DUCHENNE AND BECKER’S DYSTROPHINOPATHIES
A REVIEW OF THE LATEST THERAPEUTIC STRATEGIES AND REHABILITATION PROGRAMMES

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SUMMARY

Duchenne and Becker muscular dystrophies are X-linked progressive dystrophinopathies that result of genetic defects in dystrophin, the major structural protein of the muscle. Their clinical patterns are similar and include gradual generalized weakness and wasting of all voluntary muscles as well as several other physical symptoms. Duchenne dystrophy differs from Becker muscular dystrophy which has a later age of onset and a slower rate of progression.

The correct diagnosis is very important to avoid misclassifications and it relies both in clinical signs and in important tools such as measurement of creatine phosphokinase, electromyography, muscle biopsy and genetic testing. Therapeutic strategies for dystrophinopathies can be categorized into three groups: molecular, cellular and pharmacological therapies. Molecular therapy involves viral and non-viral approaches while cellular therapy involves myoblast and stem cell strategies. Currently, pharmacological therapies are supportive and play the main role in stopping disease’s progression. They involve a multidisciplinary approach early in the disease with the use of drugs/molecules to improve the phenotype as well as rehabilitation programs including physiotherapy or surgery.

Key Words: Dystrophinopathies; Molecular Therapy; Cellular therapy; Pharmacological Therapy.
# Table of Contents

List of Figures ........................................................................................................ vii

Introduction ........................................................................................................... 8

Pathophysiology .................................................................................................... 8

Clinical Features .................................................................................................. 10

Diagnosis ............................................................................................................... 11

Differential Diagnosis ......................................................................................... 13

Evaluation of Disease’s Progression ................................................................... 14

Treatment ............................................................................................................ 15

Molecular Therapy ............................................................................................... 15

1. Viral-mediated Gene Therapy ......................................................................... 15
   A. Adenovirus Vectors ...................................................................................... 15
   B. Helper-dependent/gutted Adenoviral Vectors ........................................... 15
   C. Adeno-associated Virus Vectors ................................................................. 16
   D. Lentivirus Vectors ....................................................................................... 16
   E. Homologous Recombination ...................................................................... 16
   F. Utrophin Genetic Up-regulation ................................................................ 16

2. Non-viral Gene Therapy .................................................................................. 17
   A. Plasmid DNA .............................................................................................. 17
   B. Antisense Oligonucleotides ..................................................................... 18
   C. Chimeric RNA/DNA Oligonucleotides ..................................................... 19

Cellular Therapy .................................................................................................. 20

1. Myoblast Transplantation ............................................................................... 20

2. Stem Cell Therapy .......................................................................................... 21

Pharmacological Therapy ..................................................................................... 22
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Anti-inflammatory Drugs .................................. 22</td>
</tr>
<tr>
<td></td>
<td>A. Steroids ................................................. 22</td>
</tr>
<tr>
<td></td>
<td>B. Other Anti-inflammatory drugs .......................... 25</td>
</tr>
<tr>
<td>2.</td>
<td>Specific Anti-cytokine drugs ................................ 26</td>
</tr>
<tr>
<td>3.</td>
<td>Anabolic Processes Stimulation ............................ 26</td>
</tr>
<tr>
<td></td>
<td>A. Myostatin Regulation ..................................... 26</td>
</tr>
<tr>
<td></td>
<td>B. Insulin-like Growth Factor I ............................ 26</td>
</tr>
<tr>
<td></td>
<td>C. β2-agonists ............................................... 27</td>
</tr>
<tr>
<td>4.</td>
<td>Antibiotics .................................................. 27</td>
</tr>
<tr>
<td>5.</td>
<td>Anti-proteases ............................................... 28</td>
</tr>
<tr>
<td>6.</td>
<td>Anti-oxidants ............................................... 28</td>
</tr>
<tr>
<td>7.</td>
<td>Pharmacological utrophin up-regulation ................... 29</td>
</tr>
<tr>
<td>8.</td>
<td>Supplements ................................................ 30</td>
</tr>
<tr>
<td>9.</td>
<td>Management of Complications ............................... 31</td>
</tr>
<tr>
<td></td>
<td>A. Respiratory ............................................... 31</td>
</tr>
<tr>
<td></td>
<td>B. Cardiac .................................................. 31</td>
</tr>
<tr>
<td></td>
<td>C. Orthopedic ............................................... 32</td>
</tr>
<tr>
<td></td>
<td>i. Ambulant Children ........................................ 32</td>
</tr>
<tr>
<td></td>
<td>ii. Non-ambulant Children .................................. 32</td>
</tr>
<tr>
<td></td>
<td>iii. Management of Scoliosis ................................ 32</td>
</tr>
<tr>
<td>Conclusion</td>
<td>......................................................... 33</td>
</tr>
<tr>
<td>References</td>
<td>.......................................................... 34</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 1: Gower’s Sign ................................................................. 10
Figure 2: Muscle Biopsy (H&E staining) ........................................ 11
Figure 3: Western Blot of Dystrophin ........................................... 12
Figure 4: Dystrophin Staining ...................................................... 13
INTRODUCTION

The muscular dystrophies are a group of inherited myogenic disorders that share a set of clinical and pathological characteristics but differ in severity, inheritance pattern, and molecular defect.

The most well known and prevalent of these disorders is Duchenne Muscular Dystrophy (DMD), followed by Becker Muscular Dystrophy (BMD), a clinically similar disorder but less severe. Both of them are X-linked progressive Muscular Dystrophies with an incidence of 1/3500 male births in DMD and 1/30000 males in BMD “Emery (1991)”.

Duchenne Muscular Dystrophy was originally described by Edward Meyron in 1851 “Meyron (1851)”, but it was only when Duchenne detailed the clinical and muscle histology in 1861 that it became a recognized independent disorder “Duchenne (1861)”. Almost 100 years later, in 1953 Becker described an allelic variant to DMD with later age of onset and slower progression; nowadays known as Becker Muscular Dystrophy “Becker (1953)”.

