and the lysosomes with fluorescein-labeled and anti-Lamp1 antibodies. The location of the complexes was conducted by confocal microscopy. After one hour at 4°C, both complexes made of lactosylated or mannosylated polylysine were seen, as expected, at the cell surface. After 10 to 15 min of incubation at 37°C, both complexes were seen inside the cells and, in agreement with the presence of a large number of mannose-specific membrane lectin at the cell surface, more mannosylated complexes were seen inside the cells, as compared with lactosylated complexes (7.2 ± 0.7 and 4.2 ± 0.5 at 30 min, respectively, $p<0.01$). After 1h, some of the complexes approached the nuclear membrane and, after 2h, some were detected inside the nucleus. The largest quantity of complexes detected inside the nucleus was observed between 4 and 8h, with lactosylated and mannosylated complexes present in the nucleus of 42% and 27% of the cells at 8h, respectively ($p>0.05$). Between 8 and 24h, a larger proportion of mannosylated complexes as compared with lactosylated complexes, were localized inside the lysosomes ($41.8\pm6.6\%$ and $19.8\pm5.2\%$ at 24h, respectively, $p<0.05$). After 24h, almost no complexes were observed in the nucleus and most of them gathered in one area of the cell. After 48h, a very small amount of complexes remained inside the cells. Our results show that a larger proportion of mannosylated than of lactosylated complexes reach the lysosomes which may explain the lower gene transfer efficiency obtained with mannosylated complexes. Moreover, the presence of mannosylated complexes in the nucleus of many cells suggests that the nuclear location of the complexes is not sufficient to allow an efficient gene transfer. Other factors are likely to be involved, amongst them a preferential dissociation of the plasmid DNA from the lactosylated polylysine in situ is currently under investigation.

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1025. Sugar-mediated intracellular trafficking of DNA complexed to glycosylated polylysines in cystic fibrosis airway epithelial cells

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Using glycosylated polylysines as vectors, we have previously shown that the uptake by immortalized cystic fibrosis airway epithelial cells (ΣCFTE cells) of glycoconjugates containing α-D-mannopyranosyl moieties was much more efficient than that of glycoconjugates containing lactosyl residues, while gene transfer using lactosylated polylysine was far more efficient than that using mannosylated polylysine (Fajac et al., Hum. Gene Ther. 1999; 10 : 395-406). These results suggested that plamid DNA complexed to glycosylated polylysines has a different intracellular trafficking according to the nature of the sugar moietie substituting the polylysine. On these bases, we intended to precisely investigate the intracellular trafficking of a plasmid DNA complexed either with lactosylated or with mannosylated polylysine. We have used a biotinylated DNA and fluorescein-labeled lactosylated or mannosylated polylysine. ΣCFTE cells were incubated in the presence of the complexes for 1 hour at 4°C in order to allow their binding to the cell membrane but to prevent their uptake by the cells. Then, the complexes still present in the supernatant were withdrawn and the cells were incubated at 37°C. At various times, from 10 min up to 48 h, the biotinylated DNA was detected with rhodamine-labeled streptavidine, the nucleus with fluorescein-labeled and anti-lamin A/C antibodies