INTRACELLULAR TRAFFICKING OF PLASMID/LACTOSYLATED POLYETHYLENIMINE COMPLEXES AFTER NASAL INSTILLATION IN MICE

Fajac, I.[1]; Aron, Y.[1]; Thévenot, G.[1]; Grosse, S.[1]; Monsigny, M.[2] *1. Physiol. Respir. Cochin, Faculté Médecine Paris 5, Paris, France; 2. Glycobiologie, CBM, CNRS, Orléans, France*

Lactosylated polyethylenimine (PEI) is the most efficient glycosylated PEI for in vitro gene transfer into airway epithelial cells. It promotes the plasmid/lactosylated PEI complex entry into the airway cells and its endosomal exit, the plasmid/lactosylated PEI complex staying all the time mostly as such. Lactosylated PEI has also been shown to keep the efficiency of the unsubstituted PEI for in vivo gene transfer into respiratory cells after nasal instillation in mice and interestingly to elicit a lower toxicity. Our aim was to study the in vivo intracellular trafficking of plasmid/lactosylated PEI complexes in respiratory cells after nasal instillation in mice. Lactosylated PEI (25 kDa; branched form) was generated by substituting about 5% of the amino groups with lactosylthiocarbamoyl units. The fluorescein-conjugated lactosylated PEI (3.75 µmol) was mixed with a biotinylated plasmid (100 µg) in a final volume of 150 µl of a (5g per 100 mL) glucose solution (N/P ratio of 12.5). These complexes were instilled intranasally into briefly anesthetized five-week-old female BALB/c mice. Three, 8 or 24 hours later, the animals were killed and the lungs were excised in order to study the intracellular localization of the complexes. The biotinylated plasmid was labeled with a streptavidin conjugate, the intracellular organites were labeled with specific antibodies and the localization of the complexes was analyzed by confocal microscopy. Three hours after nasal instillation, the plasmid DNA was always observed as a complex with the lactosylated PEI. Some complexes were shown to be localized within lysosomes (labeled by anti-LAMP1 antibodies) and some seldom observed in the nucleus (labeled by anti-lamin A/C antibodies). Eight hours after nasal instillation and in contrast with the in vitro situation, a dissociation between the plasmid DNA and the lactosylated PEI was observed in many cases. Lysosomal and nuclear localizations were higher than that observed after 3 hours. However, the nuclear localization was mostly observed for lactosylated PEI. Twenty-four hours after nasal instillation, the dissociation between the plasmid and its vector was seen for the majority of complexes. Lysosomal localization was massive, while nuclear localization was still low. Additional studies are ongoing to further analyze the trafficking of the plasmid. However, these initial results show that the intracellular trafficking after in vitro and in vivo administrations of similar complexes may vary and emphasize the need for thorough in vivo studies.

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