PATHOPHYSIOLOGY

DMD and BMD result of genetic defects in the major muscle cell structural protein known as dystrophin, being also called Dystrophinopathies. The dystrophin gene is the largest described to date, it’s located on Xp21 and it occupies almost 2% of that chromosome. Dystrophin is a physiological 427 kDa protein located in the subsarcolemmal region of the muscle fiber and it’s responsible for stabilizing the plasma membrane against mechanical stress during muscle fiber contraction “Hoffman et al. (1987)”. It is organized into four structural domains: the N-terminal actin-binding, the central rod domain, the cysteine-rich domain binding, and the carboxy-terminal “Hoffman et al. (1987)” “Koenig et al. (1988)”. Dystrophin is expressed mainly in skeletal muscle but it also can be found in smooth muscle cells, cardiac myocytes and, in lower levels, in brain.

Nonstructural roles have been described for dystrophin, making it a multifunctional protein. In one hand, the dystrophin complex serves as a signaling scaffold that is responsive to extracellular stressors and several
signaling molecules interact with it “Brenman et al. (1995); Yang et al. (1995); Abramovici et al. (2003); Oak et al. (2003)”. In the other hand, dystrophin is thought to play a role in calcium homeostasis, resulting in the aberrant hyperactivation of signaling cascades involved in the inflammatory response. This apparent sensitivity of dystrophic muscle in triggering inflammation due to aberrant calcium homeostasis may be detrimental to muscle cell survival and to the potential introduction of therapeutics “Turner et al. (1991); Iwata et al. (2003); Kumar et al. (2004)”.

Mutations in dystrophin gene comprise deletions (60-70% of cases), duplications (10-15%) and point mutations (10-15%). In one third of the cases, mutations occur de novo “Neri et al. (2007)”.

The gene-coded defects of dystrophin underlie DMD, which is marked by absence or severe reduction of the protein, and BMD with presence of dystrophin but defective one “Reitter and Goebel (1996)”. The threshold level of dystrophin mRNA and protein sufficient to maintain proper membrane organization and prevent muscle degeneration in humans is between 29% and 57%, if dystrophin is uniformly present in all muscle fibers “Neri et al. (2007)”. This lower level is similar to the one found in previous transgenic experiences on mdx mice “Wells et al. (1995)”. There is also a correlation between the amount of dystrophin expressed and the clinical severity: absence or less than 3% of normal dystrophin results in severe DMD; from 3-10% correlates with severe BMD; and from 20-30% of normal or altered dystrophin results mild BMD “Hoffman et al (1988)”.

Only one highly homologous dystrophin protein (80%), utrophin or dystrophin-related protein was described until now and it’s encoded by a gene in chromosome 6 “Khurana et al. (1990)”. In developing skeletal muscle, utrophin showed to identify the entire sarcolemmal membrane. It was reported a phenomenon of utrophin up-regulation in dystrophic muscle but this increase in utrophin levels doesn’t prevent muscle degeneration; it only delays the progression of the disease “Vainzof et al (1995)”.
**Clinical Features**

In DMD, the onset is early in childhood, at 2-6 years of life, with a waddling gait secondary to hip girdle muscle weakness. Very often parents note that the child pushes on his knees in order to stand, sign known as Gower’s sign (Fig.1) “Gowers (1988)”. Cognitive impairment is usual and about 20% of affected children have an IQ lower than 70. Calf enlargement (calf pseudohypertrophy) is common, resulting of fatty and fibrotic infiltration of degenerative calf muscles. Progressive weakness is seen in the proximal musculature, initially in the lower extremities but later on also in the upper ones. Around the age of 8 respiratory muscle’s strength and ambulation capacity start to decline, most of the patients being wheelchair bounded by the age of 10. Scoliosis appears once wheelchair dependency is achieved which further compromises pulmonary, cardiac and gastrointestinal function “Emery (1993)”. In the past, patients developed terminal respiratory or cardiac failure due to wheelchair bound and profound weakness by the early twenties; nowadays the survival increased to late twenties “Simons et al. (1998)”.

BMD is characterized by a later age of onset, normally around 12 years; distribution of muscle wasting and weakness similar to DMD but with a slower rate of progression. The loss of ambulation may vary but mostly it occurs from adolescence on, being the expectancy of life in BMD about 40 years of age with 90% of the patients alive beyond twenty years “Becker and Kiener (1955)”.

![Fig.1: Gower’s sign](image-url)
**Diagnosis**

Measurement of serum creatine phosphokinase (CPK) is an easy and sensitive test when suspecting of any muscle disease and correlates with the amount of muscle degeneration. Patients with DMD and BMD have increased levels of CPK since birth and they are 50 to 100 times higher than the reference range. The neonatal screening of CPK for boys with family history and the standard evaluation of CPK serum levels in boys with developmental impairment are recommended since they decrease substantially the diagnosis’ delay “Ciafalon et al. (2009)”.

Electromyography, even though not diagnostic, is important to exclude neurogenic causes of weakness.

Muscle biopsy, along with immunohistochemistry and Western Blot analysis, is an essential adjunctive tool because in the majority of cases it's the deciding factor for diagnosis before genetics studies. Muscle biopsy shows increased variability of fiber size, rounding of fibers, fiber splitting, increased numbers of central nuclei, hyaline fibers, muscle fiber necrosis, phagocytosis and regeneration, and replacement by fat and fibrous tissue “Hilton-Jones (2001)” (Fig.2).

![Fig.2: H&E (a) Normal muscle biopsy. (b) DMD fibers are smaller and rounded. The fiber size is variable with many hypercontracted fibers. The endomysial connective tissue is increased (fibrosis).](image)
Western blot analysis for dystrophin in muscle allows the determination of both the quantity and size of the molecule. In DMD dystrophin is absent or levels less than 3% can be found. In 80% of patients with BMD, the molecular weight of dystrophin is reduced to 20-90% of normal, 15% have reduced quantity and 5% show an abnormally large protein “Hoffman et al (1988)” (Fig.3). Muscle biopsy has the disadvantage of being an invasive procedure.

**Fig.3:** Western blot of dystrophin.
Lane 1: **BMD:** Dystrophin has reduced abundance but normal size.
Lane 2: **BMD:** Dystrophin has reduced size and abundance.
Lane 3: Normal; Dystrophin has normal size and amount.
Lane 4: DMD; Almost no protein is present.
Lane 5: DMD; Dystrophin has severely reduced abundance.

Immunostaining for dystrophin and utrophin on skin biopsies is also a reliable tool for the diagnosis of dystrophinopathies “Tanveer et al. (2009)”.

