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Novel Lipidic Gene Delivery Systems for Gene Therapy

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The ultimate goal of gene therapy is to cure both inherited and aquired diseases by genetic modifications, which include correction, addition, and replacement of the related genes. There are still a number of obstacles to be overcome before practical clinical applications. Vectors developed for gene therapy are divided into two categories, vial and nonviral. While vial vectors are generally highly efficient in gene delivery, they have several drawbacks, such as toxicity, adverse immun-stimulation, oncogenesis, and possible replication of competent virus, depending on the type of virus. Advantages of nonviral vectors are low toxicity, nonimmunogenicity, and simplicity of the systems. However, the nonviral vectors have some drawbacks including relatively low gene delivery efficiency and transient expressions. Recent years, a great deal of effort have been made to resolve the problems in both types of vector.

Among the nonviral vectors, cationic liposomes have been widely utilized for *in vitro* gene expression as well as various preclinical and clinical studies for gene therapy. Cationic lipids, a major component of cationic liposomes, are capable of binding with DNA by means of electrostatic interaction, and carrying DNA into cells through an uncleared route. To improve the liposomal gene-transferring efficiency, we have synthesized a new series of cationic lipids (Fig. 1), dimyristoyl-aspartatyl-lysine (DMDK) and dipalmitoyl-aspartatyl-lysine (DPDK), and investigated their transfection activities, comparing to that of DOTAP, one of the most commonly used cationic lipids for gene transfection. These lipids were synthesized using hydrocarbon chains and aspartate as a backbone, and lysine as a positive charge provider, which are all biocompatible. The transfection activities of various liposomal formulations, made of DMDK or DPDK, were verified in 293 cells and B16BL6 cell using luciferase gene as a reporter. The optimized transfection activities of DMDK- and DPDK-based liposomes exhibits 2-5 fold higher than that of DOTAP. Inclusion of cholesterol or DOPE as a helper lipid decreased their transfection activities. These data suggest that these cationic lipids can be utilized as a gene delivery vehicle for various clinical applications.

We have been proposed that if the infection capability of viral vectors are provided to liposomal gene delivery systems, both advantages of viral and nonviral

vectors may be combined; less toxic and more efficient gene delivery. Fusogenic liposomes (virosomes) consisting of Sendai virus envelopes have been utilized for *in vitro* and *in vivo* genetic modification of animal cells (Fig. 2). The virosomes containing DNA were prepared by quantitative reconstitution of Sendai envelope proteins, F (fusion) protein and HN (hemagglutinin-neuraminidase) protein. Generally the F-virosomes and F/HN-virosomes more efficiently transferred genes into cultured 293 transformed human kidney cells than cationic liposomes did. The F-virosomes and F/HN-virosomes exhibited the best efficiency of gene transfection into the cells at 200:1 and 100:1 weight ratios of envelope protein to lipid, respectively. The Sendai virosomes required a relatively shorter incubation period and were much less cytotoxic, compared to the cationic liposome/DNA complex. This type of Sendai virosome is relatively convenient for preparation and storage, compared to fusogenic liposomes prepared by liposome-virus fusion. Most importantly, since the constituents are quantitatively formulated, this type of virosome formulation can provide further consistent gene transfection. These types of lipidic gene delivery system will be useful for *in vitro* gene expression as well as gene therapy.