Pharmacologic and Genetic Therapy for Childhood Muscular Dystrophies

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The outstanding advances in the molecular characterization of muscle diseases, including muscular dystrophies, inflammatory myopathies, and ion channel disorders, have resulted in the identification of potential targets for pharmacologic and genetic therapy in the best characterized of these diseases. The most common myopathy in children, Duchenne muscular dystrophy (DMD), is the focus of active pharmacologic clinical trials. Genetic transfer therapy research for this and other dystrophies is rapidly moving forward. However, as new approaches for treatment are being actively investigated, the current modality of treatment for all myopathies is still in the realm of physical medicine and rehabilitation. The focus of this review is on the advances in pharmacologic and genetic therapy research in DMD and limb girdle muscular dystrophies.

Introduction

Muscle disorders in childhood are a common cause of morbidity and mortality. However, with a few exceptions, therapies available are nonspecific and rarely lead to a cure for the disease. The lack of successful treatment for most of these disorders frustrates physicians and parents alike. Because the specific diagnosis of a myopathic disorder relies on invasive procedures, the treating physician is often confronted with a reluctant parent, hesitant to have the child undergo an electromyelogram (EMG) or muscle biopsy unless the diagnosis will change the prognosis or affect the medical management of the patient. It is the role of the physician to help families understand that having a diagnosis does impact future management and prognosis. An outcome can be determined in many cases, and genetic counseling can be provided to family members. Furthermore, a definite diagnosis may be required for eligibility in genetic or pharmacologic clinical trials, which might benefit the individual and help to advance the field.

Outstanding advances have been made in the understanding of many neuromuscular diseases affecting children, including the muscular dystrophies, mitochondrial myopathies, and ion channel disorders. Identification of specific genetic defects has given rise to a broader understanding of the pathophysiology of these diseases, enabling the design of therapeutic strategies that are targeted to interrupt specific pathologic cascades. An example of this process can be seen in Duchenne muscular dystrophy (DMD). Since the discovery of the dystrophin gene [1] and protein [2], a delineation of the pathophysiologic cascade of events has been constructed in a collective scientific effort. Recently, new events leading to muscle necrosis and fibrosis in DMD have been identified and are now the focus of pharmacologic trials in animal models of the disease [3•]. Translational research to move potentially beneficial drugs into human trials has already begun. In parallel, gene and cell therapy approaches are being investigated, as are novel ways to functionally replace the missing protein.

Unfortunately, other muscular dystrophies, such as congenital muscular dystrophies, Emery-Dreifuss muscular dystrophy, facioscapulohumeral muscular dystrophy, and the limb girdle muscular dystrophies (LGMDs) lag behind in this journey, and most of them have only recently been genetically characterized. Most of the research efforts in such diseases are focused on the molecular pathophysiology of the disease, with the goal of identifying targets for experimental therapeutics.

In spite of the routine utility of pharmacologic and genetic therapies on the horizon, the current modality of treatment for muscular dystrophies is still in the realm of physical medicine and rehabilitation. The role of physical and occupational therapy, as well as respiratory rehabilitation, cannot be overemphasized. Rehabilitation therapy keeps children with myopathies functional for longer periods of time, and can prolong survival and improve quality of life. The care of children with progressive or chronic muscle disorders is best done in a multidisciplinary setting, where physicians (neurologists, physiatrists, cardiologists, pulmonary medicine), physical and occupational therapists, nutritionists, exercise physiologists, and social workers can work together for the overall well being of the child and the family. This review focuses on promising efforts towards therapies in DMD and related LGMDs, and readers are encouraged to consult other sources [4–7] for updates in respiratory management and orthosis.

Duchenne Muscular Dystrophy

Duchenne muscular dystrophy is the most common and devastating type of muscular dystrophy (incidence of one in 3500 live-born male infants worldwide). DMD is characterized by a complete loss of dystrophin, leading to progressive muscle weakness and wasting. DMD patients typically become wheelchair-bound at the age of 10 to 12 years, and succumb to respiratory failure in their late teens to early twenties due to involvement of the diaphragm and other respiratory muscles. Cardiac involvement is frequent, but it does not usually become clinically significant until the late teens, although a number of children have symptomatic heart failure that causes premature death. The primary defect in this disease is a mutation in the dystrophin gene, the largest gene known to date, which is located on the short arm of the X chromosome [1,8]. Because of the size of the gene, there is a very high spontaneous new-mutation rate, which makes eradication of the disease by genetic counseling and screening impossible.