Diagnosis can be achieved by genetic testing on a peripheral blood sample. About 96% of mutations are identified by direct sequencing of the dystrophin gene. The three main techniques are used in a 3-time approach: first polymerase chain reaction (PCR) amplification to detect large deletions; secondly detection of virtually all mutations (DOVAM) for point mutations; thirdly multiplex amplifiable probe hybridization (MAPH) to define duplications. Since the dystrophin gene is very large, most testing protocols only screen a part of the gene. Thereby, a negative genetic test doesn’t exclude the diagnosis and a muscle biopsy is required “Dent et al. (2005)”.

Genetic counseling is very important and the first line test to determine the female carrier status is a sensitive PCR for the same deletion/duplication observed in the affected child. With the possibility of germline mosaicism a negative carrier test doesn’t exclude the risk of mutation. Moreover, one third of the cases represent new mutations.

For the prenatal diagnosis, multiplex PCR can detect around 93% of deletions being simple, reliable and inexpensive. Multiplex ligation-dependent probe amplification (MLPA) is important for detecting deletions in non-hot regions/exons and duplications “Clemens et al. (1991); Li et al. (2009)”. 
There can be clinical and histopathologic overlap between muscular dystrophies and inflammatory myopathies. Pathologic findings of inflammation and major histocompatibility complex up-regulation, typical of inflammatory myopathies, occur in some muscular dystrophies. Dystrophin levels should be accessed by immunocytochemical staining in all cases “Nirmalananthana et al. (2004)” (Fig.4).

Limb-girdle muscular dystrophies are clinically similar to DMD, but occur in both sexes due to autosomal recessive and autosomal dominant inheritance. Testing for deficiency of proteins from the transmembrane sarcoglycans complex is indicated in dystrophin-positive dystrophies (Erazo-Torricelli (2004)).

Emery-Dreifuss muscular dystrophy is associated with limb contractures and cardiac arrhythmia. X-linked recessive forms are identified by emerin deficit on immunohistochemistry, autosomal dominant and autosomal recessive forms are both exclusively identified by DNA study “Erazo-Torricelli (2004)”. Spinal Muscle Atrophy is characterized by poor muscle tone and symmetric muscle weakness sparing the face and ocular muscles due to anterior horn cell loss. The definitive diagnosis relies on DNA analysis of mutations in the Survival of Motor Neuron 1 gene (SMN1) “Monani (2005)”. 

Fig.4: Dystrophin staining. (a) Normal dystrophin staining around the rim of muscle fibers. (b) Absent dystrophin in DMD. (c) DMD with one “revertant” fiber with dystrophin staining.
**EVALUATION OF DISEASE’S PROGRESSION**

Annual neurological, respiratory and cardiological assessments should be co-ordinated via a centralized rehabilitation unit.

From the time of diagnosis boys must be assessed once to twice a year by therapists with special experience in neuromuscular disorders. The interval between assessments depends on boy’s age, disease’s progression and his functional ability “Bushby et al. (2005)”.

Respiratory surveillance should include: serial spirometry accessing forced vital capacity (FVC) and peak expiratory flow (PEF) to document the progression of respiratory muscle weakness; serial measurement of nocturnal oxycapnography/polisomnography, once clinical signs of nocturnal hypoventilation; serial measurement of peak cough flow; chest and spine radiograph for monitoring scoliosis’ degree and chest deformities “Finder et al. (2004)”.

Cardiac investigation (echocardiogram and electrocardiogram) is indicated at diagnosis, every 2 years thereafter to age of 10 and then annually, or more often, if abnormalities are detected “American Academy of Pediatrics (2005)”.
TREATMENT

Therapeutic strategies for dystrophinopathies are divided into 3 groups based on their approach: molecular, cellular and pharmacological therapy.

MOLECULAR THERAPY

1. VIRAL-MEDIATED GENE THERAPY

The aim is to deliver DNA encoding dystrophin or other therapeutic genes (utrophin) to muscle. To date most dystrophin-delivery approaches are hampered by the gene’s large size (it’s larger than the cloning capacity of most current viral vectors), immune responses to the protein and viral antigens, and difficulty finding an effective delivery system. Important advances were made in identifying key regions of the gene in truncated dystrophin gene construction, optimal vectors for gene delivery and better delivery methods.

A. Adenovirus vectors

The first gene transfer vectors derived from adenovirus and, despite their success in delivering dystrophin up to 50% to myofibers, they were too small to fit the entire coding sequence of dystrophin. Functional studies of the gene in mdx mice showed that multiple regions of dystrophin can be deleted in various combinations to generate highly functional mini- and microdystrophin genes with the advantage of being within viral/plasmid cloning capacities “Fabb et al. (2002)”. One important problem was still remaining; the strong immune response they caused “Smith (1995)”.

B. Helper-dependent/gutted adenoviral vectors

To overcome some of the limitations with adenovirus vectors, helper-dependent or gutted adenoviral vectors, which are deleted for all of the viral protein coding sequences, started to be used. Although a weaker immune reaction was induced, they had to cross the basal lamina of muscle fibers reducing the efficiency of transduction. A co-deliverance of immunomodulatory molecules such as CTL4Alg was suggested to face some of these problems “Parks et al. (1996); Dudley et al. (2004); Jiang et al. (2004)”. 
C. Adeno-associated virus vectors (AAV)

Another promising viral vector is AAV given its nonpathogenicity, broad tropism and infectivity and long-term persistence. These vectors appear to be more efficient for transducing adult fibers, especially if delivered systemically together with factors that increase vascular permeability. They can only accommodate minidystrophin which gives good functional rescue when replacing dystrophin in transgenic mdx mice “Wang et al. (2000)”.

D. Lentivirus vectors

Additional viral vectors being tested for gene transfer to muscle are the HIV-derived lentiviral vectors. They have been found to stably transduce post-mitotic cells with expression lasting up to 2 months with no associated immune response “Odom et al. (2007)”.

E. Homologous Recombination (HR)

Other approach is called gene targeting, which means the replacement of an endogenous DNA segment with a homologous, exogenous segment of DNA by HR. A major advantage of the HR gene targeting approach is that the risk of random integration as is presented with retroviral mediated gene replacement can be avoided or minimized; this has been accomplished using helper-dependent Adenovirus as a vector platform “Odom et al. (2007)”.

