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GLYCOSYLATED POLYETHYLENIMINES FOR IN VIVO GENE DELIVERY IN THE MOUSE LUNG

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Branched and linear polyethylenimines (PEI) of low molecular weight have been proved to be efficient vectors for in vitro and in vivo gene transfer into respiratory cells. Glycosylated PEIs were shown to enhance in vitro gene transfer by favoring the complex entry into the airway cells and by eliciting a lower cytotoxicity as compared to the unsubstituted polymer. The aim of our study was to evaluate the in vivo efficiency of gene transfer mediated by glycosylated PEIs in the mouse lung. Several glycosylated PEIs (25 kDa; branched form) were generated by substituting about 5% of their amino groups with either lactosylthiocarbamoyl, glucosylthiocarbamoyl or mannosylthiocarbamoyl units. The requested amount of glycosylated PEI or unsubstituted PEI (25kDa, branched form or 22 kDa, linear form, ExGen®) was mixed with 50, 100 or 150 µg of a plasmid encoding either luciferase or green fluorescent protein (GFP) in a final volume of 100 to 200 µl of various formulation solutions in order to reach the requested N/P ratio. These complexes were instilled intranasally into briefly anesthetized five-week-old female BALB/c mice. At the time of the gene transfer analysis step, the animals were killed and the lungs and trachea were used to estimate the gene transfer efficiency. For all the vectors tested, no luciferase activity was observed in the trachea. In the lungs, for all 25 kDa PEI derivatives, the highest luciferase activity (around 10^4 RLU/mg proteins) was observed 48 hours after complex instillation and with complexes generated in a 5% glucose solution, with 100 µg of plasmid in a final volume of 150 µl. For each PEI derivative, the optimal N/P ratio was in the 10 to 15 range. A slightly higher luciferase activity was observed with complexes made with linear 22 kDa PEI (10^5 RLU/mg proteins) than with those made with 25 kDa branched PEI derivatives. However, the GFP expression was similar for all the vectors tested: few cells expressed GFP and they were more often observed in the lung than in the airway epithelium. Glycosylated PEIs were less toxic than unsubstituted 22 and 25 kDa PEIs which were the cause of the death of some mice. Complexes made with fluoresceinylated and lactosylated PEI were also instilled. They were found to be present in many pulmonary cells, indicating that the entry into those cells is probably not a limiting step. The identification of the type of respiratory cells which are most readily transfected and the study of the intracellular limiting steps in vivo are ongoing.

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