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Molecular Therapeutic Strategies Targeting Duchenne Muscular Dystrophy

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Abstract

Since discovery of the gene for Duchenne muscular dystrophy more than 20 years ago, scientists have worked to apply molecular principles for restoration of the dystrophin protein and correction of the underlying physiologic defect that predisposes muscle fibers to injury. Recent studies provide realistic hope that molecular therapies may help patients who have this disorder. At present only corticosteroids can improve walking ability and increase quality of life for boys with this disease. The results are modest and encumbered by side effects. We review 3 molecular therapeutic approaches that have been introduced into the clinic: (1) gene replacement therapy, (2) mutation suppression, and (3) exon skipping.

Keywords

Duchenne muscular dystrophy; gene therapy; exon skipping; mutation suppression

Introduction

The gene for Duchenne muscular dystrophy was identified more than 20 years ago.^{1,2} At the time, it appeared that new options for boys with this disease would rapidly follow. Of interest, the only beneficial treatment for the disease, corticosteroids, was first described about the same time as isolation and sequencing of the gene.³ Despite clearly expressed doubt as to the potential impact of prednisone on lives of boys with Duchenne muscular dystrophy, the past 2 decades of experience provide a long-term perspective.⁴ Unequivocally, corticosteroids result in life-changing experiences for boys with the disease. The ability to walk is prolonged and activities of daily living are improved.⁵ This comes at a price, because steroid side effects are not trivial.

To combat adverse effects, alternate corticosteroids and different dosing regimens have been introduced. The best example is deflazacort, a sodium-sparing glucocorticoid that has efficacy equal to prednisone but is associated with reduced weight gain.⁶ Some advocate administering very high-dose weekly prednisone (10.0 mg/kg/wk (divided into 2 doses given on Saturday and Sunday) as opposed to daily administration (0.75 mg/kg), with claims of equal or improved efficacy with fewer side effects.⁷ This summarizes the current state of therapy for boys with Duchenne muscular dystrophy. Planned trials will compare

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corticosteroids and dosing regimens to give a clearer picture as we move forward (see ClinicalTrials.gov).

Molecular Therapeutic Approaches

Gene Replacement Therapy

Molecular therapeutic approaches based on the initial gene discovery have been slower to evolve but they are now gaining momentum. Three principal means of altering gene expression are under active investigation. Gene therapy has vigorously been pursued in the laboratory, with initial translational clinical efforts in a handful of patients. A major obstacle for gene replacement therapy is the size of the Duchenne muscular dystrophy gene. Because it is the largest gene in the human genome, it will not fit into the best vehicle for gene transfer, adeno-associated virus.^{8,9} This means the gene has to be reduced in size by more than one-half to fit the limited (~4.7 kb) packaging capacity of adeno-associated virus. In fact, considering the final cDNA for gene transfer with its promoter and poly(A) tail, the gene insert must be reduced by almost one-third of its ideal size to be transferred to muscle by adeno-associated virus. These small or reduced-size Duchenne muscular dystrophy genes are referred to as mini- or micro-dystrophins.^{10–12} In the *mdx* mouse they are capable of robust gene expression and partial correction of the physiologic defect that predisposes dystrophin-deficient muscle to insults from eccentric contraction accompanied by a reduction in force generation.^{13–15} No matter how well mini- or micro-dystrophins work in the dystrophic mouse or dog, the real issue is the limited ability of scientists to predict the potential functional benefit that will accrue from their expression in patients with Duchenne muscular dystrophy.

Initial efforts at translating mini-dystrophin carried by adeno-associated virus to the clinic have led to additional unanticipated obstacles induced by expression of these novel dystrophin isoforms (Mendell JR, *N Engl J Med*, in press). Under specific conditions related to the patient's mutation, an immune response can be induced by transgene expression. In addition, some individuals previously exposed to adeno-associated virus have pre-existing immunity to the virus. These factors can impede gene delivery of adeno-associated virus to muscle or set the stage for rejection of muscle fibers transduced by the virus and its cargo. These are not insurmountable barriers to success but will require further study.

