DIMERIC CATIONIC LIPIDS

TECHNICAL FIELD

The present invention is directed to dimeric cationic lipid compounds useful in lipid aggregates for the delivery of macromolecules into cells.

BACKGROUND AND INTRODUCTION TO THE INVENTION

Some bioactive substances do not need to enter cells to exert their biological effect, because they operate either by acting on cell surfaces through cell surface receptors or by interacting with extracellular components. However, many natural biological molecules and their analogues, such as proteins and polynucleotides, or foreign agents, such as synthetic molecules, which are capable of influencing cell function at the subcellular or molecular level are preferably incorporated within the cell in order to produce their effect. For these agents the cell membrane presents a selective barrier which may be impermeable to them.

While these membranes serve a protective function by preventing entry of toxic substances, they can also prevent passage of potentially beneficial therapeutic agents into the body. This protective function is influenced by the complex composition of the cell membrane which includes phospholipids, glycolipids, cholesterol, and intrinsic and extrinsic proteins, as well as by a variety of cytoplasmic components. Interactions between these structural and cytoplasmic cell components and their response to external signals make up transport processes responsible for the membrane selectivity exhibited within and among cell types.

Successful intracellular delivery of agents not naturally taken up by cells has been achieved to some extent by exploiting natural delivery vehicles, such as viruses, that can penetrate a cell's membrane or are taken up by the cell's natural transport mechanisms or by natural process of intracellular membrane fusion. (Duzgunes, N., *Subcellular Biochemistry* 11:195–286, 1985).

The membrane barrier may be overcome in the first instance by viral infection or transduction. Various techniques for introducing the DNA or mRNA precursors of bioactive peptides into cells include the use of viral vectors, such as recombinant vectors and retroviruses, which have the inherent ability to penetrate cell membranes. However, the use of such viral agents to integrate exogenous DNA into the chromosomal material of the cell carries a risk of damage to the genome and the possibility of inducing malignant transformation.

Another aspect of this approach which restricts its use in vivo is that the integration of DNA into the genome accomplished by these methods implies a loss of control over the expression of the peptide it encodes, so that transitory therapy is difficult to achieve and potential unwanted side effects of the treatment could be difficult or impossible to reverse or terminate.

The membrane barrier may also be overcome by associating these agents in complexes with lipid formulations closely resembling the lipid composition of natural cell membranes. These lipids are able to fuse with the cell membranes, and in the process, the associated agents are delivered intracellularly. The structure of various types of lipid aggregates in formulations vary depending on a variety of factors which include composition and methods of forming the aggregate. Lipid aggregates include, for example, liposomes, unilamellar vesicles, multilamellar vesicles, micelles and the like, and may have particle sizes in the nanometer to micrometer range.

The lipids of these formulations may comprise an amphipathic lipid, such as the phospholipids of cell membranes, which form hollow lipid vesicles or liposomes in aqueous systems either spontaneously or by mechanical agitation. This property can be used to entrap the agent to be delivered within the liposomes. In other applications, the agent of interest can be incorporated into the lipid vesicle as an intrinsic membrane component, rather than entrapped in the hollow aqueous interior.

Liposomes have been utilized as in vivo delivery vehicles and some encouraging results were obtain when this approach was applied to intracellular expression of DNA (Mannino, R. J. and Fould-Fogerite, S., *Biotechniques* 6:682–690, 1988; Itani, T. et al. *Gene* 56:267–276. 1987; Nicolau, C. et al. *Meth. Enz.* 149:157–176, 1987; Straubinger, R. M. and Papahadjopoulos, D. *Meth. Enz.* 101:512–527, 1983; Wang, C. Y. and Huang, L., *Proc. Natl. Acad. Sci. USA* 84:7851–7855, 1987; however, the methodology has fundamental problems. An important drawback to the use of this type of aggregate as a cell delivery vehicle is that the liposome has a negative charge that reduces the efficiency of binding to a negatively charged target cell surface. Consequently, the liposome is often taken up by the cell phagocytically. Phagocytized liposomes are delivered to the lysosomal compartment, where polynucleotides are subjected to the action of digestive enzymes and degraded, which leads to low efficiency of expression.

A major advance in this area was the discovery that a positively charged synthetic cationic lipid, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), in the form of liposomes, or small vesicles, could interact spontaneously with DNA to form lipid-DNA complexes which are capable of fusing with the negatively charged lipids of the cell membranes of tissue culture cells, resulting in both uptake and expression of the DNA (Felgner, P. L. et al. *Proc. Natl. Acad. Sci., USA* 8:7413–7417, 1987 and U.S. Pat. No. 4,897,355 to Eppstein, D. et al.). Others have successfully used a DOTMA analogue, 1,2-bis (oleoyloxy)-3-(trimethylammonia)propane (DOTAP) in combination with a phospholipid to form DNA-complexing vesicles. Lipofectin™ (Bathesda Research Laboratories, Gaithersburg, Md.) is an effective agent for the delivery of highly anionic polynucleotides into living tissue culture cells that comprises positively charged DOTMA liposomes which interact spontaneously with negatively charged polynucleotides to form complexes. When enough positively charged liposomes are used, the net charge on the resulting complexes is also positive. Positively charged complexes prepared in this way spontaneously attach to negatively charged cell surfaces, fuse with the plasma membrane, and efficiently deliver functional polynuleotide into, for example, tissue culture cells.

Although the use of known cationic lipids overcomes some of the problems associated with conventional liposome technology for polynucleotide delivery in vitro, problems related to both in vitro and in vivo applications remain. Cationic lipids such as DOTMA are toxic to tissue culture cells and are expected to accumulate in the body due to their poorly metabolized ether bonds.

Another commercially available cationic lipid, 1,2-bis (oleoyloxy)-3,3-(trimethylammonia)propane ("DOTAP") differs from DOTMA in that the oleoyl moieties are linked by ester, rather than ether linkages. However, DOTAP is reported to be more readily degraded by target cells leading to low efficiency of delivery.