Effect of combined treatment with soluble activin receptor IIB and AAV-U7-mediated dystrophin exon skipping on muscle function in mdx mice


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Treatment with the soluble activin type IIB receptor (sActRIIB-Fc) has been shown to increase muscle mass and to improve muscle function in the dystrophin deficient mdx mice. This compound binds and inhibits the activity of several TGFβ signaling proteins, the most important being Myostatin, which is known to act as a negative regulator of muscle mass. The beneficial effect of sActRIIB-Fc in mdx mice points to the potential therapeutic value of sActRIIB-Fc in Duchenne Muscular Dystrophy (DMD) patients. We here hypothesized that combined treatment with sActRIIB-Fc and dystrophin exon skipping may synergistically improve mdx dystrophic muscle function by stimulating muscle growth and restoring dystrophin expression. In this study we therefore determined individual and combined effects of systemic sActRIIB-Fc treatment and local intramuscular AAV-U7-mediated dystrophin exon skipping on force generation in mdx mice. Five weeks of systemic treatment in mdx mice with sActRIIB-Fc (10 mg/kg twice weekly, n = 6) resulted in increased total weight, muscle mass and muscle fiber diameter compared to the saline treated group (n = 6). AAV-U7-mediated dystrophin restoration in dystrophic muscle resulted in a significant increase of specific tetanic force and strongly improved mdx muscle resistance to eccentric contractions. In contrast, sActRIIB-Fc treatment alone did not increase specific tetanic force, although it resulted in a modest improvement in muscle resistance to eccentric contractions. Surprisingly, the combined treatment with sActRIIB-Fc did not increase specific tetanic force, and no additive improvement in mdx muscle resistance to eccentric contractions was observed. In conclusion, our study suggests that in adult mdx mice sActRIIB-Fc does not confer an additional improvement on muscle force when combined with AAV-U7-mediated dystrophin exon skipping. Future studies will be concentrated on determining the effect of combined treatment in the mdx utrn→–/–DMD mouse model.

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Development of systemic antisense treatment in dystrophic mouse models for Duchenne muscular dystrophy


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Antisense-mediated reading frame restoration is one of the most promising therapeutic approaches for Duchenne muscular dystrophy. It uses antisense oligonucleotides (AONs) to induce exon skipping during pre-mRNA splicing of mutated dystrophin transcripts, aiming to restore the disrupted open reading frame allowing synthesis of internally deleted, partly functional Becker-like dystrophin proteins. Proof of concept has been obtained in cultured cells and mdx mice and this approach is currently tested in clinical trials by Prosensa/GSK (GSK2402968 and PRO044) and AVI Biopharma (AVI-4658). Dystrophic animal models allow detailed analysis of pharmacokinetic and pharmacodynamic effects of AONs. The 2′-O-methyl phosphorothioate (2OMePS) chemistry used in the Prosensa/GSK trials has favorable pharmacokinetic properties: the PS backbone binds serum proteins, which prevents clearance by the kidney and increases serum half life. Here, we optimized dosing and maintenance regimes using subcutaneous 2OMePS AON injections in mdx mice. In addition, we tested safety and efficacy of high dose (200 mg/kg/week) AON treatment for up to 6 months in mouse models with varying levels of severity: mdx mice (mild phenotype) and mdx mice with one utrophin allele (mdx+/–; intermediate phenotype). Treatment was well tolerated, liver and kidney weights and serum parameters were similar between AON and saline treated mice at the end of treatment. The therapeutic effect was larger in mdx+/–; mice; exon skip and dystrophin levels were higher, creatine kinase levels were more decreased and rotaror running time was more increased. Results suggest that AON levels in muscles of mdx+/– mice are higher compared to mdx mice, indicating that AON uptake is aided by disease pathology. These results indicate that long term subcutaneous treatment with 2OMePS AONs is safe and efficient in dystrophic mice, which is encouraging for long term trials in patients, recently initiated by Prosensa Therapeutics/GSK.

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