Compositions and Methods for Inhibiting Gene Expression of LPA

BACKGROUND

Lipoprotein(a) [Lp(a)] is a heterogeneous low density lipoprotein (LDL)-like particle containing a lipid core and apolipoprotein B (apoB-100) with a unique constituent, apolipoprotein (a) (apo(a)), that is attached to apoB-100 through a disulfide bond.

The apo(a) gene (*LPA*) is expressed predominantly in the liver and expression is restricted to human and non-human primates. Lp(a) levels in humans are genetically defined and do not change significantly with diet, exercise, or other lifestyle changes. *LPA* varies in length depending upon the number of Kringle KIV2 domains present and its expression is inversely correlated with the number of domains present. Normal Lp(a) levels range from 0.1-25 mg/dl, with about 25% of the population in the United States of America having Lp(a) levels of 30 mg/dl or higher.

Analysis of Lp(a) levels in multiple studies have implicated high Lp(a) levels as an independent risk factor for cardiovascular disease, stroke, and other related disorders including atherosclerotic stenosis. In addition, genome-wide association analyses have also implicated LPA as a genetic risk factor for diseases such as atherosclerotic stenosis.

When therapeutic lipoprotein apheresis is used to lower both Lp(a) and LDL levels in hyperlipidemic patients, significant reductions of cardiovascular events have been observed. Therefore there exists a need for therapeutics and treatments related to these and other LPA-related diseases.

SUMMARY

In one aspect, it is referred to a pre-filled syringe comprising a pharmaceutical composition comprising an LPA RNA interference (RNAi) agent and a pharmaceutically acceptable excipient, wherein the LPA RNAi agent comprises: (i) a sense strand and an antisense strand, wherein the antisense strand comprises the sequence of SEQ ID NO: 1280, wherein the sense strand comprises a sequence that is complementary to the sequence of the antisense strand; and (ii) a targeting group comprising an asialoglycoprotein receptor ligand, wherein the targeting group is conjugated to the sense strand. In another aspect, it is referred to the pre-filled syringe as described herein for use in therapy. In yet another aspect, it is referred to the use of the pre-filled syringe as described herein in the manufacture of a medicament for treating coronary artery disease or peripheral artery disease in a subject. Also described herein are LPA (also termed apo(a)) RNA interference (RNAi) agents (also termed RNAi trigger, or trigger) and compositions containing LPA RNAi agents for selectively and efficiently inhibiting expression of the *LPA* gene. *LPA* is the name of the gene which encodes apolipoprotein (a) (apo(a)), a key component of the lipoprotein (a) particle (Lp(a)). The LPA RNAi agents described herein can be used in the prevention or treatment or the preparation of a medicament for the prevention or treatment of diseases including, but not limited to: Berger's disease, peripheral artery disease, coronary artery disease, metabolic syndrome, acute coronary syndrome, aortic valve stenosis, aortic valve regurgitation, aortic dissection, retinal artery

occlusion, cerebrovascular disease, mesenteric ischemia, superior mesenteric artery occlusion, renal artery stenosis, stable/unstable angina, acute coronary syndrome, heterozygous or homozygous familial hypercholesterolemia, hyperapobetalipoproteinemia, cerebrovascular atherosclerosis, cerebrovascular disease, and venous thrombosis.

Each LPA RNAi agent includes at least a sense strand and an antisense strand. The sense strand and the antisense strand can be partially, substantially, or fully complementary to each other. The length of the RNAi agent sense and antisense strands described herein each can be 17 to 30 nucleotides in length. In some examples, the sense and antisense strands are independently 17 to 26 nucleotides in length. The sense and antisense strands can be either the same length or different lengths. The RNAi agents described herein, upon delivery to a cell expressing the LPA gene, inhibit the expression of the LPA gene *in vitro* or *in vivo*.

A sense strand of an LPA RNAi agent comprises a nucleotide sequence having at least 90% identity over a core stretch of at least 17 consecutive nucleotides to a sequence in an *LPA* mRNA. In some examples, the sense strand nucleotide sequence having at least 90% identity to a sequence in the *LPA* mRNA is 17, 18, 19, 20, 21, 22, or 23 nucleotides in length. An antisense strand of an LPA RNAi agent comprises a nucleotide sequence having at least 90% complementary over a core stretch of at least 16 consecutive nucleotides to a sequence in the *LPA* mRNA and the corresponding sense strand. In some examples, the antisense strand nucleotide sequence having at least 90% complementarity to a sequence in the *LPA* mRNA or the corresponding sense strand is 17, 18, 19, 20, 21, 22, or 23 nucleotides in length.

