1333. Lactosylated Polyethylenimine as an Efficient Vector for Gene Transfer into Cystic Fibrosis Airway Epithelial Cells

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Polyethyleneimine (PEI) is a synthetic cationic polymer with endosome buffering capacity that has been shown to mediate efficient in vitro and in vivo gene transfer into various mammalian cells, including airway epithelial cells. In order to target airway epithelial cells, we have developed lactosylated PEI and studied its ability to transfer genes into cystic fibrosis airway epithelial cells.

Lactosylated PEI (25 kDa; branched form) contained about 5% of its amino groups substituted with a lactosylthiocarbamoyl unit. The requested amount of lactosylated or sugar-free PEI was complexed to a plasmid (2.5 µg) encoding the green fluorescent protein (GFP) in order to obtain a putatively protonated polymer nitrogen/DNA phosphate (N/P) ratio = 10. Immortalized cystic fibrosis airway epithelial cells (ΣCFTE cells) were incubated in the presence of complexes for 1h at 37°C and the number of transfected cells was determined by flow cytometry 24 h later. Intracellular trafficking of complexes was studied using lactosylated or sugar-free fluorescein-labeled PEI complexed to a biotinylated plasmid DNA. ΣCFTE cells were incubated in the presence of complexes for 1h at 4°C, washed and incubated at 37°C in culture medium for various times from 10 min up to 48h. Cells were fixed and the biotinylated DNA was stained with rhodamine-labeled streptavidin. Intracellular organelles were labeled by immunocytochemistry and the location of complexes was analyzed by confocal microscopy.

After a single transfection, lactosylated PEI and sugar-free PEI allowed an efficient gene transfer into 33.7±4.3% and 15.5±3.2% of cells, respectively ($p<0.05$). After 3 transfections performed daily, using lactosylated PEI as a vector, 60.8±1.7% cells were transfected. In contrast, when sugar-free PEI was used as a vector, cell toxicity was too massive after 3 transfections to study gene transfer efficiency. As assessed by flow cytometry, the cellular uptake of lactosylated complexes was greater than that of complexes made with sugar-free PEI. Intracellular trafficking analyzed by confocal microscopy was similar for both types of complexes : location of complexes in endosomal compartments labeled by anti-transferrin receptor antibody (Ab) was observed from 30 min up to 2 h. Location of complexes in lysosomal compartments labeled by anti-Lamp1 Ab was observed from 2 to 8 h, with a maximal location at 4h. Nuclear membrane was labeled by anti-lamin A/C Ab. A nuclear location of complexes was maximal at 6h and was observed in 20% of the cells. However, few complexes were present in the nucleus, less than 5% of the total intracellular load.

Our results show that lactosylated PEI is more efficient for gene transfer into cystic fibrosis airway epithelial cells than sugar-free PEI. As intracellular trafficking was similar for both complexes, the greater gene transfer efficiency allowed by lactosylated PEI compared to that of sugar-free PEI may be attributed to the greater cell uptake of lactosylated complexes and to their lower cytotoxicity. Gene transfer using lactosylated PEI/plasmid complexes into human primary airway epithelial cells and in vivo in mice is under investigation.

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