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INTRACELLULAR RATE-LIMITING STEPS OF GENE TRANSFER USING GLYCOSYLATED POLYLYSINES IN CYSTIC FIBROSIS AIRWAY EPITHELIAL CELLS

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Glycosylated polylysines (pLKs) are synthetic, efficient and non-toxic vectors substituted with sugar moities which allow a receptor-mediated uptake through membrane lectins. However, gene transfer efficiency using these vectors is not sufficient to allow clinical applications and an identification of the intracellular barriers to an efficient gene transfer were investigated. Texas Red-labeled DNA was complexed either with FITC-labeled lactosylated pLK or with FITC-labeled mannosylated pLK which are known to be both taken up by cystic fibrosis airway epithelial cells (ΣCFTE29o- cells) but to differ in their gene transfer efficiency, lactosylated polylysine being the most and mannosylated pLK, the least efficient glycosylated pLK. ΣCFTE cells were incubated in the presence of the complexes for 1 hr at 4°C in order to allow their binding to the cell membrane but to prevent their uptake. Then, cells were washed and incubated at 37°C. At various times, from 10 min up to 48 hrs, cells were fixed and intracellular organites labeled by immunocytochemistry. Location of the complexes was analyzed by confocal microscopy. In accordance with the presence in majority of a mannose-specific membrane lectin at the surface of ΣCFTE29o- cells, more mannosylated complexes were present inside the cells after 30 min of incubation at 37°C, as compared with lactosylated complexes (7.2±0.7 and 4.2±0.5 complexes per cell, respectively, $p<0.01$). However, mannosylated complexes appeared to stay longer in endosomal compartments labeled by anti-transferrin receptor antibody (Ab), as compared to lactosylated complexes (from 30 min to 3 hrs and from 10 min to 30 min, respectively). At 24 hrs a larger proportion of mannosylated complexes were localized inside lysosomes labeled by anti-LAMP-1 Ab, as compared to lactosylated complexes (41.8±6.6% and 19.8±5.2%, respectively, $p<0.05$). After 30 min, some of the complexes were seen near the nuclear membrane labeled by anti-lamin A/C Ab. Between 2 and 18 hrs, both lactosylated and mannosylated complexes were detected inside the nucleus, with a maximal localization at 8 hrs (8.8±2.2% and 4.5±1.7% of the complexes, respectively). In contrast with the 5% of transfected cells usually obtained with lactosylated pLK, lactosylated complexes were seen in the nuclei of 42% of the cells. A dissociation between the plasmid and its vector was never detected by our techniques. After 24 hrs, most of the complexes were observed gathered in one area of the cell. After 48 hrs, a very small amount of the complexes were still observed inside the cells. Our results show that some limiting steps for DNA transfer using mannosylated pLK are an inefficient escape from the endosomal compartments and an important localization in the lysosomes. For both lactosylated and mannosylated complexes, the nuclear import appears to be inefficient since 90 to 95 % of the complexes were localized outside the nucleus. In addition, a nuclear localization of the complexes appears not to warrant an efficient gene transfer and nuclear rate-limiting steps are under study.

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