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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 cytometer, 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.

[PE3-7] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

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 dimethylaminoethylene) carbamoyl] cholesterol (DC-Chol) and dioleyl phophatidyl 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 complexed 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.

[PE3-8] [10/18/2002 (Fri) 13:30 - 16:30 / Hall C]

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 (pp53-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 pp53-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 assays showed growth inhibition of cancer cells transfected with pp53-EGFP/DDC complexes