INTRODUCTION

Duchenne muscular dystrophy (DMD) is an X-linked progressive neuromuscular disorder caused by the absence of dystrophin protein. Upon exhaustion of the regenerative capacity of muscle, there is an imbalance between muscle damage and repair. Insulin-like growth factor 1 (IGF-1) plays an essential role in increasing muscle regeneration and growth, reducing inflammation and fibrosis. Thus, IGF-1 is an interesting therapeutic target for enhancing muscle quality and function in muscle wasting conditions. Both in circulation and the extracellular matrix, IGF-1 is bound to one of its binding proteins (IGFBPs). These binding proteins are thought to act by inhibiting the availability of IGF-1 to its receptor, which initiates most of the actions of IGF-1.

OBJECTIVE

Antisense oligonucleotide (AON)-mediated knockdown of IGFBP1 and IGFBP3 aims to increase IGF-1 signaling and thereby it could be a strategy to slow down the disease progression by improving the secondary defects in DMD.

EXPERIMENTAL

- Exon skipping AONs were designed to skip exon 2 of both genes.
- The AONs were tested in mIMCD3 and C2C12 cell lines for IGFBP1 and IGFBP3 downregulation, respectively.
- Exon skipping was assessed by RT-PCR.
- Protein downregulation and its effects on IGF-1 downstream signaling were investigated by Western blot.

RESULTS

4 Z′-O-MePS AONs were designed to skip exon 2 of Igfbp1 and Igfbp3 genes. After testing different concentrations in lipofectamine-mediated transfection, RT-PCR results showed that at least one AON was found effective per gene. IGFBP1 AON showed almost 100% skipping efficiency whereas this was approximately 50% in IGFBP3 AON treated cells at 200 nM AON concentration. The effect of the exon 2 skipping on protein downregulation was analyzed by Western blot.

CONCLUSION

We confirm that AON-mediated downregulation of IGFBP1 and IGFBP3 leads to enhanced IGF-1 signaling, making it a potential therapy for the treatment of secondary defects in DMD. The candidate AONs will be tested in mdx mice to see the in vivo effect of this treatment.