Mutation Suppression

A different molecular approach to treatment is referred to as mutation suppression or stop codon readthrough. This form of treatment applies only to boys with mutations resulting in premature termination codons, estimated to occur in approximately 13% to 15% of the Duchenne muscular dystrophy population.^{16,17} Stop codon readthrough requires the introduction of a nucleotide sequence at the mRNA level, creating a missense mutation (in place of the premature termination codon) that permits translation of the full-length protein.¹⁸

An important question is how well this molecular mechanism can be harnessed to benefit patients who have Duchenne muscular dystrophy. It has been known for decades that aminoglycoside antibiotics possess the unique property of stop codon readthrough in pro- and eukaryotes.^{19,20} The first relevant finding to the Duchenne muscular dystrophy population was the observation that gentamicin-induced mutation suppression in the *mdx* mouse led to expression of full-length dystrophin, accompanied by reduction of serum creatine kinase (CK) and protection against contraction-induced muscle injury.²¹ These findings were highly provocative as a potential therapeutic strategy. Attempts to treat patients with gentamicin initially produced conflicting results,^{22,23} with further resolution in our recently reported study.²⁴ As part of this study, a 14-day protocol showed reduction of

serum creatine kinase by 50%, and an extended treatment regimen of once- or twice-weekly gentamicin for 6 months increased muscle dystrophin levels to 13% to 15% of normal ($P = .027$), accompanied by reduced serum creatine kinase, stabilization of muscle strength, and a slight increase in forced vital capacity. The clinical findings in this gentamicin study demonstrate stop codon readthrough in a clinical setting in Duchenne muscular dystrophy patients. While this is encouraging, functional improvements in timed-tests for walking and climbing stairs was not achieved, suggesting that current gentamicin doses would need to be increased and may be limited by potential renal and auditory toxicity.

A more favorable approach is currently under investigation using ataluren, an oral agent that is much easier and safer to administer to promote mutation suppression.^{25,26} The efficacy of ataluren in animal studies appears promising but clinical trials require further evaluation.

Exon Skipping

Exon skipping represents a third molecular approach to treating Duchenne muscular dystrophy. The concept arose from observations in Duchenne muscular dystrophy muscle biopsies showing that rare dystrophin-positive revertant fibers are the result of spontaneous second-site mutations that skip exons and restore the open reading frame, producing functional dystrophin.^{27–30} Two clinical trials have demonstrated the potential for using antisense oligonucleotides to target specific exon splice sites, leading to correction of the reading frame to yield functional dystrophin. In general, the result will be a truncated dystrophin protein analogous to that found in Becker muscular dystrophy.

In one clinical trial, the antisense oligonucleotide consisted of a 2'-*O*-methyl-modified ribose molecule with a full-length phosphorothioate backbone (2OMePS) that was tested in 4 patients receiving injections into the tibialis anterior muscle.³¹ A biopsy was performed 28 days later. The target for this study was exon 51, and muscle from these patients demonstrated sarcolemmal expression of dystrophin reaching 17% to 35% of normal amounts.

In a separate clinical trial, antisense-induced exon skipping was achieved using a phosphorodiamidate morpholino oligomer, also targeted to skip exon 51. Seven Duchenne muscular dystrophy patients received intramuscular injections to the extensor digitorum brevis, and biopsies at 3 to 4 weeks showed mean intensity of dystrophin staining increased to 26.4% of controls.³² No adverse effects were seen in either study. These exon-skipping antisense oligonucleotides—both the 2'-*O*-methyl-modified ribose molecule with a full-length phosphorothioate backbone and the phosphorodiamidate morpholino oligomer—are undergoing further assessment to evaluate the potential for systemic delivery (by subcutaneous or intravenous routes) in hopes of reaching multiple muscle groups and achieving clinically meaningful outcomes. It has been predicted that through pre-planned skipping of particular exons, as many as 60%–80% of Duchenne muscular dystrophy mutations can be corrected through this mechanism.

Conclusion

Multiple molecular strategies are currently being tested in patients with Duchenne muscular dystrophy. These therapies seek to restore the dystrophin protein and correct the underlying physiologic defect that predisposes muscle fibers to injury. While more research is needed, the findings, so far, look very promising.

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