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PATENT APPLICATION

LIPID ENCAPSULATED INTERFERING RNA

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LIPID ENCAPSULATED INTERFERING RNA

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application Nos. 60/577,961 filed June 7, 2004, 60/ 578,075 filed June 7, 2004, 60/610,746, filed September 17, 2004, and 60/679,427, filed May 9, 2005, the disclosures of each of which are hereby incorporated by reference in their entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates to compositions and methods for the therapeutic delivery of a nucleic acid comprising a serum-stable lipid delivery vehicle encapsulating a nucleic acid to provide efficient RNA interference (RNAi) in a cell or mammal. More particularly, the present invention is directed to using a small interfering RNA (siRNA) encapsulated in a serum-stable lipid particle having a small diameter suitable for systemic delivery.

BACKGROUND OF THE INVENTION

[0003] RNA interference (RNAi) is an evolutionarily conserved, sequence specific mechanism triggered by double stranded RNA (dsRNA) that induces degradation of complementary target single stranded mRNA and "silencing" of the corresponding translated sequences (McManus and Sharp, *Nature Rev. Genet.* 3:737 (2002)). RNAi functions by enzymatic cleavage of longer dsRNA strands into biologically active "short-interfering RNA" (siRNA) sequences of about 21-23 nucleotides in length (Elbashir, *et al.*, Genes Dev. 15:188 (2001)).

[0004] siRNA can be used downregulate or silence the transcription and translation of a gene product of interest. For example, it is desirable to downregulate genes associated with liver diseases and disorders such as hepatits. In particular, it is desirable to downregulate genes associated with hepatitis viral infection and survival.

[0005] An effective and safe nucleic acid delivery system is required for interference RNA to be therapeutically useful. Viral vectors are relatively efficient gene delivery systems, but suffer from a variety of limitations, such as the potential for reversion to the wild type as well as immune response concerns. As a result, nonviral gene delivery systems are receiving

increasing attention (Worgall, *et al.*, *Human Gene Therapy* 8:37 (1997); Peeters, *et al.*, Human Gene Therapy 7:1693 (1996); Yei, *et al.*, *Gene Therapy* 1: 192 (1994); Hope, *et al.*, *Molecular Membrane Biology* 15:1 (1998)). Furthermore, viral systems are rapidly cleared from the circulation, limiting transfection to "first-pass" organs such as the lungs, liver, and spleen. In addition, these systems induce immune responses that compromise delivery with subsequent injections.

[0006] Plasmid DNA-cationic liposome complexes are currently the most commonly employed nonviral gene delivery vehicles (Felgner, Scientific American 276:102 (1997); Chonn, *et al.*, *Current Opinion in Biotechnology* 6:698 (1995)). For instance, cationic liposome complexes made of an amphipathic compound, a neutral lipid, and a detergent for transfecting insect cells are disclosed in U.S. Patent No. 6,458,382. Cationic liposome complexes are also disclosed in U.S. Patent Publication No. 2003/0073640.

[0007] Cationic liposome complexes are large, poorly defined systems that are not suited for systemic applications and can elicit considerable toxic side effects (Harrison, *et al.*, *Biotechniques* 19:816 (1995); Li, *et al.*, *The* Gene 4:891 (1997); Tam, *et al*, *Gene Ther.* 7:1867 (2000)). As large, positively charged aggregates, lipoplexes are rapidly cleared when administered *in vivo*, with highest expression levels observed in first-pass organs, particularly the lungs (Huang, *et al.*, *Nature Biotechnology* 15:620 (1997); Templeton, *et al.*, *Nature Biotechnology* 15:647 (1997); Hofland, *et al.*, *Pharmaceutical Research* 14:742 (1997)).

[0008] Other liposomal delivery systems include, for example, the use of reverse micelles, anionic and polymer liposomes. Reverse micelles are disclosed in U.S. Patent No. 6,429,200. Anionic liposomes are disclosed in U.S. Patent Application No. 2003/0026831. Polymer liposomes, that incorporate dextrin or glycerol-phosphocholine polymers, are disclosed in U.S. Patent Application Nos. 2002/0081736 and 2003/0082103, respectively.

[0009] A gene delivery system containing an encapsulated nucleic acid for systemic delivery should be small (*i.e.*, less than about 100 nm diameter) and should remain intact in the circulation for an extended period of time in order to achieve delivery to affected tissues. This requires a highly stable, serum-resistant nucleic acid-containing particle that does not interact with cells and other components of the vascular compartment. The particle should also readily interact with target cells at a disease site in order to facilitate intracellular delivery of a desired nucleic acid.