F. Utrophin genetic up-regulation

In skeletal muscle, utrophin is detectable in early fetal development over the sarcolemma where during development it is gradually replaced by dystrophin. This different expression proposed that utrophin may be the embryonic/neonatal form of dystrophin thus; utrophin up-regulation would allow replacement of dysfunctional or absent dystrophin. Furthermore, this approach benefits from the lack of neoantigen production and subsequent immune response that might result with the delivery of dystrophin “Miura and Jasmin (2006)”.
2. **Non-viral Gene Therapy**

Three different methodologies have been pursued: 1) plasmid DNA to deliver dystrophin cDNA constructs; 2) antisense oligonucleotides (AONs) to induce exon skipping of the dystrophin pre-mRNA; 3) RNA/DNA chimeric oligonucleotides for genome editing and gene correction. The main disadvantage of non-viral vectors is the delivery mechanism but they also have the potential to induce immune responses, although in a lower level. It is considered that non-viral vectors will represent a less expensive and safer mode of therapy “Rando (2007)”.

A. **Plasmid DNA**

This therapy is based on the observation that naked plasmid DNA, delivered to skeletal muscle by direct injection, is taken up in the myofiber where plasmid genes are expressed. Intramuscular or intravenous/arterial injection of a therapeutic plasmid into *mdx* mice result in dystrophin expression in up to 10% of the muscle fibers “Acsadi et al. (1991); Liang et al. (2004)”.

There are several positive aspects of this therapy including already existing scaled-up production processes, applicability to DMD patients regardless of mutation, and simplicity. Still, controversial issues exist and the expected duration of plasmid gene expression is one of them; in human treatment this expression would be necessary to last for years/decades. Research leading to plasmid persistence is being made by introducing the dystrophin plasmid into a single site in the genome with the use of integrases. Ideally the integrases should be site-specific but currently they aren’t which may lead to insertional mutagenesis. Another concern regards the distribution of the vector and thus the therapeutic protein along the length of muscle fibers in a targeted muscle. Any fiber not expressing dystrophin at therapeutic levels along its length will eventually succumb; thus the importance of quantifying dystrophin expression not only in the muscle cross-sections but also along the longitudinal axis of the muscle “Molnar et al. (2004); Bertoni et al. (2006)”.
B. Antisense oligonucleotides (AONs)

The possible therapeutic benefit of dystrophin transcript splicing modifications is based on the fact that most cases of DMD are caused by deletions in the dystrophin gene that lead to frame-shift mutations in the transcript. This led to the idea of using AONs to alter splicing so that the open reading frame is restored and the severe DMD phenotype is converted into a milder BMD phenotype. Recent studies have clearly established the potential of AONs induced exon-skipping approach since dystrophin expression was enhanced with repeated injections and no immune response was elicited “McClorey et al. (2006)”. It has been estimated that 70% of patients with DMD caused by intragenic deletions could benefit from this therapy. Phase I clinical trials with AONs in humans are being conducted to test intramuscular injections of AONs for safety and efficacy “Muntoni et al. (2005)”. Recently, a study reported that the application of multiexon skipping may provide a more uniform methodology for a larger group of patients with DMD “Aartsma et al. (2004)”. This approach corrects all dystrophin isoforms and maintains the original tissue-specific gene regulation however, its application requires an accurate molecular diagnosis, a rational plan for skipping one or more exons to produce an in-frame transcript, evidence that targeting AONs to specific sequences will result in the desired transcript, and finally the design of AONs vectors specifically for that particular mutation “Wilton and Fletcher (2008)”. It also has a transient therapeutic effect and AONs therapy would require multiple treatments each year. One solution was combining AONs technology with viral expression approaches to achieve sustained expression of antisense vectors and long-term exon skipping but this entails the challenges of viral-mediated gene therapy “Goyenvalle et al. (2004)".
C. Chimeric RNA/DNA oligonucleotides

Chimeric RNA/DNA oligonucleotides can be used to direct the correction of a mutation by inducing preferential gene conversion from a mutant to a functional allele. Such genome editing potential could be applied to the correction of point mutations responsible for dystrophin deficiency in approximately 15% of patients with DMD. Injection of chimeric oligonucleotides in \( mdx \) mice showed correction at the genomic and transcript levels, and restoration of dystrophin protein expression was observed both in vivo and in vitro \("\text{Rando et al. (2000); Bertoni and Rando (2002)}\)\). To benefit more patients with dystrophin defects other than point mutations, broader applications of this approach have been envisioned. Recently, attempts to correct mutations that disrupt the translational reading frame were made by targeting the exon/intron boundaries of the mutated exon 23 in \( mdx \) mice. It resulted in skipping/removal of this exon and production of a truncated but functional protein. The use of this technique has to overcome similar problems described in the AONs therapy, such as the location of the particular splice site to be targeted \("\text{Bertoni et al. (2003)}\)\). The major challenge concerning chimeric compounds is the low efficiency observed of this technology with current vectors \("\text{Rando et al. (2000); Bertoni et al. (2005)}\)\). Current research is focusing on the enhancement of the molecular mechanisms that mediate the gene editing, including modifications of the oligonucleotides as well as enzyme systems and cellular processes involved in the repair mechanism \("\text{Bertoni et al. (2006)}\)\).

Perhaps the greatest advantage is that it results in a permanent correction of the genetic defect in myonuclei allowing sustained therapeutic benefit as long as the targeted myonuclei persist.
**CELLULAR THERAPY**

Cell-based strategies comprise two main subjects of investigation: transplantation of myoblasts from a healthy donor expressing normal dystrophin; use of stem cells (ST) obtained from the patient that must be 'genetically corrected' in vitro. Recent works suggest the most promising possibility for the treatment of DMD being a combination of different approaches, such as gene and stem cell therapy “Farini et al. (2009)”.

1. **MYOBLAST TRANSPLANTATION**

This procedure involves injecting or transplanting donor muscle precursor cells (myoblasts) into a dystrophic host. The goal is to induce expression of dystrophin through fusion of dystrophin-positive myoblasts of the donor with dystrophin-deficient muscle fibers of the host. Although shown to be promising in *mdx* mice “Ikezawa et al.(2003)”, human trials didn’t demonstrate objective benefits with low levels of dystrophin expression “Mendell et al. (1995); Partridge (2002)”. Myoblast transplantation has several limitations, including immune rejection, poor cellular survival rates (80% undergo a rapid and massive death soon after the injection) and lack of dispersion of injected cells which limits the effect to the injection area “Gussoni et al. (1997); Mouly et al. (2005); Skuk et al. (2006)”.

