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, OVCA-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 dioleyltrimethylammonopropane (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-dimethylaminoethylene] carbamoyl) cholesterol (DC-Chol)]/DOPE liposome and commercially available lipofectin. 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 OVCA-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
The aim of this study was to enhance the transfection efficiency of emulsion-mediated gene expression by using chitosan. Conventional DNA/emulsion complexes and precondensed DNA/emulsion complexes were prepared by adding either naked or precondensed plasmids to cationic emulsion. The zeta potential, TEM, and size of transfection complexes were measured. In vitro transfection efficiency for both complexes was also studied by several methods: flow cytometry, expression analysis by confocal microscope, RT-PCR, and in addition, cytotoxicity test for transfection complexes was also performed. The expression of EGFP was determined by western blot analysis. Finally, in vivo transfection efficiency was also investigated. The mean size of uncondensed DNA/emulsion and precondensed DNA/emulsion complexes were 150nm and 100nm relatively, and their zeta-potentials were positive. This shows that chitosan efficiently reduced the size of DNA/emulsion complexes. Cell viability was higher for the transfection complexes with precondensed plasmids compared to the conventional complexes. MTT assay showed that the addition of chitosan reduced the rate of cytotoxicity. The percentage of transfection efficiency increased when the condensed DNA/emulsion complexes were used instead of the conventional DNA/emulsion complexes. From the results, this study undoubtedly demonstrated that using chitosan effectively enhanced the transfection efficiency in non-viral vector system.

Cationic Emulsions with Galactosylated Chitosan as a Novel Gene Delivery System

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To improve stability and transfection efficiency, a novel combination of cationic emulsion and galactosylated chitosan was developed for targeted gene delivery. Six formulations of cationic liposome and our novel emulsion were prepared for comparison of stability and transfection efficiency. Cationic liposomes composed of 3(N-(N,N-dimethylaminoethyl)carbamoyl)cholesterol (DC-Chol) and dioleyl phosphatidyl ethanolamine (DOPE) were prepared by extrusion method and cationic emulsions composed of DC-Chol, DOPE, castor oil, and Tween 80 were prepared by sonication method. The formulations were composed with galactosylated chitosan-condensed plasmid DNA (pEGFP-C1) encoding green fluorescent protein (GFP). The transfection efficiency of the complex was assessed by measuring GFP-positive cells expressing reporter gene by flow cytometry. The physical stability of the transfection complex was evaluated using laser light scattering measurement. Cationic emulsions showed better serum stability and higher transfection activity than conventional cationic liposomes. The galactosylated chitosan contributed to increase the gene transfer to HeLa cells and physical stability of transfection complex. This formulation using cationic emulsion and galactosylated chitosan has low cytotoxicity as a conventional liposomes. In conclusion, our novel cationic emulsion formulation for gene delivery system with galactosylated chitosan is superior as compared to the current liposome formulations.

Enhanced p53 Gene Transfer to Human Ovarian Cancer Cells using Cationic Nonviral Vector, DDC

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Previously we formulated new cationic liposomes, DDC, composed of DOTAP, DOPE, and cholesterol (Chol) in 1:0.7:0.3 molar ratios, and showed that DDC efficiently deliver the plasmid DNA into ovarian cancer cell lines. Here, wild type p53 DNA was transfected into ovarian cancer cells, using the DDC as a nonviral vector and the expression and activity of p53 gene were evaluated in vitro and in vivo. The complexes of plasmid DNA (p53-EGFP) and DDC were transfected into OVCAR-3 cells. The gene expression was determined by RT-PCR and western blot analysis. The cellular growth inhibition and apoptosis of DDC-mediated transfection were assessed by trypan blue exclusion assay and annexin-V staining, respectively. The OVCAR-3 cells treated with p53-EGFP/DDC complexes, were inoculated into nude mice and tumor growth was observed. The transfection of liposome-complexed p53 gene resulted in high levels of p53 mRNA and protein expressions in OVCAR-3 cells. In vitro cell growth showed growth inhibition of cancer cells transfected with p53-EGFP/DDC complexes.