MUSCULAR DYSTROPHY

New exon-skipping strategy rescues dystrophin

Duchenne muscular dystrophy (DMD) is a recessive X-linked neurodegenerative disorder caused by mutations in the dystrophin gene. A promising strategy to treat DMD is exon skipping using antisense oligonucleotides (AONs), but approaches so far have been limited by poor tissue uptake. Now, Goyenvalle *et al.* demonstrate that a tricyclo-DNA (tcDNA)-AON rescues dystrophin expression and function in all affected tissues in mouse models of DMD.

Exon skipping using AONs coaxes the cell's transcriptional machinery to skip over a targeted exon, to restore an open reading frame and allow expression of a truncated but functional protein. However, in DMD, recent clinical trials testing either 2′-O-methyl-modified oligoribonucleotides (2′OMes) or phosphorodiamidate morpholino oligomers (PMOs) have failed to show marked clinical benefit, probably owing to insufficient dystrophin rescue. Goyenvalle and colleagues therefore set out to investigate the therapeutic potential of a new DNA analogue, tcDNA — a conformationally constrained oligonucleotide analogue that, compared to natural DNA, has three additional carbon atoms between C5′ and C3′ that result in improved pharmacological properties.

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First, the authors intravenously administered a tcDNA-AON targeting *Dmd* exon 23 to a mouse model of DMD (mdx mice, which carry a nonsense mutation in exon 23 of the *Dmd* gene) for 12 weeks. The tcDNA-AON was stable and detected in all tested skeletal muscles as well as the heart and brain. Quantitative PCR revealed effective skipping of exon 23 to levels 5- to 6-fold higher than those achieved with 2′OMe- and PMO-AONs. Importantly, this translated into a greater rescue of dystrophin protein levels, particularly in the diaphragm and heart (where levels reached 50% and 40%, respectively), than found in wild-type mice. Notably, exon 23 skipping and dystrophin protein expression were seen in the CNS of only those animals treated with tcDNA-AON.

Restoration of dystrophin expression significantly improved the mdx mouse phenotype. The specific force of tibialis anterior muscles was essentially normalized and maintained up to 80% following eccentric contractions (used to measure the structural integrity of muscle fibres). A significant improvement in respiratory function was also noted, to a greater extent than that seen with 2′OMe- or PMO-AON treatment. In addition, echocardiography revealed that the tcDNA-AON significantly improved the ventricular ejection fraction and shortening fraction.

The tcDNA-AON also had beneficial effects in the CNS. Tonic immobility (freezing) resulting from a restraint-induced fear response — a highly reproducible behavioural phenotype of mdx mice that is controlled by central mechanisms — was the same in tcDNA-AON-treated mdx and wild-type mice, whereas the responses of the 2′OMe- and PMO-AON-treated mdx mice did not differ from those of untreated mdx mice.

Intravenous tcDNA-AON treatment for 20 weeks was similarly effective in a more severe mouse model of DMD (mice lacking both utrophin and dystrophin). It partially rescued dystrophin protein expression in all affected tissues, leading to significant phenotypic improvements.

This study shows for the first time an exon-skipping strategy that enables dystrophin rescue and functional improvements in all tissues affected by the lack of dystrophin in mouse DMD models. tcDNA-AON may therefore represent a novel therapeutic strategy for genetic diseases eligible for exon-skipping approaches.

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