A recent study concluded that fetal myogenic cells can be successfully isolated and expanded in vitro from human fetal muscle biopsies. Cells from a 16-week-year old fetus showed higher growth capacities when compared to cells from 13 and 30 year old donors. This form of treatment is controversial due to ethical issues regarding the use of fetal tissue “Hirt-Burri et al. (2008)”.
2. STEM CELL THERAPY

Different types of stem cells have been explored for the treatment of DMD and the ideal scenario of a bone-marrow derived stem cell that circulates to reach all muscles has proved clinically irrelevant to date “White and Grounds (2003)”. Satellite cells represent the oldest known adult stem cell niche; they reside beneath the basal lamina of skeletal muscle fibers and their two critical properties are self-renewal and capacity to turn into various lineages of cells. Satellite cells exhibit substantial phenotypic and functional heterogeneity, evident through differences in their cell-surface marker expression, induction of myogenic transcription factors, and in vivo and in vitro proliferation characteristics “Rouger et al. (2004)”.

In late 1990s, were described cells likely to be more primitive than satellite cells within muscle, nowadays called side population (SP) “Montanaro et al. (2004)”. These cells demonstrated potential plasticity to myogenic and hematopoietic lineages suggesting that to undergo myogenic commitment they require the environment provided by myogenic cells “Asakura et al. (2002); Camargo et al. (2003)”. They can be systemically delivered, intravenously or intra-arterially, and then they extravasate from vessels into skeletal muscle, engraft into muscle tissue and deliver their genetic material to host myofibers. The mechanisms by which SP can extravasate from vessels and engraft in skeletal muscle were studied and enhancement of progenitor cell engraftment was achieved with intra-arterial SP transplantation “Perez et al. (2009)”.

Blood vessels were identified as a promising source of novel stem cells called mesangioblasts. They can be delivered through blood and have a striking capacity to form muscle and repopulate diseased skeletal muscles. Mesangioblast therapy was studied in mice and in dogs with health improvement of the dystrophic animals “Galvez et al. (2006); Sampaolesi et al. (2006)” but in human treatment controversy still exists “Davies and Grounds (2006)”.

A promising recent study showed that it is possible to transduce SP cells from a dystrophic mouse with a lentiviral vector expressing human microdystrophin, and reconstitute a few dystrophic fibers with the human protein following intra-vascular delivery “Bachrach et al. (2004)”.
Presently supportive therapies are the main tool in reducing morbidity, increasing quality of life and prolonging lifespan. Pharmacological approaches involve the use of drugs/molecules to improve the disease phenotype by targeting specific components of the pathophysiology. Since the targets are specific to a pathological defect, a combination of approaches is required which enhance the probability of drug interactions and adverse effects.

1. **Anti-inflammatory Drugs**

   **A. Steroids**

   The relevant steroids in neuromuscular practice, prednisone/prednisolone and deflazacort, have a predominant glucocorticoid action. They showed potential for providing temporary improvement in two outcome measures: prolongation of ambulation and enhancement of muscle strength and function. Steroids demonstrated to stabilize muscle strength and function from six months up to two years. Long-term pulmonary and cardiac benefits were also reported but still need to be confirmed “Manzur et al. (2008)”.

   Corticosteroids have a catabolic effect on muscle (non-exercised muscle) preserving existing muscle fibers and reducing inflammation though, their exact mechanism of action in dystrophic skeletal muscle is unknown. Several possibilities based mainly on observations in mouse models were proposed and include: differential regulation of genes in muscle fibers “Muntoni et al. (2002)”; decrease in the number of cytotoxic/suppressor T cells “Kissel et al. (1991)”; reduction of cytosolic calcium concentrations “Vandebrouck et al. (1999)”; increasing laminin expression and myogenic repair “Anderson et al. (2000)”; retarding muscle apoptosis and cellular infiltration “Kojima et al. (1999)”; inhibition of muscle proteolysis “Rifai et al. (1995)”; protecting against mechanically induced fiber damage “Jacobs et al. (1996)”; enhancing dystrophin expression “Hardiman et al. (1993)”; shift the fiber type towards the fast-twitch fibers “Fisher et al. (2005)”; increasing muscle levels of taurine and creatine “McIntosh et al. (1998)”.

   The first randomized, double-blind placebo controlled study on the use of corticosteroids in DMD was reported in 1989. It compared a 6-month trial of
daily prednisone at doses of 0.75mg/kg and 1.5mg/kg where the 0.75 mg/kg daily dose had the most favorable profile “Mendell et al. (1989)”. Later on, the same group compared doses of 0.3mg/kg/day and 0.75mg/kg/day to placebo and the lower dose of 0.3 mg/kg daily was less beneficial “Griggs et al. (1991)”. Daily administration versus alternate day dosing was also studied and it was found that daily dosing at 0.75mg/kg maintained the effects longer than 2.5mg/kg on alternate days “Fenichel et al. (1991)”.

Deflazacort is an oxazolone derivative of prednisone and the therapeutic equivalence is about 1.2 mg deflazacort to 1.0 mg prednisone. A study compared two treatment deflazacort protocols, one using 0.6mg/kg/day for the first 20 days of the month and the other 0.9 mg/kg/day, determining the more effective dose being 0.9 mg/kg/day. This study confirmed the long-term benefits of deflazacort on mobility in both treatment protocols compared to boys not treated with deflazacort. At the present deflazacort is only available in some countries “Biggar et al. (2004)”.

Several comparison studies of prednisone and deflazacort have been performed and one of the latest shows similar benefits to both treatments. Deflazacort was also much more expensive and many families chose the prednisone regimen. Regarding to side-effects, excessive weight gain was more common with prednisone and asymptomatic cataracts with deflazacort “Balaban et al. (2005)”.

Controversies still exist regarding to starting age of corticosteroids, clinical criteria to start it, which corticosteroid, which dose and which regimen, and when to discontinue corticosteroids. Immunizations schedule is generally thought to be a reason to hold off initiating corticosteroids until 4 years of age. Another reason is that under 2 years of age children are still gaining motor skills making steroids unrecommended. Usually clinicians should propose initiation of steroids at age 4–8 years, when a plateau phase (there is no longer progress in motor skills and prior to decline) can be identified. Steroids are still advised once a decline in motor skills is achieved but the benefits may be significantly reduced “Bushby et al. (2010)”.