Dystrophin is a subsarcolemmal protein critical in membrane stabilization and prevention of contraction-induced cell membrane damage. Alteration or absence of this protein results in abnormal membrane tearing [9•]. The pathophysiologic cascade includes myofiber necrosis, fibrosis, and wasting. Much is known at present about the pathophysiologic cascade that is triggered by the lack of dystrophin, in part due to the natural existence of homologous dog, mouse, and cat models of DMD that have been used for studies in pathophysiology, as well as pharmacologic and genetic therapies. Figure 1 represents a diagram of some of the known events that could be altered by pharmacologic intervention.

Different basic and clinical research groups are pursuing promising therapeutic paths for DMD. These include cell and gene therapy, up-regulation of utrophin (a protein similar to dystrophin existing at the neuromuscular junction) to compensate for the lack of dystrophin in the muscle, genetic manipulation to alter the translation of the mutated dystrophin gene, and pharmacologic interventions. We discuss each of these in the following section.

Cell therapy

Experiments in developing treatments for the myopathies are varied, and myoblast transplantation in dystrophinopathies was one of the earliest approaches in which direct alteration of muscle was performed. In this method, muscle precursor cells from a healthy donor are injected into the muscle of an affected individual or dystrophin-deficient animal model. The premise is that the transplanted cells will produce enough normal dystrophin to restore levels to a therapeutic amount, thereby improving muscle function and strength. Unfortunately, there are many hurdles to the success of this technique, not the least of which is a poor survival rate of approximately 10% of injected cells, presumably due to an immune response by the host. Migration of injected cells is generally only a few millimeters from the injection site and not enough to effectively cause an increase in strength. One nonacademic laboratory in Tennessee has made claims that myoblast transfer has restored strength in some individuals with Duchenne and Becker dystrophy; however, their published reports have come under fire from other scientists for lack of adequate experimental controls and for nonstandard methods of dystrophin quantitation. Recently, the Food and Drug Administration (FDA) shut down this organization, citing several protocol violations and inappropriate medical supervision of patients.

New methods for systemic delivery of myoblasts may improve the efficiency of successfully transplanted cells. A promising technique is delivery through the circulatory system under high-pressure conditions following bupivacaine for induction of muscle regeneration and hyaluronidase for breakdown of the basal lamina barriers. The immunogenicity of myoblasts may be reduced by harvesting cells directly from the patient and transfecting myoblasts with a normal dystrophin gene in vitro. The patient's own myoblasts then would be reinjected, thereby reducing immune response [10•]. Unfortunately, a contributing factor to the limited survival of transplanted cells is likely to be the immunogenicity of dystrophin itself, which presents as a neoantigen in patients who are dystrophin-deficient [11]. The remaining problems, including overcoming or outwitting an immune response, systemic delivery, and finding the best cell type and donor, must all be solved in the laboratory before this methodology should be utilized in a clinical setting.

Gene transfer

Delivery of a sufficient quantity of a normal gene into an individual whose own gene(s) are defective would presumably produce enough of the missing protein for the individual to regain muscle strength and function. However, gene transfer techniques face the same obstacles as myoblast transfer therapy: systemic delivery, immunogenicity, and the need to transfect a high enough quantity of cells. Additionally, the size of muscle cytoskeleton genes is prohibitive for some gene therapy approaches, and the best method for returning genes to muscle remains debatable. The use of viral vectors seems to be more promising than alternative methods, including nonviral delivery systems (*eg*, liposomes) and the injection of "naked" DNA. Of the viral vectors used for gene transfer research, the third-generation adenovirus ("gutted" adenovirus) and the adeno-associated virus (AAV) appear to be closest to being used in an actual clinical application. The third-generation adenovirus is replication incompetent and requires a helper virus to package normal cDNA. Importantly, this vector is able to carry the entire dystrophin gene, in part due to the excision of most of the viral genes. The design of this virus is an improvement over other vectors, due to reduced immunogenicity and consequent improvements in the duration of protein expression. There are, however, two significant drawbacks to the use of currently available adenoviral vectors. One is that they have difficulty

