Development of Novel Oligonucleotides Delivery Platforms for Inflammatory Bowel Disease Therapeutics

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Antisense oligonucleotides (ASO) are short sequences of nucleic acids ranging between 12 to 25 bases in length. ASOs can modulate gene expression in multiple ways, paving the way for several new therapeutic modalities. However, one of the main hurdles preventing ASOs from widespread use is the high dosages they require to achieve efficacy that may induce adverse effects and result in an unfavorable risk-benefit profile. A possible approach to lower the ASO doses is their encapsulation in lipid nanoparticles (LNPs) that can deliver them to the desired target organ. Here, we report the successful use of LNPs to deliver ASOs for treating intestinal inflammation in mice. Initially, LNPs formulated with eight different novel ionizable lipids were screened for stability and toxicity in multiple cell lines. To assess the potency of the formulations, LNPs were formulated with locked-nucleic acids (LNA), a modified ASO inducing a splice switching of the hypoxia-inducible factor 1 subunit alpha (Hif1α) mRNA to create a completely synthetic copy, allowing for precise detection of LNA-related activity. The same selection process was used to test the *in vivo* activity of three successful formulations upon intravenous administration. We demonstrated that LNA-ASOs-loaded LNPs enabled a 30-fold dose reduction in comparison to unformulated LNA-ASOs. Finally, the most potent formulation (lipid 15-formulated LNPs) was tested for its therapeutic potential in a colitis-induced murine model by encapsulating an ASO specific for TNFα, a key cytokine in Inflammatory Bowel Diseases (IBD) pathogenesis. The TNFα specific LNA-ASO-LNPs significantly alleviated disease burden, reduced pro-inflammatory cytokines, and showed a good safety profile while retaining dose efficiency in the colitis-bearing mice. These results highlight the great potential of using LNPs to precisely deliver LNA-ASOs as therapeutics for inflammatory diseases such as IBD and ultimately may open new avenues as novel therapeutic modalities.