Intratracheal Gene Delivery into the Mouse Model of Cystic Fibrosis Using B-H Polypex

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ABSTRACT SUMMARY

We have been conducted to develop a new method of intratracheal gene delivery using polyplex nanomicelles consisting of PEG-P[Asp-(DET)] and plasmid DNA for intractable lung disease. The nanomicells used in the previous study showed high gene introduction efficiency, this gene delivery method has been showed a useful tool for intratracheal administration [1]. In this paper, in order to develop the vector for the treatment of cystic fibrosis, the model animals were tested to deliver the therapeutic gene and to reduce inflammatory response for the future clinical use. The nanomicells having N/P ratios in block-homo combinational polyplex (B-H polymer) were examined in vitro and in vivo. High expression levels of CFTR mRNA were seen in the pulmonary epithelial cells from CFTR-KO mice, resulting in decreased expression of IL-6. The lung tissue having pneumonia induced by LPS administration in CFTR-KO mice showed high expression of CFTR, and decreased cytokine expression after introduction of CFTR gene by B/H polymer. By using B/H polymer, sufficient and safe introduction of therapeutic gene was observed.

INTRODUCTION

Cystic fibrosis (CF) is a genetic disorder of the autosome recessive heredity and has the genetic mutation in a CFTR gene. The main cause of death of the patient is a respiratory tract disorder caused by abnormality of the water metabolism (dyspnea, infectious disease). In the present time, since there is no useful therapy, gene therapeutic approach is highly expected. For functional improvement of CF pathologic condition, transduction of CFTR gene to tracheal epithelial cells in patients, repetition of intratracheal administration and safety gene delivery are demanded.

Lung tissue has been known to have a strong immune defense and the mucosa is mechanically protected to exogenous substances by mucinous components. Therefore, immune response induced by intratracheal administration is a suggestive issue. Although there are some reports in therapeutic approaches using viral vectors (adenovirus, Sendai virus and so on), immunogenicity of the vector itself and difficulty of repetition treatment are considered to be the problem. We have been attempted gene delivery using PEG-P[Asp-(DET)] as a non-viral delivery systems to lung tissue.

Although PEGylated polyplexes appeared to be safe, an excess ratio of polycation to pDNA was needed to obtain sufficient transgene expression, which may cause toxicities shortly after gene introduction. In the present study, we investigated the combined use of two polymers, PEG-block-PAsp(DET) (B) and homo PAsp(DET) (H) across a range of mixing ratios to construct polyplexes. Gene expression and toxicity in mice for CF model treated with CFTR gene in B/H polymer were measured. In addition, acute inflammatory response of the lungs was evaluated in LPS-induced pneumonia of CFTR-KO mice receiving B-H polyplex nanomicelles and plasmid DNA.

EXPERIMENTAL METHODS

In vitro gene delivery

The tracheal epithelial cells were obtained from CFTR-KO mice and cultured. CFTR expression vector was transfected into the cells by polyplex nanomicells with block
polymer or B/H polymer (1:1) with N/P ratio of 8. Gene expression of CFTR and IL-6 was measured by Real-Time PCR.

In vivo gene delivery

In order to induce pneumonia usually occurs in CFTR patients which worsens their prognosis, lipopolysaccharide (LPS) was administered. Two days after, CFTR gene was intratracheally transfected with B/H polyprex nanomicelle (N/P=8). The repeated administration was also tested. The treated lungs were excised to measure the gene expression of CFTR and inflammatory cytokines and to have histopathological examination. The AST, ALT, BUN and Cre in the blood were also measured.

RESULTS AND DISCUSSION

In vitro gene delivery

Gene expression levels of CFTR transfected into the pulmonary epithelial cells from CFTR-KO mice by B/H polymer showed higher than block polymer with N/P ratio of 8. The expression levels of IL-6 mRNA increased after 4h and 8h, and then decreased. Compared to the cells transfected with reporter gene, the cells expressed lower levels of IL-6 mRNA. These results suggest that introduction of CFTR gene by B/H polymer ameliorated the inflammatory response in the epithelial cells from the CFTR-KO mice.

In vivo gene delivery

After administration of LPS intraperitoneally, massive pneumonia was observed (Fig. 1). By administration of CFTR gene into LPS treated CFTR-KO mice by B/H polymer, significant amount of CFTR gene expression was observed. The expression levels of cytokines showed decrease compared with that the control vector was transfected. The survival rate was improved in the animals transfected with CFTR suggesting that introduction of CFTR as a therapeutic delivery showed favorable effect in CFTR mouse models. In order to use clinically, the repeated transfection was introduced efficiently without any additional inflammation.

CONCLUSION

By using B/H polymer, more efficient gene transfer was obtained with lower N/P ratio, which decreased inflammatory response in the gene transfected lung. These findings may pave the way for clinical application.

REFERENCES


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