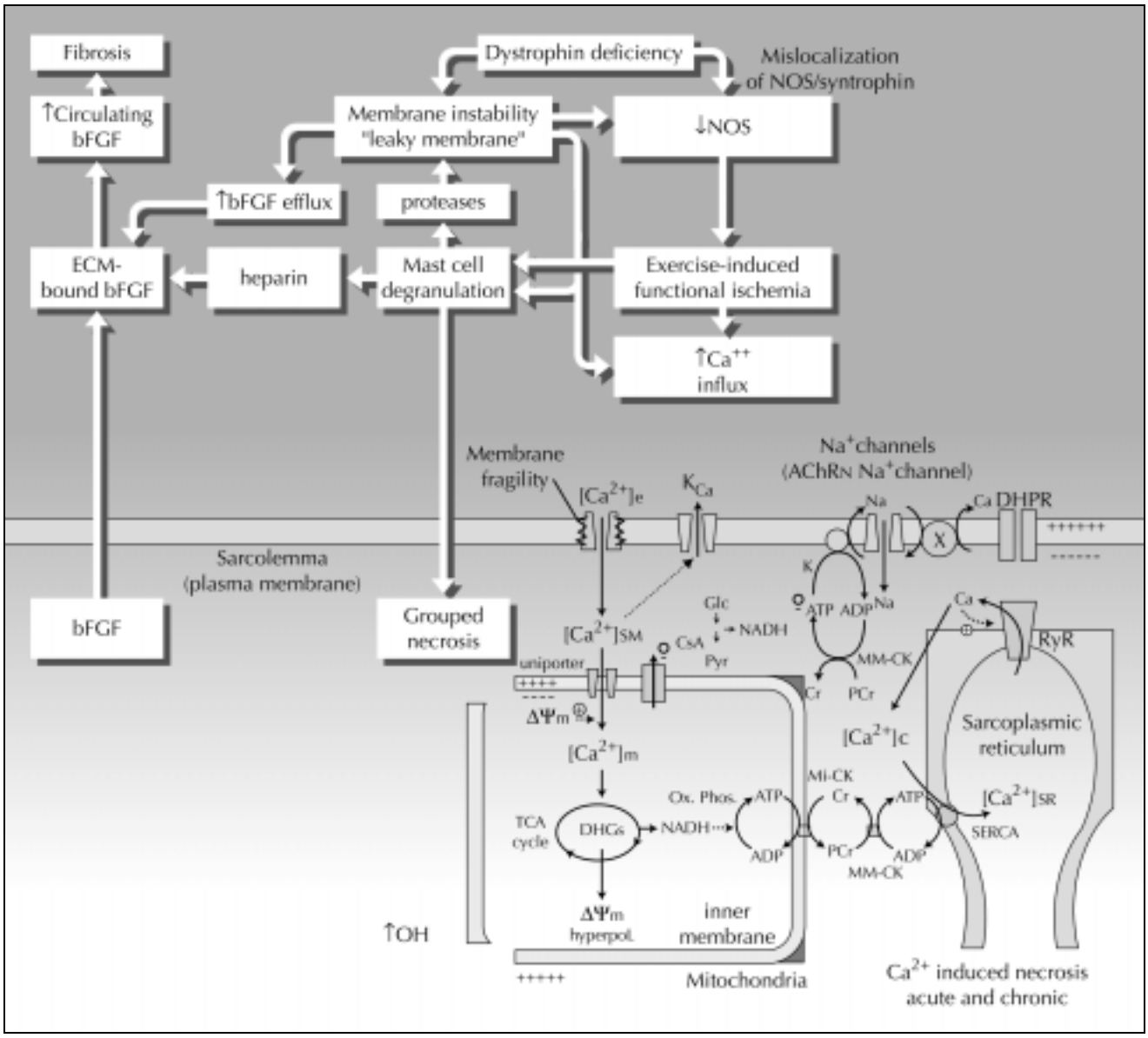

Figure 1. Pathophysiologic cascades in dystrophic muscle. The lack of dystrophin results in a leaky membrane. The primary cascade is excessive influx of calcium that enhances oxidative metabolism of key Ca^{2+}-activated dehydrogenases. An additional chronic cascade is an efflux of fibroblast growth factor (bFGF), resulting in increased muscle fibrosis and decreased regeneration of muscle cells. A recently defined pathway is mislocalization and decreased nitrous oxide synthase (NOS), resulting in muscle ischemia, perhaps enhanced during exercise. Finally, mast cell degranulation resulted in group necrosis, and increased fibrosis has been defined. (ECM—extracellular matrix.)

transfecting mature muscle cells, and the second is that they also fail to integrate into the host's genome, requiring repeated administrations. These hurdles must be overcome before adenovirus can be tested in a clinical setting.