In some examples, one or more LPA RNAi agents are delivered to target cells or tissues using any oligonucleotide delivery technology known in the art. Nucleic acid delivery methods include, but are not limited to, by encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres, proteinaceous vectors or Dynamic Polyconjugates™ (DPCs (see, for example WO 2000/053722, WO 2008/0022309, WO 2011/104169, and WO 2012/083185, each of which is incorporated herein by reference). In some examples, an LPA RNAi agent is conjugated to a targeting group. In some examples, the targeting group can include a cell receptor ligand, such as a galactose cluster, including a galactose cluster comprising an N-acetyl-galactosamine trimer.

In some examples are described pharmaceutical compositions comprising one or more LPA RNAi agents. In some examples, an LPA RNAi agent is optionally combined with one or more additional (i.e., second, third, etc.) therapeutics. An additional therapeutic can be another LPA RNAi agent (e.g., an LPA RNAi agent which targets a different sequence within the LPA target). An additional therapeutic can also be a small molecule drug, antibody, antibody fragment, and/or vaccine. The LPA RNAi agents, with or without the one or more additional therapeutics, can be combined with one or more excipients to form pharmaceutical compositions.

In some examples, compositions for delivering an LPA RNAi agent to a liver cell, particularly hepatocytes, *in vivo* are described, comprising: an LPA RNAi agent conjugated to a targeting group. In some examples, the targeting group is a asialoglycoprotein ligand.

Also described are methods of treating a human subject having a pathological state or at risk of developing a pathological state mediated at least in part by LPA expression, the methods comprising the step(s) of administering to the subject a therapeutically effective amount of an LPA RNAi agent or LPA RNAi agent-containing composition. The method of treating a subject with an LPA RNAi agent or LPA RNAi agent-containing composition can optionally be combined with one or more steps of administering one or more additional (i.e., second) therapeutics or treatments. The LPA RNAi agent and additional therapeutics can be administered in a single composition or they may be administered separately. Examples of additional therapeutics include, but are not limited to, HMg Co-A reductase inhibitors (statins), ezetimibe, PCSK-9 inhibitors, CTEP inhibitors, therapies targeting ANGPTL3, therapies targeting APOC3, and niacin.

Use of the described LPA RNAi agents can be used in methods for therapeutic prevention or treatment of diseases including, but not limited to: Berger's disease, peripheral artery disease, coronary artery disease, metabolic syndrome, acute coronary syndrome, aortic valve stenosis, aortic valve regurgitation, aortic dissection, retinal artery occlusion, cerebrovascular disease, mesenteric ischemia, superior mesenteric artery occlusion, renal artery stenosis, stable/unstable angina, acute coronary syndrome, heterozygous or homozygous familial hypercholesterolemia, hyperapobetalipoproteinemia, cerebrovascular atherosclerosis, cerebrovascular disease, and venous thrombosis. Such methods comprise administration of an LPA RNAi agent as described herein to a subject, e.g., a human or animal subject.

The pharmaceutical compositions can be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration can be made by any way commonly known in the art, such as, but not limited to, topical (e.g., by a transdermal patch), pulmonary (e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer, intratracheal, intranasal), epidermal, transdermal, oral or parenteral. Parenteral administration includes, but is not limited to, intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; subdermal (e.g., via an implanted device), intracranial, intraparenchymal, intrathecal, and intraventricular, administration. In some examples, the pharmaceutical compositions described herein are administered by subcutaneous injection.

The described LPA RNAi agents and/or compositions can be used in methods for therapeutic treatment of diseases, including but not limited to: Berger's disease, peripheral artery disease, coronary artery disease, metabolic syndrome, acute coronary syndrome, aortic valve stenosis, aortic valve regurgitation, aortic dissection, retinal artery occlusion, cerebrovascular disease, mesenteric ischemia, superior mesenteric artery occlusion, renal artery stenosis, stable/unstable angina, acute coronary syndrome, heterozygous or homozygous familial hypercholesterolemia, hyperapobetalipoproteinemia, cerebrovascular atherosclerosis, cerebrovascular disease, and venous thrombosis. Such methods comprise administration of an LPA RNAi agent as described herein to a subject, e.g., a human or animal subject.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.