Systemic side-effects of steroid therapy in dystrophinopathies include those commonly seen with chronic steroid use. There have been reports of the following adverse effects: weight gain, behavioral changes, cushingoide
appearance, excessive hair growth, acne, osteoporosis/fractures, hyperglycemia/glycosuria, hypokalemia, hypertension, gastrointestinal side-effects, increased appetite, cataracts, sepsis and death “Manzur et al. (2008)”.

Maintenance of a daily schedule is appropriate when the child’s motor function is stable or in decline and if any corticoid side-effects are manageable and tolerable. A regimen in alternate days is an option if unmanageable side-effects develop in a daily dose regimen and they don’t ameliorate with dose reduction. In case of intolerable side-effects despite the regimen, a dose reduction of approximately 25–33% is suggested and a clinical reassessment in 1 month is necessary to determine whether side-effects have been controlled or not. If the adjustments to dose and/or schedule regimens prove ineffective in making any significant side-effects sufficiently manageable, then it is necessary to discontinue therapy despite the state of motor function. Management for weight gain should be accompanied by dietary advice and if obesity is of concern (>10-20% over estimated normal weight for height over a 12-month period) switching treatment from prednisone to deflazacort should be considered. Behavioural changes should be supported by psychological input and advice on bone health should be provided alongside with monitoring of fracture frequency. Vertebral fractures can be treated with intravenous bisphosphonates under the guidance of an expert in bone metabolism however there isn’t sufficient evidence to recommend the use of prophylactic oral bisphosphonates. Prophylaxis relies on advice about calcium and vitamin D intake through diet and sunshine with supplements of vitamin D3, if 25-hydroxy vitamin D is <32 nmol/L, and calcium to reach an intake of 1000 mg/day. Concomitant treatment with non-steroidal anti-inflammatory agents should be avoided and the use of antacids is recommended in the presence of gastrointestinal complaints. Annual ophthalmological examination and blood pressure/glucose intolerance surveillance at each clinic visit are also advised “Manzur et al. (2008)” “Bushby et al. (2010)”.
B. Other anti-inflammatory drugs

Other immunosuppressive drugs demonstrated benefits in \textit{mdx} mice leading to increasing recognition for the damaging role of inflammation in DMD. Clinical trials on dystrophic muscle with the use of cyclosporine proved beneficial through direct actions on skeletal muscle by inhibiting calcineurin and ameliorating impaired mitochondrial metabolism. This demonstrated that cyclosporine treatment significantly normalized many functional, histological, and biochemical endpoints by acting on events that are independent or downstream of calcium homeostasis. Muscle strength increased with a minimal dose of 5 mg/kg/day, however, cyclosporine exerts multiple dose-dependent effects and a correct dosage must be established especially when administered to young dystrophic patients during muscle development \cite{Sharma1993, DeLuca2005}.

Another potential anti-inflammatory drug that has attracted attention is the phosphodiesterases inhibitor pentoxifylline. Pentoxifylline is claimed to have an anti-inflammatory, anti-oxidant and anti-fibrotic action; it reduces Tumor Necrosis Factor-alpha (TNF-\(\alpha\)) production in vitro, reduces fibrosis and may also play a role in normalizing blood flow in dystrophic muscle. It was also shown that pentoxifylline counteracts the abnormal activity of calcium channels responsible for high sarcolemmal calcium permeability of dystrophic myofibers, suggesting a possible amelioration of dystrophic condition through alternative pathways. Despite this apparent modulation of many different targets, it was proposed a unique mechanism of action residing in pentoxifylline ability to inhibit specifically several isoforms of phosphodiesterases in different tissues. Until now no significant side-effects were reported with the use of pentoxifylline but, considering the function of phosphodiesterase enzymes and the key role of cyclic nucleotides for modulating vital functions, it’s important to use it carefully with special attention to dose and administration routine \cite{Burdi2009}.

Oxatomide and cromolyn are other anti-inflammatory drugs that block mast cell degranulation. They have shown benefits in \textit{mdx} mice, indicating that mast cell products, including tumor necrosis factor-alpha (TNF-\(\alpha\)), have detrimental effects in dystrophic muscle. However, further studies must be performed to corroborate this hypothesis \cite{Granchelli2000, Radley2006}.
2. SPECIFIC ANTI-CYTOKINE DRUGS

A new approach involves the modulation of specific cytokines, which has been very successful clinically in some severe inflammatory disorders. TNF-α is a key pro-inflammatory cytokine that stimulates the inflammatory response and its specific pharmacological blockade may reduce muscle necrosis. Satisfactory results in dystrophic muscle were obtained with the use of two different antibodies against TNF-α, infliximab and etanercept. In 2004, a study with infliximab-treated mdx mice showed reduction in the onset, intensity of necrosis and dystrophopathology “Grounds and Torrisi (2004)”. Later studies demonstrated that etanercept reduced effectively plasma CPK with physiological benefits in muscle strength and chloride channel function “Hodgetts et al. (2006); Pierno et al. (2007)”. This new strategy has the important benefit of few related side-effects due to its target high specificity.

3. ANABOLIC PROCESSES STIMULATION

A. Myostatin regulation

Myostatin is a member of the Transforming Growth Factor-beta family (TGF-β) and it’s a potent, negative regulator of functional muscle mass. Deletions of the myostatin gene cause muscle cell hypertrophy “Schuelke et al. (2004)” and in the mdx mouse, blocking myostatin lead to an increase in body weight as well as muscle mass, size and strength. Inhibition of the myostatin gene by injecting blocking antibodies is predicted to improve the disease phenotype in a variety of myopathies. In treated animals a significant reduction in muscle fiber degeneration and serum CPK levels was observed but the exact mechanism whereby myostatin improves muscle function is not yet known. Studies showed that an increase in the expression of utrophin appears not to be involved in the process “Bogdanovich et al. (2002)”.

