LIPID-NUCLEIC ACID PARTICLES PREPARED VIA A HYDROPHOBIC LIPID-NUCLEIC ACID COMPLEX INTERMEDIATE AND USE FOR GENE TRANSFER

This application is a continuation of and claims the benefit of U.S. Continuation application Ser. No. 09/431,594, filed Nov. 1, 1999, the disclosure of which is incorporated by reference. Application Ser. No. 09/431,594 is a continuation of Ser. No. 08/660,025, filed Jun. 6, 1996, U.S. Pat. No. 5,976,567, which is a continuation-in-part of U.S. application Ser. No. 08/485,458 now U.S. Pat. No. 5,705,385 and of U.S. application Ser. No. 08/484,282, now U.S. Pat. No. 5,981,501 both filed on Jun. 7, 1995.

FIELD OF THE INVENTION

This invention relates to lipid-nucleic acid particles which are useful for the introduction of nucleic acids into cells, and methods of making and using them. The invention provides a circulation-stable, characterizable delivery vehicle for the introduction of plasmids or antisense compounds into cells. These vehicles are safe, stable, and practical for clinical use.

BACKGROUND OF THE INVENTION

Gene transfer into genetically impaired host cells in order to correct the genetic defects has vast potential for successfully treating a variety of thus far hitherto untreatable medical conditions. There are currently six major non-viral methods by which genes are introduced into host cells: (i) direct microinjection, (ii) calcium phosphate precipitation, (ii) DEAE-dextran complexes, (iv) electroporation, (v) cationic lipid complexes and (vi) reconstituted viruses and virosomes (see Chang, et al., *Focus* 10:88 (1988)).

Most reported examples of gene transfer have been performed in vitro. In vivo gene transfer is complicated by serum interactions, immune clearance, enzymatic degradation of the genes, toxicity and biodistribution. In in vivo administration, selection is not possible, and a reasonably high frequency of transformation is necessary to achieve sufficient expression to compensate for a defective endogenous gene.

The in vivo gene transfer methods under study in the clinic consist almost entirely of viral vectors. Although viral vectors have the inherent ability to transport nucleic acids across cell membranes and some can integrate exogenous DNA into the chromosomes, they can carry only limited amounts of DNA. In addition, their use poses significant risks. One such risk is that the viral vector may revert to a pathogenic genotype either through mutation or genetic exchange with a wild type virus.

In view of these limitations and risks, alternative non-viral-based gene transfer methods have been developed. These methods use often plasmid vectors, which are small circular sequences of DNA, as vectors for DNA delivery. However, most plasmids do not possess the attributes required for intracellular delivery and therefore sophisticated delivery systems are required.

Cationic lipid complexes are presently the most effective generally used means of introducing non-viral nucleic acids into cells. A number of different formulations incorporating cationic lipids are commercially available. These include:(i) LIPOFECTION® (which uses 1,2-dioleyloxy-3-(N,N,N-trimethylamino)propane chloride, or DOTMA, see Eppstein, et al., U.S. Pat. No. 4,897,355); LIPOFECTAMINE® (which uses DOSPA, see Hawley-Nelson, et al., Focus 15(3):73 (1993)); and LIPOFECTACE® (which uses N,N-distearyl-N,N-dimethyl-ammonium bromide, or DDAB, see Rose, U.S. Pat. No. 5,279,833). Others have reported alternative cationic lipids that work in essentially the same manner but with different efficiencies, for example 1,2-dioleoyloxy-3-(N,N,N-trimethylamino) propane chloride, or DOTAP (see Stomatatos, et al., Biochemistry 27: 3917–3925 (1988)); glycerol based lipids (see Leventis, et al., Biochem. Biophys. Acta 1023:124 (1990); lipopolyamines (see, Behr, et al., U.S. Pat. No. 5,171,678) and cholesterol based lipids (see Epand, et al., WO 93/05162, and U.S. Pat. No. 5,283,185). It has been reported that DOTMA and related compounds are significantly more active in gene transfer assays than their saturated analogues (see, Feigner, et al., WO91/16024). However, both DOTMA and DOSPA based formulations, despite their efficiency in effecting gene transfer, are prohibitively expensive. DDAB on the other hand is inexpensive and readily available from chemical suppliers but is less effective than DOTMA in most cell lines. Another disadvantage of the current lipid systems is that they are not appropriate for intravenous injection.

Lipid-based vectors used in gene transfer have generally been formulated in one of two ways. In one method, the nucleic acid is introduced into preformed liposomes made of mixture of cationic lipids and neutral lipids. The complexes thus formed have undefined and complicated structures and the lipofection efficiency is severely reduced by the presence of serum. A second method involves the formation of DNA complexes with mono- or poly-cationic lipids without the presence of a neutral lipid. These complexes are often prepared in the presence of ethanol and are not stable in water. Additionally, these complexes are adversely affected by serum (see, Behr, *Acc. Chem. Res.* 26:274–78 (1993)).

An examination of the relationship between the chemical structure of the carrier vehicle and its efficiency of gene transfer has indicated that the characteristics which provide for effective gene transfer would make a carrier unstable in circulation (see, Ballas, et al., *Biochim. Biophys. Acta* 939:8–18 (1988)). Additionally, degradation either outside or inside the target cell remains a problem (see, Duzghines, *Subcellular Biochemistry* 11:195–286 (1985)). Others who have attempted to encapsulate DNA in lipid-based formulations have not overcome these problems (see, Szoka et al., Ann. Rev. Biophys. Bioeng. 9:467 (1980); Deamer, U.S. Pat. No. 4,515,736, and Legendre, *Pharm. Res.* 9:1235–1242 (1992)).

Ideally, a delivery vehicle for a nucleic acid or plasmid will have the following characteristics: a) ease of preparation, b) capable of carrying a large amount of DNA per particle to enable gene transfer of all sizes of genes and reduce the volume of injection, c) homogenous, d) reproducible, e) is serum stable with minimal serum interactions and shields DNA from extracellular degradation, and f) is capable of transfecting target cells in such a way that the DNA is not digested intracellularly.

The present invention provides such compositions and methods for their preparation and use.

SUMMARY OF THE INVENTION

The present invention comprises novel, lipid-nucleic acid particles. The invention also comprises methods of making and using these particles.

In some embodiments, the particles are made by formation of hydrophobic intermediate complexes in either detergent-based or organic solvent-based systems, followed by removal of the detergent or organic solvent. Preferred embodiments are charge-neutralized.