8 Abstract

At the moment, there are two main strategies for gene transfer: viral and nonviral gene transfer. In particular among the advantages of synthetic gene carriers over recombinant viruses is the lack of immune response due to the absence of viral peptides and proteins. In addition, uncontrolled insertion of genes into the genome after viral gene transfer may lead to mutagenesis. Such side effects have not been observed for synthetic gene carriers indicating an improved safety profile as compared to recombinant viruses. However, *in vivo* gene transfer efficiency of synthetic gene carriers is far lower as compared to recombinant viruses. This problem also exists concerning gene transfer into the lung. In this work different strategies were followed to enhance nonviral gene transfer into mice lungs and to improve the kinetic of gene expression. Additionally, the influence of gene transfer on lung function of mice was determined.

One strategy of gene transfer to the lung is to use PEI-pDNA complexes, which can be applied via aerosol or intranasal instillation. It was shown that stability of PEI-pDNA complexes is dependent on the pH. The highest possible concentration is 1mg/ml. The pDNA-clearance out of lungs was 3.5-times higher after intranasal instillation of PEI-pDNA complexes to mice compared to the clearance after aerosol application. Thus, clearance is dependent on the way of application.

Furthermore, a quick increase of the amount of cells in BALF was observed after intranasal instillation. 90% of cells in BALF were alveolar macrophages. Activated alveolar macrophages were increased only moderately 24 h after aerosol application. After intranasal instillation, activation of macrophages was enhanced and longer lasting. Activated macrophages probably contributed to the higher pDNA-clearance via phagocytosis.

Luciferase expression as a sign for effective gene transfer was observed only 24 h after intranasal instillation. After aerosol application, the highest expression rate was detected after 72 h and

remained at high levels up to day seven. This result is consistent with the lower clearance rate after aerosol application. Interestingly, luciferase expression could be further enhanced in experiments, which used CpG-depleted pDNA for aerosol gene delivery. 1 h and 24 h after application of CpG-depleted pDNA, luciferase expression was 10-times higher compared to the expression after aerosol delivery of pDNA containing CpG motives. After 72 h gene expression still was 3-times higher. Thereafter, the same levels of expression were detected after aerosol delivery of CpG-depleted pDNA and pDNA containing CpG-motives.

The results of these experiments suggest the following advantages of aerosol application of PEI-pDNA complexes - also regarding a possible clinical usage: i) Reduced clearance rates and, therefore, longer lasting gene expression may result in reduced frequencies of application. That might increase the compliance rate. ii) Reduced doses are possible. This might result in reduced cell infiltration and macrophage activation in the lung (fewer side effects). iii) The inhalation of medical aerosols is widely used avoiding invasive treatment of lung disorders.

For the first time, this work examined the influence of PEI-pDNA complexes on the lung function of mice. Lung histology of mice has shown signs of inflammation 24 h after aerosol application as well as after intranasal instillation. However, inflammation was more severe after intranasal instillation compared to aerosol application. In control experiments mice inhaled water and CpG-depleted pDNA, respectively. In this case, inflammation could not be detected by histology.

Concerning lung function parameters, 1 h after aerosol application and 1 h after intranasal instillation of PEI-pDNA complexes or water, compliance of lungs was reduced. This may be due to the hypoosmotic effect of PEI-pDNA complexes formulated in distilled water. 24 h after aerosol application of PEI-pDNA complexes, the compliance of mice lungs was comparable to values of untreated control mice.

At later time points after aerosol application of PEI-pDNA, compliance was reduced again. This may be due to an inflammatory effect caused by the treatment with PEI-pDNA complexes. As described above this was also observed in histological examinations and may be caused by CpG motives in the pDNA used for the treatment. After aerosol application of CpG-depleted PEI-pDNA complexes, lung function of mice was less impaired. Hence, the usage of CpG-depleted pDNA also resulted in improved lung function parameters, beside increased gene expression levels.

Improved lung function parameters were also found with aerosol application compared to intranasal instillation. After intranasal instillation of PEI-pDNA complexes and distilled water, respectively, the compliance of mice lungs was reduced at every time point of measurement. The restricted lung function in this case may be caused by the high amount of hypoosmotic liquid, which damages the lung tissue, also detected in histology.

Reduced compliance after intranasal instillation and at late time points after aerosol application of PEI-pDNA complexes containing CpG-motives is also reflected in reduced values of further lung function parameters as elastance and tissue damping. However, the resistance of the airways was not changed significantly after aerosol application and intranasal instillation, respectively, compared to values of control mice. Altogether impairment of lung function is mainly observed in lung tissue itself and not in afferent airways.