B. Insulin-like growth factor I (IGF-I)

IGF-I is an example of a positive regulator of muscle growth. Although the presence of IGF-I can’t correct the primary defect in muscular dystrophy, it’s possible that it can play an important role defending against the secondary symptoms of the disease. Several different studies of normal muscle showed
that IGF-I is effective in increasing muscle mass and strength, promoting muscle regeneration, and preventing apoptosis. It was also described that muscle-specific IGF-I expression is beneficial to dystrophic muscle by preventing fibrosis in addition to promoting functional hypertrophy. The combined effect of enhanced repair and decreased wasting might lead to greater functional capacity over time, where there is a reduction in the proportion of maximal effort needed to produce a required force making the muscle less likely to be damaged by normal activity. As so, the increased rate of muscle regeneration may be a therapeutic alternative to replace the missing or defective dystrophin complex member in the dystrophinopathies “Barton et al. (2002)”.

C. β2-agonists

Beta-2 adrenergic agonists were shown to induce muscle hypertrophy and prevent atrophy after physical and biochemical insults in animals. A clinical trial in a boy with DMD showed that albuterol produced a modest increase in muscle strength without significant side-effects “Fowler et al. (2004)”. Recent studies reported that formoterol has more powerful anabolic effects on skeletal muscle than the older generation of β2-agonists, but inconsistency still exists regarding to adverse effects of this therapy “Harcourt et al. (2007)”. 

4. ANTIBIOTICS

Approximately 15% of DMD cases and most BMD cases are due to the formation of a premature stop codon within the coding sequence of dystrophin. Gentamicin is an aminoglycoside antibiotic that interferes with the ability of the ribosome to recognize a given stop codon allowing translation to continue past (suppress) a mutation in a given gene. This seems to be the only current pharmacological strategy aiming to correct the primary defect. Gentamicin-treated mdx mice showed both an increase in dystrophin expression, up to 10–20%, and functional improvement. Protection against contractile injury was also reported as a result of sarcolemmal dystrophin localization “Barton-Davis et al. (1999)”. Concerning to human gentamicin trials, although an initial decrease in CPK levels was noted, there was no increase in dystrophin expression as in animal’s results “Wagner et al. (2001); Dunant et al. (2003)”.
5. Anti-proteases

Dystrophin deficiency results in elevated Ca$^{2+}$ influx and impaired Ca$^{2+}$ homeostasis, which in turn activates calpain proteases that may account for myofiber degeneration. A pioneer study suggested a potential therapeutic effect with a new compound, BN 82270 (Ipsen), which is a membrane-permeable prodrug of a chimeric compound (BN 82204) dually acting as calpain inhibitor and anti-oxidant. It demonstrated increase in muscle strength, reduction in serum CK and fibrosis of the mdx mice diaphragm “Burdi et al. (2006)”. However, recent data showed that calpain enzymes perform only limited proteolysis of target substrates being unlikely the predominant contributor of muscle protein breakdown. Despite this, calpain enzymes play an important role initiating the breakdown of myofibrillar proteins by releasing protein fragments that become suitable substrates for the ubiquitin-proteasome system. Two classes of muscle-cell permeating dual calpain/proteasome inhibitors were developed and studied. Tripeptidic calpain/proteasome inhibitors showed superior proteasome inhibition when compared to dipeptidic inhibitors as well as higher potency in inhibiting cellular Ca$^{2+}$-induced proteolysis induced by Ca$^{2+}$ influx. Given their inhibitory activity and lower cellular toxicity, these compounds have a potential benefit in dystrophinopathies “Briguet et al. (2008)”.

6. Anti-oxidants

Several findings suggest that oxidative stress might be involved in the dystrophic process. Free radical injury may contribute to loss of membrane integrity in muscular dystrophies and dystrophic muscle cells have an increased susceptibility to reactive oxygen intermediates “Rodriguez et al. (2003)”.

Coenzyme Q10 (CoQ10; also called ubiquinone) is a powerful antioxidant and mitochondrial respiratory chain co-factor. Previous double blind trials have shown some efficacy but further studies are needed. At the present a phase III clinical trial is currently assessing the effect of CoQ10 (serum level greater than 2.5 μg/ml) and prednisolone (0.75 mg/kg/day) in wheelchair-dependent patients with DMD “http://www.clinicaltrials.gov/“.

Green tea polyphenols are also powerful antioxidants and along with its active constituents may improve dystrophinopathies prognosis. In an
experiment varying concentrations of green tea extract were given to mice for four weeks, beginning at birth. It was determined that the extract significantly reduced the degradation of certain muscles and noted that higher doses correlated with greater inhibition of decline. There was also biochemical evidence that green tea extract reduced oxidative stress in muscle cells “Dorchies et al. (2006)”.

It has also been suggested a role for nuclear factor (NF)-κB in muscle degeneration. The effects of blocking NF-κB by inhibition of oxidative stress/lipid peroxidation on the dystrophic process in mdx mice were studied using a strong antioxidant and lipid peroxidation inhibitor, IRFI-042. It was shown that IRFI-042 reduced muscle necrosis and enhanced regeneration by blunting NF-κB DNA-binding activity and TNF-α expression in the dystrophic muscles “Messina et al. (2006)”.

7. PHARMACOLOGICAL UTOPHIN UP-REGULATION

The high degree of structural similarity between dystrophin and utrophin led to the speculation that utrophin might be able to replace dystrophin in DMD and reconstitute the dystrophin associated protein complex (DAPC) “Tinsley and Davies (1993)”. Two promoters (A and B) have been described at the 5’ end of the utrophin gene with utrophin A being the expressed one at the neuromuscular junction; the expression of utrophin B is mainly confined to the vascular endothelia “Weir et al. (2002)”.

The utrophin promoter A is transcriptionally regulated in part by heregulin-mediated, extracellular signal-related kinase-dependent activation of the GABPα/β transcription factor complex. Studies showed amelioration of phenotype but not as dramatic as that reported by using transgenic means. Yet, it has a fundamental advantage of obviating the immune and delivery problems associated with germ-line modification and/or somatic gene therapy “Krag et al. (2004)”.

Stimulation of Calcineurin is thought to increase extrasynaptic expression of utrophin-A leading to restoration of DAPC and attenuation of the dystrophic pathology “Chakkalakal et al. (2004)”.
Treatment of *mdx* mice with nitric oxide donors or substrates of nitric oxide synthase (NOS) such as L-arginine increased the expression of utrophin with concomitant morphological corrections of muscle. Neuronal nitric oxide synthase (nNOS) catalyzes the production of nitric oxide and citrulline from the substrate L-arginine. Studies show that the loss of nNOS is a major contributing factor to many of the pathological changes in DMD “Voisin et al. (2005)”.