Adeno-associated virus has advantages where the adenovirus is deficient: it is nonpathogenic and nonimmunogenic, its natural tropism is towards muscle, and it integrates stably into chromosomes, replicating along with the host's genome. Experiments using AAV for gene transfer have been conducted with encouraging results in a δ-sarcoglycan–deficient hamster model for a subset of the LGMDs [12•]. Using this vector, expression in muscle appears to be long lasting, and functional rescue is achieved [12•]. A human clinical trial for individuals with sarcoglycanopathies was published as a protocol [13]; however, it is unclear if the recent FDA decision to temporarily close some human gene therapy experiments has affected this trial. The major drawback to using AAV for myopathies with large genes is the limited viral carrying capacity. To overcome this problem in conditions like the dystrophinopathies, methods are being researched to place larger genes into this vector.

Modification of gene translation

Another approach to treating certain genetic diseases is to correct for genetic mutations by forcing the patient's own

genetic replication enzymes to use alternate sequences to produce a functional protein. Nature has already proven to us that its machinery will make an attempt to correct for genetic mutations with phenomena like revertant fibers in Duchenne dystrophy, but finding a pharmacologic or other intervention to exploit this effectively enough to restore muscle function is challenging and mutation-specific. Aminoglycosides are effective as antibiotics because they force the replication machinery of bacteria to read through translational stop codons and cause the production of abnormal and lethal proteins. Use of gentamicin in the genetic mouse model for DMD has shown promising results; presumably because the premature stop codon mutation in the dystrophin gene of *mdx* mice is ignored as a result of gentamicin administration [14•]. The quantity of normal-sized dystrophin in the mice after treatment was 15%, which is in the Becker muscular dystrophy range for humans. Human clinical trials have been conducted on Duchenne and Becker patients with premature stop codon-point mutations, and have so far failed to reproduce the results seen in the mice. Further efforts to cause exon skipping of either the mutation-carrying exon or an abnormally duplicated exon are being used in other genetic diseases, and any successes would likely be applicable to the myopathies as well. A severe limitation to this potential therapy for myopathies is the risk of systemic toxicity, and extended treatments may not be feasible in chronic, progressive conditions.

Utrophin up-regulation

Utrophin is a protein similar in structure to dystrophin, and when expressed in higher-than-normal levels, is able to functionally replace the absent dystrophin protein. Methods for increasing utrophin expression in dystrophin-deficient skeletal muscle include pharmacologic up-regulation of the protein and utrophin gene transfer techniques using the vectors described earlier. Utrophin resides at the neuromuscular junction (NMJ) in normal mature muscle, and two drugs that induce expression of NMJ proteins have proven successful in inducing higher levels of utrophin in cultured myogenic cells. These drugs, agrin and heregulin [15,16], have yet to be investigated for efficacy and dose-response in animal models of DMD. First-generation adenoviral vectors have been used to deliver a truncated version of utrophin to *mdx* mice, with successful transduction of about one third of the muscle fibers. Dystrophin-associated proteins were restored and functional rescue of muscle was attained [17•]. Utrophin gene transfer has the same hurdles in Duchenne patients as dystrophin gene therapy, excepting that utrophin is not a foreign protein to individuals with dystrophin-deficiency, so one would predict a less significant patient immune response.