Summarizing, compared to intranasal instillation aerosol application of PEI-pDNA complexes is more convenient due to better clearance and kinetic profiles, less influence on lung function parameters, fewer side effects and higher gene expression rates. These results can even be improved if CpG-depleted pDNA is used for the treatment. Additionally, the histology of mice lungs was not changed after aerosol application of CpG-depleted PEI-pDNA.

To further improve gene delivery into the lung the targeted aerosol delivery to the lung with aerosol droplets comprising superparamagnetic iron oxid nanoparticles (SPION) – so called nanomagnetosols – in combination with a target directed magnetic gradient field was examined.

Targeted aerosol delivery to the affected lung tissue may improve therapeutic efficiency and minimize unwanted side effects. PEI-pDNA complexes were formulated together with SPION, whereas no bond between PEI-pDNA complexes and SPION could be detected. It was shown, that magnetic deflection of SPION in mice lungs was possible and a targeted and increased deposition of pDNA was achieved. Lung function was not deteriorated additionally by the usage of SPION.

In mice wearing a permanent magnet fixed on their fur above the lungs and which were placed into a aerosol chamber, a 4-times increased gene expression could be detected after aerosol application of SPION together with PEI-pDNA (in a ratio of 125:1) compared to mice without a permanent magnet. However, expression was 7.5-times higher after aerosol application of PEI-pDNA without SPION and without magnet. In *in vitro* experiments using a lung cell line the optimal ratio of 15:1 (SPION/pDNA) for gene expression after delivery with magnetic forces was determined. At higher and lower ratios, respectively, gene expression was reduced.

In further *in vivo* experiments using SPION/pDNA in a ratio of 15:1 a 1.5-times higher deposition of pDNA could be detected in mice lungs wearing a permanent magnet compared to mice without a magnet. However, deposition in mice lungs was again 5-times higher after application of PEI-pDNA without SPION and without any magnetic field. After aerosol delivery of PEI-pDNA together with SPION gene expression could not be detected. It is possible that the results of *in vitro* experiments are not transferable to the situation *in vivo* and another ratio of SPION/pDNA could provide better results.

In future studies, which are already ongoing using pigs as animal model, SPIONs should be optimized and the influence of SPION on the clearance of pDNA by alveolar macrophages should be examined in detail. In this work a higher clearance rate of pDNA out of lung tissue – maybe due to the combination with SPION - was observed. The abolishment of the adverse effects due to the combination of SPION with PEI-pDNA complexes observed in this work could afterwards result in higher and targeted deposition of pDNA after aerosol application in combination with a target directed magnetic gradient field. This would be a great improvement compared to existing methods.

Furthermore, in this work a new method to increase gene transfer efficiency was established, which is based on the usage of the glucocorticoid receptor (GR) as transcription factor. After activation of GR by dexamethasone (or comparable ligands), GR is transported from the cytoplasm into the nucleus of a cell. pDNA containing a glucocorticoid responsive element (GRE) can bind to GR in the cytoplasm. Therefore, binding of the GR to its GRE and activation by its ligand (dexamethasone) can be used for the active transport of pDNA from the cytoplasm into the nucleus.

In *in vitro* experiments an increased number of cells expressing EGFP was detected after the application of pEGFPLucGRE$_2$ and dexamethasone compared to pEGFPLuc and dexamethasone. In *in vivo* experiments using mice, which received pEGFPLucGRE$_2$ complexed with liposomes containing dexamethasone intravenously, an increased expression of luciferase (2.5-times) in lungs could be detected compared to mice which received pEGFPLuc-liposomes containing dexamethasone. In further experiments PEI-pDNA complexes were applied to mice via aerosol. After application of pEGFLucGRE$_2$ gene expression increased about 4.7-times compared to pEGFPLuc, if dexamethasone was injected intraperitoneally 2 h after aerosol application.

Thus, it is possible to increase gene transfer and gene expression *in vivo* by glucocorticoid receptor induced transport of plasmids containing GRE into the nucleus. In future studies this method could be improved by the optimization and detailed examination of decomplexation of pDNA complexes in the cytoplasm. Afterwards, the interval between the application of gene transfer complexes and dexamethasone could be further improved. Furthermore, the anti-inflammatory effect of dexamethasone should be examined regarding a synergistic effect on gene transfer efficiency into the lung.

Taken together this work shows, that it is possible to improve non viral gene transfer efficiency into the lung of mice in a compliant way by targeted aerosol application in combination with a magnetic gradient field and/or the usage of glucocorticoid receptor as transcription factor. One challenge in the future will be to transfer the method of aerosol application of gene transfer complexes with or without magnetic gradient field or the application of GR as transcription factor

to a big animal model and finally to patients and to optimize these methods. In particular, CpG-depleted pDNA should be used because tolerance and gene transfer efficiency are increased.