim 1, wherein the bile acid sequestrant is colestipol.
- 10 13. The method of claim 1, wherein the FXR antagonist is guggulipid.
14. The method of claim 1, wherein the substituted-isoxazole derivative is 3-{2-Chloro-4-[3-(2,6-dichlorophenyl)-5-naphthalen-2-ylisoxazol-4-ylmethoxy]benzoylamino}benzoic acid or 3-{4-Biphenyl-4-yl-3-(2,6-dichlorophenyl)-isoxazol-4-ylmethoxy]-2
- 15 chlorobenzoylamino}benzoate.
15. A method for modulating lipid metabolism in a subject in need thereof comprising administering an effective amount of a) a bile acid sequestrant; and b) a Farnesoid X Receptor (FXR) antagonist to said subject.
- 20 16. The method of claim 15, wherein the modulation of lipid metabolism comprises increasing reverse cholesterol transport, decreasing Low-Density Lipoprotein Cholesterol (LDL-C) or reducing the risk of atherosclerosis, or a combination thereof.
- 25 17. The method of claim 15, wherein said subject is a human.
18. The method of claim 17, wherein said subject is a patient having a cardiovascular disease.
- 30 19. The method of claim 18, wherein said cardiovascular disease associates with atherosclerosis.
20. The method of claim 19, wherein said subject is a patient having atherosclerosis.

21. The method of claim 19, wherein said subject is a patient having gallstones.
22. The method of claim 15, wherein said bile acid sequestrant and FXR antagonist are administered to the subject intravenously, intradermally, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, intramuscularly, intraperitoneally, subcutaneously, subconjunctival, intravesicularly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, *via* a catheter, and/or *via* a lavage.
23. The method of claim 15, wherein the subject is administered the bile acid sequestrant and the FXR antagonist in a pharmaceutical or nutritional composition.
24. The method of claim 23, wherein the pharmaceutical composition takes the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain about 10% to about 95% of active ingredient.
25. The method of claim 15, wherein the subject is administered with the bile acid sequestrant and the FXR antagonist at the same time.
26. The method of claim 15, wherein the subject is administered with an amount of bile acid sequestrant and an amount of FXR antagonist.
27. The method of claim 15, wherein the bile acid sequestrant is colesevelam, cholestyramine, colestipol, or a saponin, or a combination thereof.
28. The method of claim 27, wherein the bile acid sequestrant is colesevelam.
29. The method of claim 27, wherein the bile acid sequestrant is cholestyramine.
30. The method of claim 27, wherein the bile acid sequestrant is colestipol.

31. The method of claim 15, wherein the FXR antagonist is a natural compound, wherein the natural compound is a compound or substance isolated from or produced by a living organism found in nature.
- 5 32. The method of claim 15, wherein the FXR antagonist is a synthetic compound.
33. The method of claim 15, wherein the FXR antagonist is a FXR anti-sense RNA or a FXR iRNA or a combination thereof.
- 10 34. The method of claim 31, wherein the FXR antagonist is a guggulipid, a stigmasterol, a tuberatulide, lithocholate, oleanolic acid or a combination thereof.
35. The method of claim 15, wherein the FXR antagonist is guggulipid, stigmasterol, a FXR anti-sense RNA, or a FXR iRNA, or a combination thereof.
- 15 36. The method of claim 32, wherein the FXR antagonist is CDRI 80/574, QRX-401, or a substituted-isoxazole derivative.
37. The method of claim 32, wherein the substituted-isoxazole derivative is 3-{2-Chloro-
20 4-[3-(2,6-dichlorophenyl)-5-naphthalen-2-ylisoxazol-4-ylmethoxy]benzoylamino}benzoic
acid or 3-{4-Biphenyl-4-yl-3-(2,6-dichlorophenyl)-isoxazol-4-ylmethoxy}-2
chlorobenzoylamino}benzoate.
38. The method of claim 33, wherein the FXR antagonist is a FXR anti-sense RNA.
- 25 39. The method of claim 33, wherein the FXR antagonist is a FXR iRNA.
40. The method of claim 34, wherein the FXR antagonist is guggulipid.
- 30 41. The method of claim 34, wherein the FXR antagonist is stigmasterol.
42. The method of claim 15, wherein the bile acid sequestrant is saponin and the FXR antagonist is guggulipid.

43. The method of claim 15, further comprising a pharmaceutically acceptable carrier.

44. The method of claim 15, wherein the FXR antagonist binds FXR.

5 45. A method for modulating lipid metabolism in a patient in need thereof comprising administering an effective amount of a bile acid sequestrant, wherein said patient has already been administered a FXR antagonist.

