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Role of dynamin-dependent and clathrin-dependent uptake pathways in nonviral gene delivery studied by chemical and genetic means

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Introduction: Endocytosis is known to be a major cell uptake mechanism for non-viral gene delivery vehicles. Several mechanisms of endocytosis have been described and it seems that not all of them are equally beneficial in terms of gene delivery efficiency. According to the literature the preferential cell uptake pathway is both carrier and cell type dependent. Rational design of effective and safe gene delivery vectors requires deeper understanding of the cellular uptake mechanisms of gene delivery vehicles. The purpose of our study was to clarify the role of dynamin-dependent cell uptake pathways, including both clathrin-dependent and caveolae-dependent endocytosis, in non-viral gene delivery. *Methods*: The studies were performed with three widely used non-viral gene delivery systems: cationic polymer branched polyethyleneimine (PEI), cationic lipid N-(1-(2,3-dioleoyloxy)propyl)-N,N,N-trimethyl ammonium methylsulfate (DOTAP) and calcium phosphate (CaP) precipitates. The internalization pathways of these gene delivery vehicles were studied by using genetically modified cell lines: HeLaK44A cells with inducible block of dynamin-dependent endocytosis and BHK21-tTA cells with inducible block of clathrin-dependent endocytosis. As an alternative approach chemical blockers chlorpromazine, dansylcadaverine, nystatin and dynasore were used to inhibit specific endocytic pathways. Relevant concentration of each inhibitor was determined by MTT cell viability assay. Size of the complexes was measured, and expression of marker protein at different timepoints from 0 to 72 hours after exposure to complexes was determined in intact cells and cells with blocked endocytic pathway(s). Results: The obtained data indicated that in both HeLaK44A and BHK21-tTA cell lines for DOTAP-based nanoparticles clathrin-dependent endocytic pathway seemed to be predominantly responsible for successful gene delivery, whereas for efficient PEI-mediated transfection caveolae-mediated pathway was important. In HeLaK44A cells block of dynamin-dependent endocytosis resulted only in moderate (40–50%) decrease of transfection efficiency of both PEI and DOTAP complexes. This suggests that other pathways, not dependent of dynamin, participate in the uptake of both PEI- and DOTAP-based nanoparticles in this cell line. In HeLaK44A cells blockage of dynamin-dependent endocytosis by genetic means increased transfection efficiency of Ca-phosphate precipitates 4-fold whereas chemical blockage of dynamin-dependent pathway by dynasore reduced transfection efficiency of Ca-phosphate precipitates almost completely. However, in general, the results obtained by using genetic means were comparable with results obtained by using chemical inhibitors.

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Enhanced intracellular delivery by guanidinium functionalized ROMP-polymers

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Intracellular delivery of therapeutic molecules has always been a challenge due to the poor permeability of cell membrane to large, negatively charged macromolecules and their restricted biodistribution. In the past decades, cell penetrating peptides (CPPs) are shown to improve the intracellular delivery of bioactive molecules and among the CPPs, arginine-rich peptides are highlighted as the most effective subclass. In the light of this information, we designed and synthesized guanidinium functionalized polyoxanorbornenes which can adopt cell penetrating activity and show superior uptake properties compared to peptide analogues (i.e. nonaarginine, R9). The structure–activity relationship was studied by mono-guanidinium and di-guanidinium functionalized monomers and a specific trend was observed for each cell line studied. In addition to intracellular uptake profiles of molecules, their exceptional ability to deliver bioactive cargo, such as DNA, siRNA and intact proteins, into both adherent and suspension cell lines, as well as in primary cells has been demonstrated. A non-covalent complexation approach was utilized for the delivery of bioactive molecules, instead of covalent attachment. Non-covalent interactions are highly favored over covalent attachment of cargo, in terms of simplicity, efficiency of delivery and stability of bioactive cargo. Furthermore, structural requirements and optimal experimental conditions have been investigated for an efficient intracellular delivery agent.

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Engineering functional chitosan for delivery of drugs or RNAs

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In the last decade, considerable studies on preparation of nanocarriers with cationic liposomes or polymers have been reported for intracellular delivery of DNA and siRNA [1]. Particle uptake has been proven through several kinds of endocytosis pathways, but the uptake efficiency varies depending on the property of carrier materials, particle size, and cell types. Using biocompatible and biodegradable chitosan (CTS) as carrier material, we designed and synthesized functional chitosan derivatives (such as amphiphilic CTS, ligand-targeted CTS), and then developed different technologies to prepare CTS nanoparticles for the potential application of loading, delivering and releasing anti-cancer drugs or RNA therapeutics (siRNA and microRNA). In one system, we initially conjugated a fatty acid (LA) to CTS to obtain amphiphilc CTS-LA, and then synthezised CTS-LA-TM by quaternization. Subsequently nanoparticles with size less than 200 nm can be easily formed by self-assembly of CS-LA-TM in biological solution or neutral solution [2]. These loaded PTX with encapsulation efficiency of 60–90% and showed sustained release in 1 week without burst release. Alternatively,