Effect of Combined Systemic and Local Morpholino Treatment on the Spinal Muscular Atrophy Δ7 Mouse Model Phenotype

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ABSTRACT

Background: Spinal muscular atrophy (SMA) is a fatal motor neuron disease of childhood that is caused by mutations in the *SMN1* gene. Currently, no effective treatment is available. One possible therapeutic approach is the use of antisense oligos (ASOs) to redirect the splicing of the paralogous gene *SMN2*, thus increasing functional SMN protein production. Various ASOs with different chemical properties are suitable for these applications, including a morpholino oligomer (MO) variant with a particularly excellent safety and efficacy profile.

Objective: We investigated a 25-nt MO sequence targeting the negative intronic splicing silencer (ISS-N1) 10 to 34 region.

Methods: We administered a 25-nt MO sequence against the ISS-N1 region of SMN2 (HSMN2Ex7D [-10-34]) in the SMAΔ7 mouse model and evaluated the effect and neuropathologic phenotype. We tested different concentrations (from 2 to 24 nM) and delivery protocols (intracerebroventricular injection, systemic injection, or both). We evaluated the treatment efficacy regarding SMN levels, survival, neuromuscular phenotype, and neuropathologic features.

Results: We found that a 25-nt MO sequence against the ISS-N1 region of SMN2 (HSMN2Ex7D [-10-34]) exhibited superior efficacy in transgenic SMAΔ7 mice compared with previously described sequences. In our experiments, the combination of local and systemic administration of MO (bare or conjugated to octaguanidine) was the most effective approach for increasing full-length SMN expression, leading to robust improvement in neuropathologic features and survival. Moreover, we found that several small nuclear RNAs were deregulated in SMA mice and that their levels were restored by MO treatment.

Conclusion: These results indicate that MO-mediated SMA therapy is efficacious and can result in phenotypic rescue, providing important insights for further development of ASO-based therapeutic strategies in SMA patients. (*Clin Ther.* 2014;36:340–356)

Key words: morpholino oligomer, SMAΔ7 mice, spinal muscular atrophy, survival motor neuron, therapy.

INTRODUCTION

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disease characterized by the degeneration of motor neurons in the spinal cord.[1] It results in progressive muscle weakness and atrophy

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and is one of the most common genetic causes of infant mortality.[1] SMA occurs due to mutations in the *SMN1* gene, which lead to reduced SMN protein levels.[2] The SMN protein, gemins 2 to 8, and the unr-interacting protein together form the SMN complex, which is required for small nuclear ribonucleoprotein particle (snRNP) assembly and metabolism.[3–5] Spliceosomal snRNPs (U1, U2, U4, U5, U6, U11, U12, U4atac, and U6atac) combine with numerous splicing factors to form the spliceosome, which mediates the removal of introns from primary mRNA transcripts.[6,7] Thus, the SMN reduction that occurs in SMA directly affects snRNP assembly, leading to snRNPs to chiometric disequilibrium and ultimately resulting in misprocessing of certain pre-mRNAs.[8] Interestingly, this change in small nuclear RNA (snRNA) levels is cell type specific because the same snRNPs are not identically affected in all cell types.

In addition to *SMN1*, the human genome harbors the paralogous gene *SMN2*, which essentially differs from *SMN1* by a single C-to-T transition in exon 7 that modifies a splicing modulator and causes exon 7 exclusion in 90% of SMN2 mRNA transcripts.[9] The SMN protein lacking exon 7 does not oligomerize efficiently and is rapidly degraded, reducing SMN levels. The *SMN2* gene also produces 10% full-length SMN protein,[9] which is a major modulator of SMA clinical phenotype. Patients with a lower SMN2 copy number have severe SMA (type I, infantile), whereas patients with higher SMN2 copy numbers have a milder form (types III-IV, juvenile or adult onset).[9]

Currently, no effective therapies are available for SMA. Because SMA is caused by reduced SMN protein levels, most treatments have focused on increasing the amount of SMN, with strategies that include *SMN2* transcription promotion with drugs or small molecules and *SMN1* gene replacement using viral vectors carrying wild-type *SMN1*.[10] In transgenic mice with a severe SMA phenotype (SMAΔ7 mice), treatment with adenoassociated viral vectors encoding the human wild-type SMN protein results in phenotypic rescue with a log-fold increase in median survival (from weeks to more than a year).[11–14] This result is quite remarkable because this animal model has previously invariably survived no more than 2 weeks and has historically been refractory to any therapeutic attempt.[10]

An alternative promising molecular approach for SMA treatment is the modulation of SMN2 mRNA splicing to restore functional protein production.[15] Such an effect can be achieved with antisense oligos (ASOs), which are nucleotide acids analogs that can bind mRNA intronic and exonic sites and thus modify splicing events.[15] Numerous regions are involved in SMN2 splicing regulation, one of which is the negative intronic splicing silencer (ISS-N1), a 15-nucleotide splice-silencing motif located downstream of *SMN2* exon 7. ASOs targeting the ISS-N1 region promote the inclusion of exon 7 without off-target effects.[16,17] It has been hypothesized that hybridization of ASOs to the ISS-N1 region displaces trans-acting negative repressors and/or unwinds a cis-acting RNA stem-loop that interferes with the binding of U1 small nuclear RNA at the 5′ splice site of exon 7.[16,18]

Preclinical and clinical studies have examined 2 types of ASO that differ in chemical structure for use in treating human diseases through mRNA regulation: (1) the 2'-*O*-methyl-modified phosphorothioate (2OMePS) oligonucleotides or the more stable variant 2′-O-(2-methoxyethyl)-modified (MOE) phosphorothioate oligonucleotides and (2) the morpholino oligomers (MOs). In the MO ASO, the phosphorothioate-ribose backbone is replaced with a phosphorodiamidate-linked morpholine backbone that is refractory to metabolic degradation. MOs feature low toxicity and have produced encouraging results in clinical trials, such as that for Duchenne muscular dystrophy.[15] In addition, impressive phenotype rescue has been observed in mouse models of SMA after ASO-mediated SMN up-regulation in the central nervous system (CNS) using ASO-10-27 with MOE chemistry,[18] indicating promising potential as a treatment for SMA patients. The name ASO-10-27 is based on its position relative to the exon 7 donor site. Another recent work found the successful correction of SMN2 splicing with ASO-10-29 based on MO chemistry, with associated improvement of the SMA phenotype.[19]

These findings suggest that ASO-induced interference with splicing will likely be one of the first molecular therapies for SMA to reach clinical development. Indeed, ASO from ISIS Pharmaceuticals (Carlsbad, California) is already in a Phase II trial. Given their excellent safety and efficacy profile, MOs are among the most promising candidates for this purpose. However, several critical issues remain to be resolved, including the optimal type of MO chemistry and sequence and the modality of administration. It is unclear whether local injection is sufficient to rescue