10 46. A method for modulating lipid metabolism in a patient in need thereof comprising administering an effective amount of a FXR antagonist following administration of a bile acid sequestrant.

15 47. A method for increasing reverse cholesterol transport in a subject in need thereof comprising administering an effective amount of a) a bile acid sequestrant; and b) a Farnesoid X Receptor (FXR) antagonist to the subject.

20 48. A method for decreasing LDL-C in a subject in need thereof comprising administering an effective amount of a) a bile acid sequestrant; and b) a Farnesoid X Receptor (FXR) antagonist to the subject.

49. A method for reducing the risk of atherosclerosis in a subject in need thereof comprising administering an effective amount of a) a bile acid sequestrant; and b) a Farnesoid X Receptor (FXR) antagonist to the subject.

25 50. A pharmaceutical composition comprising a) a bile acid sequestrant; and b) a Farnesoid X Receptor (FXR) antagonist.

51. The pharmaceutical composition of claim 50, further comprising a pharmaceutically acceptable carrier.

30 52. The pharmaceutical composition of claim 50, taking the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain about 10% to about 95% of active ingredient, preferably about 25% to about 70%.

53. The pharmaceutical composition of claim 50, containing an amount of bile acid sequestrant and an amount of FXR antagonist.

54. The pharmaceutical composition of claim 50, wherein the bile acid sequestrant is colesevelam, cholestyramine, or colestipol or a combination thereof.

55. The pharmaceutical composition of claim 54, wherein the bile acid sequestrant is colesevelam.

56. The pharmaceutical composition of claim 54, wherein the bile acid sequestrant is cholestyramine.

57. The pharmaceutical composition of claim 54, wherein the bile acid sequestrant is colestipol.

58. The pharmaceutical composition of claim 50, wherein the FXR antagonist is a natural compound.

59. The pharmaceutical composition of claim 50, wherein the FXR antagonist is a synthetic compound.

60. The pharmaceutical composition of claim 50, wherein the FXR antagonist is a FXR anti-sense RNA or a FXR iRNA or a combination thereof.

61. The pharmaceutical composition of claim 58, wherein the FXR antagonist is guggulipid or stigmasterol, or a combination thereof.

62. The pharmaceutical composition of claim 50, wherein the FXR antagonist is guggulipid, stigmasterol, a FXR anti-sense RNA, or a FXR iRNA, or a combination thereof.

63. The pharmaceutical composition of claim 60, wherein the FXR antagonist is a FXR anti-sense RNA.

64. The pharmaceutical composition of claim 60, wherein the FXR antagonist is a FXR iRNA.

5 65. The pharmaceutical composition of claim 61 wherein the FXR antagonist is guggulipid.

66. The pharmaceutical composition of claim 61, wherein the FXR antagonist is stigmasterol.

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In Vivo Macrophage Reverse Cholesterol Transport