A recent work showed that the utrophin 5’ end confers IRES (internal ribosome entry sites)-mediated translational control during regeneration of skeletal muscle fibers. Some viral and eukaryotic cellular mRNAs can be translated via internal initiation making the stabilization of utrophin or the post-transcriptional control of utrophin expression additional targets for therapeutic intervention “Miura et al. (2005)”.

### 8. Supplements

Creatine is an “energy precursor” naturally produced by the body. Transformed by the body into phosphocreatine, it enters muscle cells and promotes protein synthesis while reducing protein breakdown. Data suggested that supplemental creatine can improve muscle performance and strength, decrease fatigue, and slightly improve bone mineral density. Glutamine is involved in many metabolic processes and it is an important energy source for many cells. A recent study showed inconclusive results but it couldn’t exclude a disease-modifying effect of creatine and glutamine in younger DMD “Escolar et al. (2005)”.

Taurine is abundant in normal skeletal muscle and is believed to exert both long- and short-term control over the functionality of ion channels. Supplementation of taurine counteracts exercise-induced weakness after chronic exercise and ameliorates macroscopic chloride conductance (an index of degeneration-regeneration) in *mdx* mice muscles “De Luca et al. (2003)”.

A combination of creatine monohydrate, conjugated linoleic acid, alphalipoic acid and betahydroxy-beta-methylbutyrate improved strength and decreased fatigue in the *mdx* mice. This combination was better than any individual supplement alone and the combination with prednisone provided the best results “Payne et al. (2006)”.
9. MANAGEMENT OF COMPLICATIONS

A. Respiratory

Effective airway clearance is critical to prevent atelectasis and pneumonia that can lead to respiratory failure and death. Cough peak flows correlate directly to the ability to clear secretions from the respiratory tract and the use of assisted cough technologies is recommended whether there’s clinical signs of ineffective clearance, the peak cough flow is less than 270 L/minute or when maximal expiratory pressures are less than 60cm H$_2$O. Mechanical insufflation-exsufflation has shown to be more effective than manual techniques.

Another major complication of dystrophinopathies is a sleep-disordered breathing. The main recommendation is to use nasal continuous positive airway pressure (CPAP) in patients with obstructive sleep apnea syndrome but without nocturnal hypoventilation; and to use nasal intermittent positive pressure ventilation (NIPPV) along with bilevel positive airway pressure (BiPAP) generator or mechanical ventilator to treat sleep-disorder breathing and nighttime hypoventilation. Avoidance of supplemental oxygen is crucial because it can suppress respiratory drive and worsen the defect. Daytime ventilation should be considered when measured PCO2 exceeds 50 mmHg or when hemoglobin saturation remains < 92% while awake. Non-invasive techniques like mouth-piece intermittent positive pressure ventilation are preferred but tracheostomy remains an option when others are contraindicated “Finder et al. (2004)”.

B. Cardiac

Dilated Cardiomyopathy (DCM) occurs in up to 90% of boys with DMD aged ≥18 years. It becomes symptomatic only in a minority and it’s the cause of death in 20% of the cases. Recently, positive results were found with the use of angiotensin converting enzyme inhibitors, mainly perindopril, and/or beta-blockers in patients with early cardiomyopathy. However optimal timing for introducing therapy for DCM remains undetermined “Bouhouch et al. (2008)”.
**C. Orthopedic**

The main goal in the treatment of DMD is to maintain ambulation for as long as possible and to anticipate and manage the associated complications, such as joint contractures and scoliosis. There are several physical interventions recommended according to the stage of the disease.

**i. Ambulant children:** active, active-assisted or passive daily stretching at the ankle, knee and hip are necessary to prevent contractures. Moderate levels of active exercise, particularly hydrotherapy or swimming, are recommended. Night ankle-foot-orthoses (AFOs) should be provided at the loss of ankle dorsiflexion, when there is loss of normal grade of dorsiflexion and before the foot can only achieve plantagrade. Daytime AFOs are not suggested for ambulant children as they compromise their ability to walk. KAFOs (knee-ankle-foot-orthoses) can be considered to delay contracture development and prolong ambulation in the late ambulatory stage. Surgical lengthening of early contractures is also an option and it has been performed in children as young as 4-7 years. Surgical intervention is thought to prolong ambulation by 1 to 3 years “Eagle (2002); Bushby et al. (2010)”.

**ii. Non-ambulant children:** regular passive or active assisted stretching are necessary, both in lower and upper extremities. Night and daytime AFOs should be supplied as well as KAFOs (not well tolerated by night) in order to prevent painful contractures and foot deformity. Surgical intervention is only recommended to alleviate specific symptoms, such as pain and pressure, since it’s generally ineffective as a routine procedure to correct deformities “Muntoni et al. (2006); Kinali et al. (2007)”.

**iii. Management of scoliosis:** Scoliosis usually manifests after loss of walking, shows rapid progression coincident with the pubertal growth spurt and has a negative impact on respiratory function, feeding, seating and comfort. It has been showed that daily steroid treatment along with the use of KAFOs reduces the risk of scoliosis “Kinali et al. (2007)”. Surgery is usually scheduled once the Cobb angle measured on scoliosis films is between 20-40º but the optimal timing for surgical intervention is while lung function is satisfactory and before cardiomyopathy becomes severe enough to risk arrhythmia under anesthesia “Muntoni et al. (2006)”.

CONCLUSION

Research into therapeutic strategies for dystrophinopathies has progressed rapidly over the past decade. The development of practical gene- and cell-based therapies is an ongoing process that is still in its initial stages. It is only through additional investigation that the drawbacks of these promising approaches will be overcome and ultimately lead to the development of therapeutic strategies effective in stemming the progressive and multifactorial nature of DMD. Until these therapies become clinically available, supportive pharmacological treatments are the main weapons against the disease’s progression with rehabilitation playing a core role in reducing morbidity, increasing quality of life and prolonging lifespan of affected children.

Finally, it is probable that dystrophinopathies with such a complex pathogenesis will only be defeated by a multidisciplinary approach aimed to replace or correct the mutated gene product as well as counteract the devastating consequences of the primary mutation on muscle structure and function.
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