Pharmacologic therapy

The first real progress in pharmacologic treatment of DMD was the study of the natural history of the disease and establishment of criteria for clinical trials in 1983 [18]. The study proved that the natural progression of the disease was linear, and validated a muscle strength score (average of the summation of the rates from manually testing 34 muscle groups) as a reliable outcome measure for clinical trials. Following that study, a large prednisone trial was conducted based on the assumption that an immune response was responsible for the dystrophic process. That trial showed that prednisone improves muscle strength by 25% at 3 months, and maintained this effect for at least the 6 months of the study [19]. A subsequent study showed the benefit to be long term, lasting at least 3 years [20]. The mechanism of action of prednisone is not understood, as another potent anti-inflammatory drug (azathioprine) was not beneficial, in spite of a similar effect to prednisone in dramatically decreasing inflammatory cells in muscle biopsies of the study's patients [21,22]. Since then, prednisone, at a dose of 0.75 mg/kg/d, has been an established treatment for DMD, slowing the progression of the disease and prolonging ambulation [21]. However, because of the significant side effects of chronic steroid use, including weight gain, osteoporosis, cataracts, hypertension, and diabetes, this therapy is somewhat controversial. Because most patients demonstrate an improvement in strength and slowing of the disease process, many feel the beneficial effects outweigh the risk of side effects. Indeed, side effects can be adequately managed by careful monitoring of blood glucose, blood pressure, and renal function. Osteoporosis, although present in children with DMD on or off steroids, usually does not result in pathologic fractures. Cataracts, if present, remain asymptomatic. Weight can be managed by a sensible and healthy diet, together with an increase in physical activity, mainly by noneccentric exercise, such as swimming. Behavior changes, including hyperactivity and insomnia, can be avoided or reduced by giving prednisone in the morning, and with natural existing homeopathic herbal supplements with anxiolitic and sleep-inducing effects.

It is well known that steroids' side effects are reduced in patients who are given steroids on a schedule that is less than daily. However, a study of prednisone comparing a daily versus an alternate-day dose schedule in DMD failed to show any benefit in muscle strength in the alternate-day dose group compared with the no-treatment and daily-treatment groups [20]. Others have claimed a lower side-effect profile and improvement in strength with 10 days on schedule, 10 days off schedule, or twice a week at a high-dose schedule (Duvowitz and Connoly, personal communications). However, those treatment modalities have yet to be confirmed by prospective, randomized, large studies. Until such data is available, daily prednisone should be the treatment of choice for DMD.

The other controversy concerns the age at which steroids should be started. The Prednisone trial [19] included children as young as 5 years of age. Younger children have not been included in trials because their ability to cooperate with muscle and functional testing is highly variable.

The arguments in favor of starting steroids as soon as the diagnosis is made relies on trying to preserve muscle integrity when there is less damage and for as long as possible. On the other hand, prednisone might help improve muscle regeneration by recruiting satellite cells. The pool of satellite cells is limited, and if all are used early on, it might result in decreased regeneration potential in the future. At our center in Washington, DC, we start children on steroids if they are showing overt muscle weakness. In our experience, the younger the child is started on steroids, the fewer the side effects of weight gain and hyperactivity. This may be due to several factors, including the patient's better parental control on diet and lifestyle and the increased level of physical activity the young children have, which further increases as they are placed on prednisone. Many other neurologists start steroid treatment when the child is becoming nonambulatory, or can no longer get up from the floor, at about 8 to 9 years of age.

Another less toxic steroid that is chemically similar to prednisone is deflazacort. Deflazacort has similar beneficial effects and has fewer side effects than prednisone, particularly with respect to osteoporosis and weight gain [23,24•,25]. Suggested dosage is 0.9 to 1.2 mg/kg/d. However, this drug has not been approved for use by the FDA and, therefore, is not available in the United States, requiring patients to import the drug from other countries. The hassle of going through this is only justified if the child has unwanted side effects from prednisone that cannot be managed with a reduction of the dose, because lower doses of daily prednisone (0.3 mg/kg/d) are still effective [19], although less so than the full dose.

Multiple, small, clinical trials in DMD conducted in the premolecular era resulted in only two promising compounds other than prednisone. Cyclosporin (5 mg/kg/d) showed improvement in strength tested on an isolated muscle [26]. Larger studies have not been done, and this drug is not currently used in clinical practice. The anabolic steroid oxandrolone has been shown to increase and maintain muscle strength in a 3-month pilot study [27]. A larger study has been completed, but the results are not yet published.

The understanding of the pathophysiologic cascades initiated by dystrophin deficiency has dramatically increased over the past few years and has resulted in a rapidly growing body of literature. Newly identified potential targets for rational therapeutics include modulation of Ca++ influx, enhancement of mitochondrial function, supplementation of glutamine deficiency, and mast cell stabilization [9•,28–31]. Indeed, several compounds thought to impact on these targeted pathways were recently demonstrated to increase strength in the *mdx* mouse model of DMD [3•]. These compounds included glutamine (muscle-derived conditionally essential aminoacid), creatine (energy metabolism), pentoxyfilline (anti-inflammatory drug), oxatomide (mast cell stabilizer), and IGF-1 (muscle growth factor). More recent studies by the same group showed similar results with coenzyme Q10 (co-factor for complex I and II in the mitochondrial respiratory chain), pyridoxine, carnitine, EDTA, taurine, and IL-1ra (Granchelli, personal communication).

