Crossing the blood−brain barrier to deliver antisense oligonucleotides therapeutics using DNA/lysine nanopieces

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Abstract

Background & Aim: Antisense oligonucleotides (ASOs) are single−stranded deoxyribonucleotide oligomers that knock down gene expression at the post−transcriptional level. ASOs can treat a vast array of neurodegenerative diseases but cannot bypass the blood−brain barrier (BBB), necessitating invasive and inefficient intrathecal injections for clinical applications. A drug vehicle that can cross the blood−brain barrier after intravenous injection and degrade safely after delivery would greatly improve the treatment of brain disease. Janus base nanopieces (NPs) are a family of drug vehicles formed by two joined DNA bases attached to a positively charged amino acid tail. Bases non−covalently assemble into long strands of nanotubes that wrap around nucleic acid cargo to disguise negative charge. Here, we used NPs to intravenously deliver ASOs across the BBB in mice and tested their efficiency by knocking down the ubiquitously expressed noncoding RNA MALAT1 via RNase H−mediated degradation.

Methods: A^T Janus bases with a lysine tail were synthesized and mixed with ASOs, self−assembling into NPs after sonication and vortex. Optimal formulation and storage conditions were determined through an iterative process of cell transfection in vitro. Wild type mice were injected with bolus ASO/NP solutions at either 5%, 10%, 50%, or 100% dosage and were euthanized at 3 days, 1 week, or 2 weeks after administration. RNA was isolated from brain samples and MALAT1 mRNA expressions were determined using qPCR.

Results: Nanopieces improved the functional delivery of ASOs across the blood−brain barrier. Most efficient knockdown was observed at 100% dose after 1 week, decreasing MALAT1 RNA levels by 79% to 94% in all brain regions compared to untreated mice.

Conclusion: This model demonstrates that Janus base nanopieces are a potent answer to one of the greatest challenges in neurological ASO development, the blood−brain barrier.

Clinical Implications: Further studies will seek to combine NPs with therapeutically useful ASOs for eventual clinical use in treating neurodegenerative diseases.