Modulation of the immune response via the NF-κB signaling pathway in rheumatoid arthritis *in vitro* models

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Statement of Purpose: Rheumatoid Arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the joints. Currently, the cause of the disease is unknown and a cure does not exist, leaving treatment options focused on mitigating symptoms and slowing disease progression. Traditional treatments include chemotherapeutics and immuno-suppressants, as well as corticosteroids and NSAIDs. Methotrexate (MTX) is considered to be the gold standard for RA treatment; however, this drug is associated with severe side effects and high degrees of variable efficacy between patients. Furthermore, the mechanism of action of MTX in RA treatment is not fully understood.

Decoy oligonucleotides (ODN) for NF-κB, a transcription factor highly involved in regulating inflammation, have been suggested a potential treatment option. However, despite moderate efficacy with local administration, ODNs and other small nucleic acid based therapeutics, including siRNA and aptamers, have not been effective with clinical systemic administration. The two main barriers to effective ODN administration are instability due to high enzymatic degradation and a low degree of membrane permeability due to their negative charges. We have previously reported a polysaccharide based nanoparticle system for delivery of ODNs in a cystic fibrosis *in vitro* model [1]. Here, we have modified the nanoparticle system for RA treatment with the incorporation of MTX, as we expect the drug will act synergistically with the decoy ODN.

Methods: Nanoparticles were prepared as previously described via ionic complexation of N-trimethyl chitosan (TMC) with polysialic acid (PSA) in the presence of TPP. Following complexation, ODNs were added to the mixture to facilitate surface coating and stirring was continued. MTX loaded nanoparticles were prepared by including MTX in the PSA mixture during ionic complexation. A pellet of nanoparticles was obtained by centrifugation at 3000 RPM for 15 minutes. Size and zeta potential were determined using a Malvern Zetasizer Nano.

To conduct *in vitro* studies, nanoparticles coated with ODN (NPODN), nanoparticles loaded with MTX (NPMTX), and nanoparticles loaded with MTX and coated with ODN (NPODNMTX) were resuspended in serum free DMEM media at a concentration of 1 mg/ml and added to SW982 synovial sarcoma cells. ODN and MTX alone (1.0 mg/m) were used as controls. After 4 hours of treatment, the media was removed and replaced with fresh DMEM media containing 10% FBS. 24 hours subsequent to media replacement, cells were stimulated with interleukin-1β (IL-1β, 1.0 ng/ml), a proinflammatory cytokine. Supernatant samples were collected at 24 and 48 hours, and ELISA was used to determine the levels of interleukin-6 (IL-6) and interleukin-8 (IL-8) secreted. All values were expressed relative to unstimulated control cells. One way ANOVA followed by Holm-Sidak step-down comparisons was used to determine significance with an alpha of 0.05.

Results: ODN coated PSA-TMC nanoparticles, with and without encapsulated MTX, were successfully formed. Nanoparticles without methotrexate possessed a size and zeta potential of 166 ± 5 nm and 23 ± 4 mV, respectively, while those with methotrexate possessed a size and zeta potential of 184 ± 5 nm and 30 ± 6 mV, respectively. The polydispersity index was similar to the low value previously obtained for uncoated PSA-TMC nanoparticles [2], suggesting the addition of ODN to the surface and encapsulation of MTX does not affect nanoparticle stability.

After IL-1β stimulation, SW982 cells had significantly lower levels of IL-6 (**Fig. 1**) and IL-8 (**Fig. 2**) secretion when treated with NPODNMTX at 24 and 48 hours relative to untreated control cells. In addition, NPODN and NPMTX treatment yielded in significantly lower levels of IL-6 (**Fig. 1B**) and IL-8 (**Fig. 2B**) at 48 hours. MTX and ODN alone had minimal, variable efficacy on reduction of inflammatory proteins that was resolved with the addition of the PSA-TMC nanoparticle system.

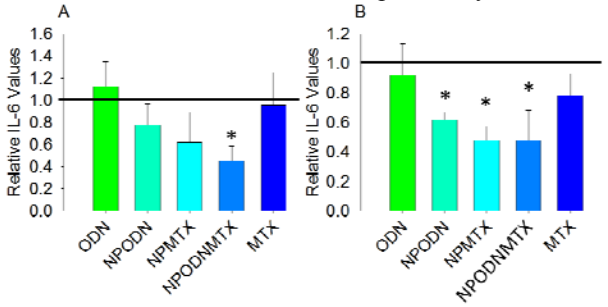

Fig. 1. IL-6 secretion by treated SW982 cells at 24 (A) and 48 (B) hours. . * indicates $p<0.05$ relative to untreated control cells.

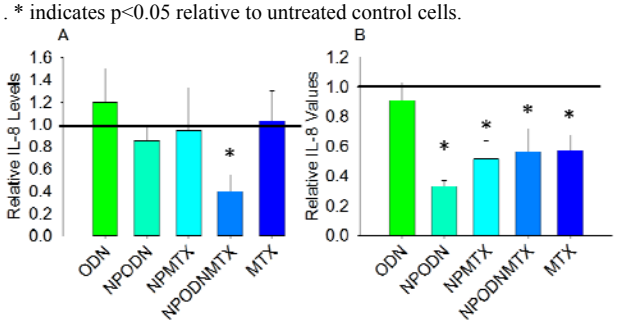

Fig. 2. IL-8 secretion by treated SW982 cells at 24 (A) and 48 (B) hours. * indicates $p<0.05$ relative to untreated control cells.

Conclusions: The results presented here suggest a time dependent anti-inflammatory effect of nanoparticle mediated ODN treatment on SW982 cells. Furthermore, the activity of the decoy ODN appears to enhance the efficacy of MTX, particularly at shorter time points. In the future, the research described herein will be extended to primary RASF cells.

References

1. Wardwell PR. JBMR Part A. 2014:Epub ahead of print.
2. Zhang N. NanoLife. 2012;2:EPub.