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Cationic lioposomes with folic acid as targeting ligand for gene delivery

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ABSTRACT

In our previous Letter, we have carried out the synthesis of a novel DDCTMA cationic lipid which was formulated with DOPE for gene delivery. Herein, we used folic acid (FA) as targeting ligand and cholesterol (Chol) as helper lipid instead of DOPE for enhancing the stability of the liposomes. These liposomes were characterized by dynamic laser scattering (DLS), transmission electron microscopy (TEM) and agarose gel electrophoresis assays of pDNA binding affinity. The lipoplexes were prepared by using different weight ratios of DDCTMA/Chol (1:1, 2:1, 3:1, 4:1) liposomes and different concentrations of FA (50–200 μg/mL) combining with pDNA. The transfection efficiencies of the lipoplexes were evaluated using pGFP-N2 and pGL3 plasmid DNA against NCI-H460 cells in vitro. Among them, the optimum gene transfection efficiency with DDCTMA/Chol (3:1)/FA (100 μg/mL) was obtained. The results showed that FA could improve the gene transfection efficiencies of DDCTMA/Chol cationic liposome. Our results also convincingly demonstrated FA (100 μg/mL)-coated DDCTMA/Chol (3:1) cationic liposome could serve as a promising candidate for the gene delivery.

Gene therapy requires safe and efficient carriers to transfer expressible genetic materials to target cells. The most extensively used delivery tools can be divided into two general categories: viral and non-viral vectors. Development of non-viral vectors has been one of the primary areas of research because of several significant advantages, including greater carrier capacity, safety, ease of large-scale production, stability, potential to incorporate targeting ligands, and unlimited size.[1] Cationic liposomes have been widely used as non-viral gene delivery vehicles. However, unlike viral analogues that have evolved to overcome cellular barriers and immune defense mechanisms, non-viral gene carriers consistently exhibit lower transfection efficiency compared with viral ones.[2] In our previous study, we synthesised a novel cationic lipid *N*-[1-(2,3-didodecylcarbamoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium iodide (DDCTMA) which contained C_{12} hydrocarbon chains, quaternary ammonium head, carbamate linkage between hydrocarbon chains and quaternary ammonium head (as shown in Fig. 1) for liposome mediated gene delivery.[3]

Some cationic liposomes showed transfection ability when used alone, but in many cases helper lipids are required to be incorporated into the formulations to yield high transfection efficiency. Among helper lipids, DOPE has been researched very extensively for it often presents super synergistic effect when used in cationic liposomes.[4] The results of our previous studies showed that DDCTMA/DOPE liposome had better capacity for gene delivery as a safe and effective gene delivery vector. In this Letter, in order to improve the stability and efficiency of gene delivery of the DDCTMA liposome, cholesterol (Chol) was used as helper lipid for preparing a series of novel DDCTMA/Chol liposomes. As compared with DOPE, Chol was a more effective helper lipid for DDCTMA liposome. And then, in order to enhance targeted delivery of cancer cells, folic acid (FA) was used to endow DDCTMA/Chol/pDNA lipoplexes with the active-targeting capacity. FA is a member of the B family of vitamins and it is metabolized to active form that is poor to export substrates and accumulate in cells where it sustains key metabolic reactions. FA is required in the synthesis of nucleotide bases, amino acids, and other methylated compounds, and consequently, it is required in larger quantities by proliferating cells. FA binds folate receptor (FR) at a high affinity to mediate transport into the cytoplasm of cells. The expression of FR is up-regulated in a range of human tumors, including ovarian, lung and colorectal cancer.[5–10] FA as a tumor-active-targeted ligand has several advantages including high stability, low molecular weight, ease of accessibility and high affinity to FR. Therefore, FR has emerged as a potential marker for response to treatment of human carcinomas with the drug.[11–16] Then, cationic liposomes further modified by tumor-active–targeted ligands such as small compounds like FA have been widely investigated.

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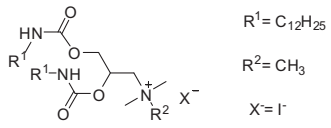

Figure 1. The structure of DDCTMA.

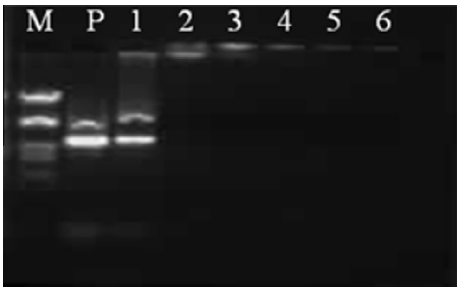

Figure 2. Gel electrophoresis of cationic liposomes (DDCTMA/Chol = 3:1)/pGFP-N2 complexes at various weight ratios. Lane M: marker (λ DNA/EcoR I + Hind III Markers from SABC), lane P: naked plasmid DNA (2 μg) and lanes 1 → 6: lipoplexes of plasmid DNA (2 μg) with progressively increasing proportions (N/P, 1:1, 2:1, 3:1, 4:1, 5:1 and 6:1) of cationic liposomes.

In this study, liposomes composed of DDCTMA/Chol (3:1) and FA were prepared according to our previous study. DNA degradation is a limiting factor in gene therapy, whereas liposomes can facilitate pDNA entry into cells and also protect pDNA from degradation by DNase. To identify the appropriate ratio of liposomes to DNA for condensation, we evaluated their ability to promote DNA condensation and to inhibit its migration in agarose gel electrophoresis. The results showed that the pDNA formed two major bands, corresponding to supercoiled pDNAs and open circular pDNAs. After the addition of liposomes, pDNA could be retarded at different N/P weight ratios (Fig. 2). The results of DNA electrophoresis demonstrated that DDCTMA/Chol cationic liposomes could form DNA-liposome complexes. It was also observed that the increasing trend in the DNA-binding ability was obvious with the increase of lipid-to-DNA ratios. The liposome with the N/P ratios of over 2:1 could completely retard pDNA.

