

DEVELOPMENT OF LIPOSOMAL GENE TARGETING FOR THERAPEUTIC INTERVENTION OR PREVENTION OF RESTENOSIS

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Restenosis after balloon angioplasty is a serious clinical problem arising in cardiology. Restenosis is caused by the induction of uncontrolled proliferation of vascular smooth muscle cells following damage of the endothelial lining of the vessel by balloon dilation. A gene therapy approach may provide the means to control the mechanisms responsible for restenosis. Several viral systems (adenoviruses, Semliki Forest virus, herpes simplex virus) have been shown to be able to transfer genes into vascular smooth muscle cells both in vitro and in vivo. However, viral systems have some disadvantages, including lack of cell-specificity, late onset of transgene expression and immunogenicity.

In our studies we are in search for efficient non-viral methods to transfect vascular smooth muscle cells in vivo, in vascular lesions. We tested various liposomal transfection systems in vitro using A7r5 cells (rat embryonic vascular smooth muscle cells). Generally these cells are difficult to transfect. Nonetheless, we achieved considerable transfection of A7r5 cells, when transfecting intensively proliferating cells at 25-50% confluency. Different polycation/DNA/liposome complexes were used as plasmid DNA (reporter gene EGFP) carriers. All three polycations - poly-L-lysine (PLL), polyethylenimine (PEI), protamine sulfate (ProtS) - used in our experiments were able to form complexes with DNA, that could subsequently be complexed with negatively charged liposomes. For our studies we have chosen two different liposomal formulations: DOPE/POPS/Chol (30:30:40) and DOPE/POPS/Chol/DSPE-PEG/DOTAP (25:30:40:3:2) with size about 9 nm. The size of polycation/DNA/liposome complexes varied from 170 nm to 380 nm, depending on composition of the formulation. Particles were size-stable during 3 month storage at 4°C. At 37°C, the size of the complexes was stable in the presence of 50% FCS cell culture medium (DMEM) or FCS alone, while in the presence of FCS free DMEM or 10% FCS DMEM, formation of aggregates was observed. Polycation/DNA/liposome complexes had higher transfection efficiency (1-10%, depending on experimental conditions) than the complexes which did not contain liposomes (0.1-4%). Moreover, the complexes, stored for 3 months at 4°C, were still able to transfect A7r5 cells. In the case of PLL/DNA/liposome

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(PLL:DNA 2.5:1 w/w; lipid:DNA 6:1 w/w) and ProtS/DNA/liposome (ProtS:DNA 6:1 w/w; lipid:DNA 12:1 w/w) complexes, the highest transfection efficiency in vitro was observed (serum free conditions). Additionally, in the presence of calcium, ProtS containing complexes were 6 to 40-fold more effective in transfection. Currently, conditions during complex formation are further optimized in order to obtain higher transfection efficiency. A first series of in situ and in vivo experiments in rats with complexes showing the highest transfection activity is in preparation.