Reversible permeability enhancement of DNA-loaded chitosan oligosaccharide nanoparticle across Calu-3 epithelial cell model for airway gene delivery

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ABSTRACT SUMMARY

DNA-loaded chitosan oligosaccharide nanoparticles were formulated using ionic gelation method. Trans-epithelial electrical resistance (TEER) and permeability experiments reveal a transiently significant, reversible permeability change across Calu-3 cell layers, an airway epithelial cell line. One possible mechanism involves the tight junction opening effect, which allows safe and efficient pulmonary gene delivery system development.

INTRODUCTION

Natural biodegradable mucoadhesive polymer chitosan can translocate the tight junction proteins ZO-1 and occludin, and thereby open the tight junction complexes to facilitate paracellular pathway of drug transport in addition to the conventional transcellular route. However, relatively little work has been done to study the potential of the low molecular, water soluble chitosan as nano drug carriers compared to the more commonly used high molecular weight chitosan.

The human lung adenocarcinoma originated Calu-3 cell line can produce many differentiated, functional human airway epithelial cell features and is one of the few respiratory cell lines that are known to form tight junctions in vitro. Pulmonary epithelium Calu-3 cells have thus become a principal representative cell model of airway epithelial barrier which mimic the major barrier hindering efficient intranasal or pulmonary drug delivery. Increased applications of Calu-3 cell layers in recent years have continuously validated its particular suitability for the airway drug delivery and toxicological research.

Some morphological and permeability differences have been observed when Calu-3 cells are grown under liquid-covered culture (LCC) or air-interfaced culture (AIC). In brief, Calu-3 cells under AIC condition tend to exhibit more morphologically similarity to the airway epithelium than under LCC condition. Herein we demonstrate the great potential of low molecular weight chitosan formulated DNA-loaded nanoparticles induced epithelium permeability enhancement on Calu-3 cell model under both LCC and AIC conditions.

EXPERIMENTAL METHODS

Herring sperm DNA-loaded chitosan oligosaccharide (oligoCS, 3 kDa) nanoparticles were prepared with cross-linker thiamine pyrophosphate (TPP) by ionic gelation approach at a weight ratio of chitosan: DNA: TPP = 50:1:25 which showed no significant cytotoxicity at oligoCS concentration of 1 mg/ml (data not shown).

Calu-3 cells were first seeded on the Transwell inserts at a density of 10,000 cells per well, air-liquid interface was created on day two. After 10 to 14 days of AIC or LCC cell culture, Calu-3 cell layers were confluent and eligible for TEER and permeability experiments.

TEER was measured using Millicell® ERS-2 Epithelial Volt-Ohm Meter (Millipore Corporation, USA) up to 48 h after oligoCS solution or nanoparticles were added. Polar lucifer yellow dye was used as a membrane and junctional integrity marker for paracellular route permeation study. fluorescein isothiocyanate (FITC) labeled DNA loaded oligoCS nanoparticles were prepared using the method reported by Hu et al. Lucifer yellow together with oligoCS solution or nanoparticles, or FITC-labeled nanoparticles (1 mg/ml) were added to the apical side of the cell layer respectively and incubated throughout the entire
24 h experiment when basolateral solutions were sampled at different time points for fluorescence intensity detection. Finally, the permeability of lucifer yellow or FITC-labeled nanoparticles was calculated accordingly.

RESULTS AND DISCUSSION

Permeability experiments of FITC-labeled nanoparticles and lucifer yellow (Figure 1) clearly showed that they could be transported across the Calu-3 monolayer due to the significantly improved cell permeability under both AIC and LCC conditions. Both oligoCS solution and DNA-loaded oligoCS nanoparticles were able to reduce the TEER rapidly (Figure 2), probably by opening the tight intercellular junctions. This penetrating enhancing effect was temporary and reversible since the TEER returned back to 80% of the original baseline level within 48 h, providing an implication that no permanent damage was occurred to the integrity of cell membrane and tight intercellular junctions. Comparing to the oligoCS solution, the oligoCS nanoparticles exerted a greater extent of cell permeability enhancement at the equivalent concentration with its additional advantage of efficient DNA incorporation, ensuring controlled nucleic acid release across epithelial surface of the airway.

CONCLUSION

Significant permeability enhancement on Calu-3 cell layers was observed after DNA-loaded oligoCS nanoparticles administration, along with a follow-up desired cell permeability recovery within 48 h. The result implies that the tight intercellular conjunction complexes could be temporarily open to aid the paracellular drug absorption, which suggests a potential of safe and specific lung targeted nucleic acid drug delivery development.

REFERENCES

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![Figure 1. The permeability% of DNA-loaded FITC-labeled oligoCS nanoparticles and lucifer yellow across Calu-3 cell monolayer (n=3).](image1)

![Figure 2. Reversible TEER of oligoCS nanoparticles and solution on Calu-3 cell layers (n=2).](image2)