The liposomes and lipoplexes were subjected to the measurement of particle size and zeta potential by using the nano particle analyzer (HORIBA scientific, nano partica, Japan). The size distribution of DDCTMA/Chol liposome, DDCTMA/Chol/pGFP-N2, DDCTMA/Chol/pGFP-N2/FA (50 μg/mL), DDCTMA/Chol/pGFP-N2/FA (100 μg/mL), DDCTMA/Chol/pGFP-N2/FA (150 μg/mL), DDCTMA/Chol/pGFP-N2/FA (200 μg/mL) were characterized to be in the range of 70–165 nm. As shown in Figure 3A, the size of DDCTMA/Chol liposome was smaller than DDCTMA/DOPE liposome, and after the addition of FA, the size of lipoplexes was increased to around 150 nm. It has been proved that this particle size range was suitable for gene delivery.[7] The zeta potential of liposomes and lipoplexes were an indirect measure of the surface charge, it can be used to evaluate the interaction of the liposomal cationic surface charges with the anionic charges of DNA. The zeta potential of the liposome DDCTMA/Chol was 90 mV, much higher than that of the liposome DDCTMA/DOPE (Fig. 3B). It can also be concluded that with the addition of FA, the zeta potential of lipoplexes decreased, as FA has negative charge.

TEM was used to visualize directly the size and morphology of liposomes and lipoplexes. The negatively stained TEM images confirmed the formation of liposomes and lipolexes (Fig. 4). Smooth spherical morphology and a uniform size distribution were observed. The mean diameter of DDCTMA/Chol liposome was a little more than half of the DDCTMA/DOPE liposome (Fig. 4A and B), which was probably caused by cholesterol on the compression and stability of liposomes. The diameters of the DDCTMA/Chol/pGFP-N2 lipoplexes ranging from 50 to 100 nm (Fig. 4C) appeared to be consistent with the results determined by Zetasizer measurement. After the addition of FA (50–200 μg/mL), the diameters of lipoplexes increased to the range of 100–150 nm

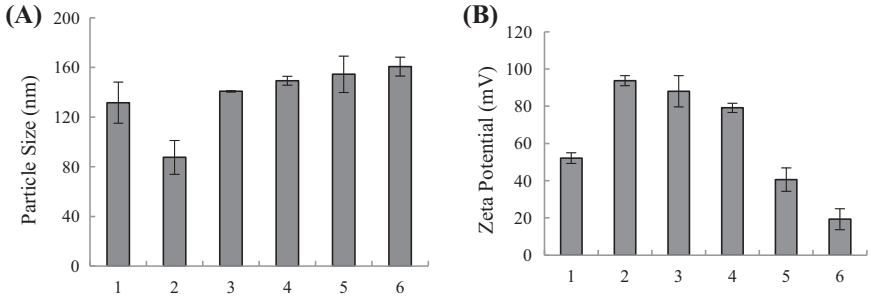

Figure 3. Particle sizes and zeta potentials of liposomes and lipoplexes. Liposomes (20 μL) were diluted in 1 mL distilled water. Particle sizes (A) and Zeta potentials (B): 1. DDCTMA/DOPE (control); 2. DDCTMA/Chol; 3. DDCTMA/Chol/pGFP-N2/FA (50 μg/mL); 4. DDCTMA/Chol/pGFP-N2/FA (100 μg/mL); 5. DDCTMA/Chol/pGFP-N2/FA (150 μg/mL); 6. DDCTMA/Chol/pGFP-N2/FA (200 μg/mL). The PDIs of liposomes were 0.310, 0.295, 0.253, 0.262, 0.277, and 0.284, respectively.

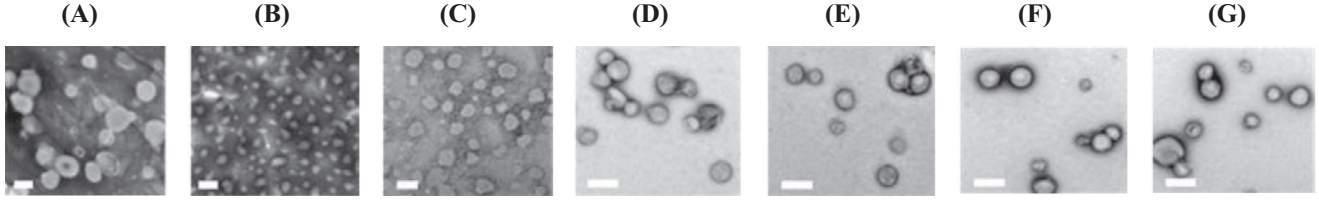

Figure 4. The negatively stained TEM images. *Scale bar* (A, B, C = 100 nm; D, E, F, G = 200 nm). (A) DDCTMA/DOPE; (B) DDCTMA/Chol; (C) DDCTMA/Chol/pGFP-N2; (D) DDCTMA/Chol/pGFP-N2/FA (50 μg/mL); (E) DDCTMA/Chol/pGFP-N2/FA (100 μg/mL); (F) DDCTMA/Chol/pGFP-N2/FA (150 μg/mL); (G) DDCTMA/Chol/pGFP-N2/FA (200 μg/mL).