cholate, a newly formulated ultradeformable cationic liposome has been prepared. The average particle size of this formulation was approximately 80nm. The in vitro transfection efficiency of plasmid DNA was assessed by the expression of green fluorescent protein (GFP) in four cell lines, OVCAR-3 (human ovarian carcinoma cells), HepG-2 (human hepatoma cells), H-1299 (human lung carcinoma cells) and T98G (human brain carcinoma cells). The optimal ratio of DNA to liposome for maximal transfection efficiency was 1:14 (w/w) in all the cell lines except the human brain carcinoma cells. The same formulation was tested for in vivo transfection efficiency by complexing it with genetic material(GFP) and applying them on dorsal skin of mice non-invasively. It was found that genes were transported into several organs once applied on intact skin, especially kidney was the organ with the most GFP.

[PE3-4] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

Polyethylene glycol (PEG)-modified cationic liposome mediated gene delivery

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In this study, we modified the cationic liposomes by polyethylene glycol (PEG)-grafted or PEG-added methods. The PEG-grafted transfection complexes were prepared by adding the plasmid DNA to the PEG-grafted cationic liposomes, composed of PEG and cationic lipids. PEG-added transfection complexes were prepared by adding the PEG to the mixture of cationic lipids and plasmid DNA. The particle sizes of PEG-modified transfection complexes did not change during storage compared to conventional transfection complexes. In the presence of serum, the expression of green fluorescent protein of conventional liposome/DNA decreased but PEG-modified transfection complexes maintained high transfection efficiency in the presence of serum. The transfection efficiency of conventional transfection complexes was significantly decreased in storage but the PEG-modified transfection complexes maintained their transfection properties after 2 weeks. After administration of the conventional, PEG-grafted and PEG-added liposome/DNA complexes into the mice by tail vein, the plasmid DNA in the blood was analyzed by PCR. PEG-added transfection complexes were showed even longer plasmid DNA circulation than PEG-grafted or conventional cationic liposomes. These results suggest that the PEG-added transfection complexes could be a promising nonviral vector because it is easy to make and has a high transfection efficiency and stability in vitro and in vivo.

[PE3-5] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

Nonviral Vector for Efficient Gene Transfer to Human Ovarian Adenocarcinoma Cells

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Various strategies have been attempted to design efficient protocols for ovarian cancer gene therapy but there has been little progress in their clinical application. In this study, we formulated and evaluated a new cationic liposome composed of dioleoyltrimethylaminopropane (DOTAP), 1,2-dioleoyl-3-phosphophatidylethanolamine (DOPE), and cholesterol (Chol) (DDC) for plasmid DNA transfer into ovarian cancer cells. The DDC liposome was prepared by mixing DOTAP, DOPE, and Chol using extrusion method. Plasmid DNA (pEGFP-C1) and DDC were complexed at various ratios to find the optimum condition and the percentage of transfected cells was determined by flow cytometric analysis. The transfection efficiency of the DDC liposome was compared with 3 [N-(N,N-dimethylaminoethylene) carbamoyl] cholesterol (DC-Chol)/DOPE liposome and commercially available lifopectin. The optimal transfection of plasmid DNA was achieved at a 1:4 (w/w) ratio of DDC to DNA. The DDC/DNA complex exhibited higher transfection efficiency in human ovarian cancer cell lines compared to that in other types of cell lines. Flow cytometry revealed that of formulations, the DDC/DNA complex exhibited an over 4-fold increase in GFP expression levels in OVCAR-3 cells, further confirmed by confocal microscopy and RT-PCR. These results suggest that new cationic liposome could be a promising nonviral vector for treating ovarian adenocarcinoma because of its selective high gene transfer ability in ovarian cancer cells.

[PE3-6] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

Enhancement of Emulsion-mediated Gene Expression by Using Chitosan as a Pre-Condensing Agent