Immunomodulation of Cystic Fibrosis Epithelial Cells via NF-κB Decoy Oligonucleotide Coated Polysaccharide Nanoparticles

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Statement of Purpose: Recently, small nucleic acids in the form of siRNA, transcription factor decoys, and aptamers have been explored for use as therapeutic and diagnostic agents. However, advances in treatment are hindered by a lack of stability in physiological environments and a low degree of cellular penetration. Nanosized carrier systems have been suggested to improve stability and cellular uptake of small nucleic acids. Zhang et al. recently reported the preparation of a polysaccharide-based carrier system via complexation of polysialic acid (PSA) and N-trimethyl chitosan (TMC) with a small size (~100 nm) and positive surface amenable to ODN delivery [1]. Here we explore the use of Nuclear Factor Kappa B (NF-κB) decoy oligonucleotide (ODN) delivery to cystic fibrosis (CF) cells via PSA-TMC nanoparticles. NF-κB plays a crucial role in transcription and activation of pro-inflammatory mediators and chemo-attractants and is suspected to be a critical factor in diseases hallmarked by inflammation, including CF. PSA-TMC nanoparticles were coated with NF-κB decoy ODN, and the ability of the ODN-coated nanoparticles to reduce the inflammatory response associated with cystic fibrosis *in vitro* was demonstrated.

Methods: Nanoparticles were prepared as previously described via ionic complexation of N-trimethyl chitosan (TMC) with polysialic acid (PSA) in the presence of TPP [1]. After complexation ODN was added to the mixture and stirring was continued, allowing association of negative ODN with the positive PSA-TMC surface. A pellet of nanoparticle was obtained by centrifugation at 3000 RPM for 15 minutes. ODN loading capacity and loading efficiency were determined by preparing nanoparticles coated with a Rhodamine labeled ODN. A calibration curve was generated based on fluorescent intensity, and supernatant fluorescence readings were used to determine the amount of ODN associated with the nanoparticles in the pellet. Size and zeta potential were determined using a Malvern Zetasizer Nano. Once isolated, ODN-PSA-TMC nanoparticles were resuspended in serum-free LHC-8 media and administered to IB3-1 CF lung epithelial cells at a concentration of 1 mg/ml. Lipofectamine 2000 transfection reagent was used as a control. Additional controls included ODN alone, PSA-TMC alone and PSA-TMC coated with a non-coding oligonucleotide. All conditions were incubated for 4 hours, removed, and replaced with fresh LHC-8 media. 24 hours after media replacement, cells were stimulated with either interleukin-1β (IL-1β, 2.5 ng/ml) or lipopolysaccharides (LPS, 10 μg/ml). Supernatant samples were collected at 24 and 48 hours, and ELISA was used to determine levels of interleukin-6 (IL-6) and interleukin-8 (IL-8) secreted. Additionally, cell number at the time of sample collection was determined by DAPI staining.

Results: ODN coated PSA-TMC nanoparticles were successfully formed, and exhibited a size and zeta potential of about 160 nm and 28 mV, respectively. Although there was a slight increase in size, as expected, the polydispersity index was not different from uncoated PSA-TMC, indicating that the addition of ODN on the surface does not affect nanoparticle stability. The loading capacity and loading efficiency were determined to be 0.766 μg/mg and 76.6%, respectively. Efficacy studies based on pro-inflammatory protein levels showed ODN-PSA-TMC is an effective means of ODN delivery. ODN-PSA-TMC administration resulted in significantly lower levels of IL-8 after 48 hours in the presence of both IL-1β and LPS stimulation as shown in Fig. 1 and 2. ODN-PSA-TMC effectively lowered IL-6 levels in response to IL-1β stimulation as well; however, the decrease was not great enough to be considered significant. A significant decrease in IL-6 is observed when LPS is the inflammatory stimulant.

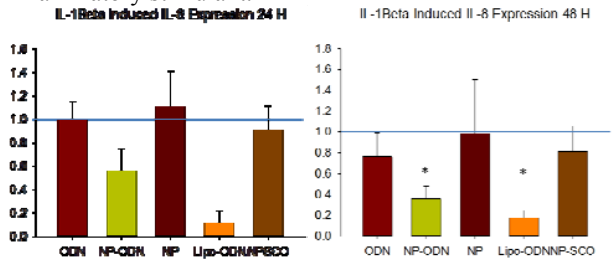

Fig.1. IL-8 levels in response to IL-1β, * represents $p<0.05$

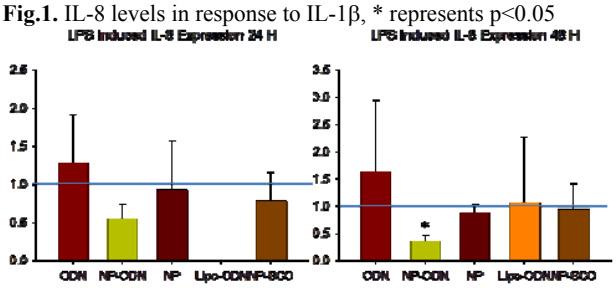

Fig.2. IL-8 levels in response to LPS, * represents $p<0.05$

Conclusions:

PSA-TMC coated with ODN are stable nanoparticles with good loading ability. Unlike cationic transfection reagents, including Lipofectamine 2000, PSA-TMC-ODN complexes were not cytotoxic, and a exhibited a time dependent effect on ODN mediated cytokine reduction in response to administration. Furthermore, TMC itself may have an anti-inflammatory effect when combined with LPS induced inflammation. The exact mechanism behind this anti-inflammatory effect is not known, although the idea has been proposed previously[2].

References

1.Zhang N., B.R., *Synthesis and characterization of polysialic acid-N trimethyl chitosan nanoparticles for drug delivery.* NanoLife, 2012.

2.Ji Q., et.al., *Modulation of pro-inflammatory mediators in LPS-stimulated human periodontal ligament cells by chitosan and quaternized chitosan.* Carbohydrate Polymers, 2013. **92**: p. 824-829.