The Cooperative International Neuromuscular Research Group (CINRG) was formed in the spring of 1999, with the purpose of translating basic research in therapeutics to use in pediatric neuromuscular diseases. This multicenter group is based on the cooperative group model that has proved so successful for the field of oncology, and employs standardized protocols and a centralized data and safety review infrastructure. With the first focus on DMD, CINRG aims to test the human clinical efficacy and safety of drugs that demonstrate beneficial effects in the *mdx* mouse model [3•] and have acceptable safety profiles in humans. CINRG is currently conducting a trial of glutamine and creatine for DMD, and expects to test two other compounds, oxatomide and coenzyme Q10, within the next 6 months. Many other clinical trials are planned within the next 3 years for children with DMD.

The importance of conducting large-scale, clinical research trials with standardized protocols cannot be overemphasized; however, there are some important caveats. Clinical research of pharmacologic or genetic potential treatments is risky, very costly and time consuming, raises hopes in children and parents involved, and requires an investment of time and resources with uncertain return. With this in mind, clinical trials must be rationally designed, meticulously controlled, and well conducted so the investigators can reach important clinical conclusions that will be valid and well accepted once they are published. CINRG is set up to run high-quality trials in an efficient manner, with multiple experienced investigators initiating peer-reviewed protocols based on animal trials.

Limb Girdle Muscular Dystrophies

The limb girdle muscular dystrophies are a heterogeneous group of disorders inherited as autosomal recessive or autosomal dominant. They present with typical symmetric proximal muscle weakness. Severity varies, and they can present early on in life and have a severe course, or present late in life with a milder outcome. Some muscles might be specifically affected, and some patients have associated cardiomyopathy. Most of the known LGMDs are due to a defect in a muscle membrane protein (sarcoglycans), or proteins associated with the cytoskeleton (calpain-3, titin).

The clinical definition of these diseases has been possible with the advances in molecular diagnosis, but a complete understanding of pathophysiology is still lagging behind, as are pharmacologic treatments. Attempts to replace the missing gene are underway, and appear more promising than gene transfer in DMD. Contrary to the dystrophin gene, the genes for these proteins are much smaller and can easily be introduced into the musculophilic viral vector.

Pharmacologic therapy has not been established for LGMD. Due to the similarities in clinical manifestations and progression of LGMD with the dystrophinopathies, some of us have used empirical treatment with steroids. Few case reports in the literature [32,33] are concordant with our experience with four children (two 8-year-old girls with α- and δ-sarcoglycan deficiency, and two siblings with LGMD due to unidentified genetic or biochemical defects) who have shown objective and continuous responsiveness to steroids. Given the lack of much-needed prospective clinical trials for these diseases, a trial of steroids following objective muscle strength and functional evaluation is indicated in children presenting with severe weakness due to sarcoglycan deficiency or other unidentified LGMD.

A pilot study of creatine for muscular dystrophies included six patients with sarcoglycanopathies [34]. This study showed a mild but significant increase in muscle strength. However, no long-term, controlled study has proven its benefit in LGMD.

Conclusions

The management of Duchenne muscular dystrophy and limb girdle muscular dystrophy is best done in a multidisciplinary setting. Pharmacologic therapy includes prednisone or deflazacort in conjunction with physical, occupational, and respiratory rehabilitative therapy. Translational research is moving safe and efficacious compounds in the *mdx* mouse model to human trials. Targeted therapies are being tested in an attempt to block the pathophysiologic changes caused by dystrophin and other protein deficiency. Parallel efforts are moving gene transfer experiments for LGMD to the human phase. Alternative approaches to up-regulate utrophin and to restore protein production by reading through or skipping mutations are also moving forward. With this explosion of knowledge and research, the prognosis for DMD and related dystrophies may be more promising than it has since the gene and protein were discovered. Some day, we may be able to provide a pharmacologic cocktail that will halt disease progress and restore muscle strength, and possibly even restore the missing protein.

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