[0010] Recent work has shown that nucleic acids can be encapsulated in small (about 70 nm diameter) "stabilized nucleic acid-lipid particles" (SNALP) that consist of a single plasmid encapsulated within a bilayer lipid vesicle (Wheeler, *et al.*, *Gene Therapy* 6:271

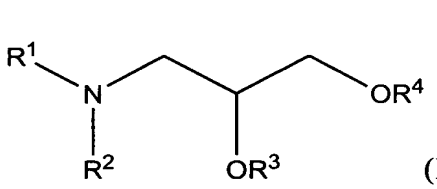
(1999)). These SNALPs typically contain the "fusogenic" lipid dioleoylphosphatidylethanolamine (DOPE), low levels of cationic lipid, and are stabilized in aqueous media by the presence of a poly(ethylene glycol) (PEG) coating. SNALP have systemic application as they exhibit extended circulation lifetimes following intravenous (i.v.) injection, accumulate preferentially at distal tumor sites due to the enhanced vascular permeability in such regions, and can mediate transgene expression at these tumor sites. The levels of transgene expression observed at the tumor site following i.v. injection of SPLP containing the luciferase marker gene are superior to the levels that can be achieved employing plasmid DNA-cationic liposome complexes (lipoplexes) or naked DNA.

[0011] Thus, there remains a strong need in the art for novel and more efficient methods and compositions for introducing nucleic acids, such as interfering RNA, into cells. In addition, there is a need in the art for methods of treating or preventing disorders such as hepatitis by downregulating genes associated with viral infection and survival. The present invention addresses this and other needs.

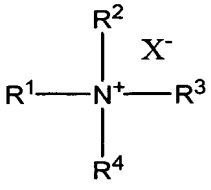
BRIEF SUMMARY OF THE INVENTION

[0012] The present invention comprises novel, stable nucleic acid-lipid particles (SNALP) encapsulating one or more interfering RNA molecules, methods of making the SNALPs and methods of deliverubg and/or administering the SNALPs.

[0013] In one embodiment, the invention provides for a nucleic acid-lipid particle comprising an interfering RNA and a cationic lipid of Formula I or II and having the following structures:



(I), and



(II),

wherein R^1 and R^2 are independently selected from the group consisting of: H and C_1-C_3 alkyls; and R^3 and R^4 are independently selected from the group consisting of alkyl groups having from about 10 to about 20 carbon atoms, wherein at least one of R^3 and R^4 comprises at least two sites of unsaturation. In a preferred embodiment, that cationic lipid is selected from 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA) and 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA). In a preferred embodiment, the interfering RNA molecule is fully encapsulated within the lipid bilayer of the nucleic acid-lipid particle such that the nucleic acid in the nucleic acid-lipid particle is resistant in aqueous solution to

degradation by a nuclease. In a preferred embodiment, the nucleic acid particle is substantially non-toxic to mammals. The nucleic acid lipid particles may further comprise a non-cationic lipid, a bilayer stabilizing component (*i.e.*, a conjugated lipid that prevents aggregation of particles, a cationic polymer lipid, a sterol (*e.g.*, cholesterol) and combinations thereof.

[0014] In some embodiments, the interfering RNA is a small-interfering RNA molecule that is less than about 60 nucleotides in length or a double-stranded RNA greater than about 25 nucleotides in length. In some embodiments the interfering RNA is transcribed from a plasmid, in particular a plasmid comprising a DNA template of a target sequence.

[0015] In one embodiment, the non-cationic lipid is selected from distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoyl- phosphatidylethanolamine (POPE) and dioleoyl- phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidyethanolamine (SOPE), a sterol (*e.g.*, cholesterol) and a mixture thereof.

[0016] In one embodiment, the conjugated lipid that inhibits aggregation of particles is one or more of a polyethyleneglycol (PEG)-lipid conjugate, a polyamide (ATTA)-lipid conjugate, and a mixture thereof. In one aspect, the PEG-lipid conjugate is one or more of a PEG-dialkyloxypropyl (DAA), a PEG-diacylglycerol (DAG), a PEG-phospholipid, a PEG-ceramide, and a mixture thereof. In one aspect, the PEG-DAG conjugate is one or more of a PEG-dilauroylglycerol (C_{12}), a PEG-dimyristoylglycerol (C_{14}), a PEG-dipalmitoylglycerol (C_{16}), and a PEG-distearoylglycerol (C_{18}). In one aspect, the PEG-DAA conjugate is one or more of a PEG-dilauryloxypropyl (C_{12}), a PEG-dimyristyloxypropyl (C_{14}), a PEG-dipalmityloxypropyl (C_{16}), and a PEG-distearyloxypropyl (C_{18}).

[0017] The nucleic acid-lipid particles of the present invention are useful for the therapeutic delivery of nucleic acids comprising an interfering RNA sequence. In particular, it is an object of this invention to provide *in vitro* and *in vivo* methods for treatment of a disease in a mammal by downregulating or silencing the transcription and translation of a target nucleic acid sequence of interest. In some embodiments, an interfering RNA is formulated into a nucleic acid-lipid particle, and the particles are administered to patients

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