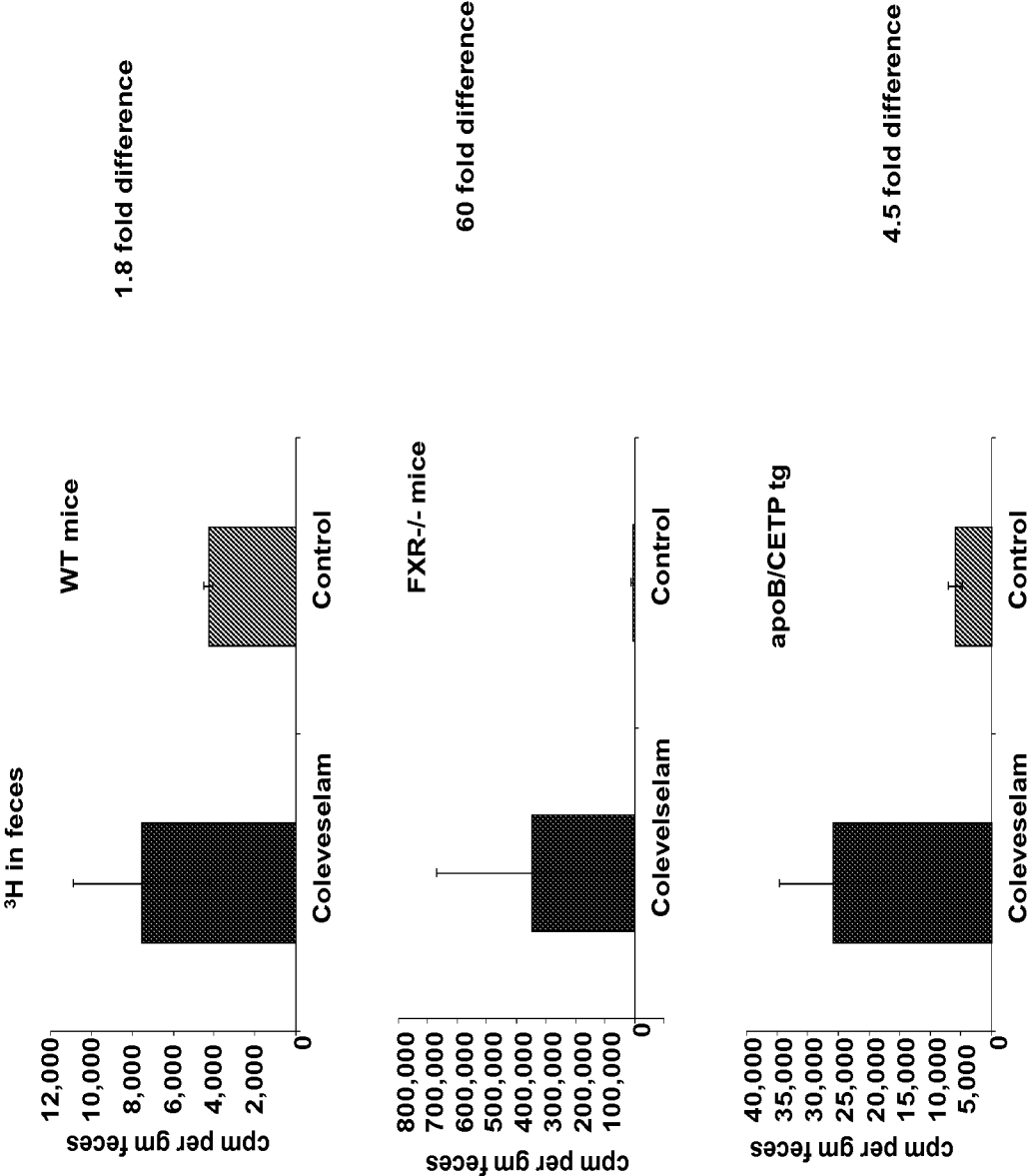


FIGURE 1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/23742

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/00 (2012.01)

USPC - 514/7.41

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A61K 38/00 (2012.01)

USPC - 514/7.41

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/7.1;424/139.1,528 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PubWest, Google Patent, Google Scholar, WIPO (stigmasterol, benzoate, RNA, iRNA, LDL, atherosclerosis, colestipol, guggulipid, colessevelam, cardiovascular, gallstones, FXR, antagonist, sequest, bile, acid, oleanolic, lipid, metabolism, farnesoid, guggulipid, saponin, siRNA, anti-sense, isoxazole, derivatives, benzoic, dichlorophenyl)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 2008/0132519 A1 (MARTIN, et al.) 05 June 2008 (05.06.2008) entire document, especially para[0016], [0053]-[0054], [0056], [0063, [0066], [0256]-[0257], [0291], [0298], [0346]-[0351], [0353] and [0376]-[0377].	15-27, 29-30, 43-54, 56-57 ----- 1-14, 28, 31-42, 55, 58-66
Y	US 2004/0253327 A1 (NIAZI, et al.) 16 December 2004 (16.12.2004) entire document, especially para[0019]-[0021].	1-14, 31, 34-35, 40-42, 58, 61-62, 65-66
Y	US 2007/0244048 A1 (MARSH, et al.) 18 October 2007 (18.10.2007) entire document, especially para[0086] and [0090].	10, 28, 32, 36-37, 55, 59
Y	US 2010/0172870 A1 (ANDRE, et al.) 08 July 2010 (08.07.2010) entire document, especially para[0072] and [0244].	33, 38-39, 60, 63-64
Y	Kainuma, et al. Design, synthesis, and evaluation of non-steroidal farnesoid X receptor (FXR) antagonist. Bioorg. Med. Chem. ePub 31 January 2007, 15:2587 2600; pg 2587 and 2598.	14, 37
Y	US 2009/0137516 A1 (HARTMAN, et al.) 28 May 2009 (28.05.2009) entire document, especially para[0037].	41, 66
Y	US 2007/0173495 A1 (PALANI, et al.) 26 July 2007 (26.07.2007) entire document, especially par[0002] and [0300].	42

☐ Further documents are listed in the continuation of Box C.

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

08 May 2012 (08.05.2012)

Date of mailing of the international search report

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