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Duchenne muscular dystrophy: an historical treatment review

Distrofia muscular de Duchenne: revisão histórica do tratamento

Lineu Cesar WERNECK[1], Paulo José LORENZONI[1], Renata Dal-Prá DUCCI[1], Otto Hernández FUSTES[1], Cláudia Suemi Kamoi KAY[1], Rosana Herminia SCOLA[1]

ABSTRACT

In this review, we discuss the therapies used in the treatment of patients with Duchenne muscular dystrophy since the first description of the disease. A short description is given of the various theories based on disease pathogenesis, which give the substrates for the many therapeutic interventions. A brief review of the methods of evaluation used in therapeutic trials is made. Of all the treatments, the only drugs that are still considered able to modify the course of the disease are the corticosteroids (prednisone/prednisolone/deflazacort). Other drugs (coenzyme Q10 and creatine) have had a little effect in a few functions without adverse reactions. Idebenone seems to improve the respiratory function in the long term. The trials with mRNA transcription, through nonsense mutations or exon 51 skipping, show some beneficial results in a few functional tests, but they are limited to a small set of DMD patients.

Keywords: Muscular dystrophy, Duchenne dystrophy, muscular diseases.

RESUMO

Nesta revisão são discutidas as terapêuticas empregadas no tratamento da distrofia muscular de Duchenne desde a descrição da doença. Apresentamos as diversas teorias que fundamentaram as intervenções terapêuticas, com uma breve descrição dos tipos de avaliação empregados nos ensaios terapêuticos. Dentre todos os tratamentos, a única medicação que até agora modificou o curso da doença foram os corticosteroides (prednisona/prednisolona/deflazacort). A coenzima Q10 e creatina tiveram algum efeito pequeno em algumas funções e evolução da doença sem efeitos colaterais. O idebenone mostrou efeito benéfico na função respiratória em longo prazo. As tentativas de intervir no gene da distrofina utilizando técnicas de transcrição do mRNA através das mutações sem sentido e técnicas que pulam o exon 51 mostram resultado muito discreto em algumas provas funcionais e limitado a uma parcela pequena de casos.

Palavras-chave: Distrofia muscular, Distrofia muscular de Duchenne, doenças musculares.

Duchenne muscular dystrophy (DMD) is a progressive hereditary muscular disease with X-linked recessive inheritance, occurring mainly in males. Muscular impairment is initially in the proximal muscles of the lower limbs with reduced muscle strength and progressive contractures with gait impairment. Subsequently, with reduced muscle strength in the upper limbs, the patients develop contractures in the arms, and impairment of the respiratory and heart muscles. Although there is no effective therapy, most patients currently walk until age 13, some even until age 20, and some survive for more than 30 years due to ventilatory and cardiac support[1].

The DMD is caused by the absence of a protein called dystrophin that binds to F-actin and sarcolemma proteins allowing flexibility of the plasma membrane during muscle contraction and maintaining the fiber structure (Figure 1).

With the advancement and diffusion of molecular biology techniques, the diagnosis of DMD has become easy and, nowadays, a muscle biopsy is performed only in selected cases. A molecular diagnosis has many therapeutic implications, as we will see later in this paper. According to the literature, most cases of DMD (~80%) have deletions or duplications of one or more exons by disrupting messenger ribonucleic acid (mRNA) transcription into ribosomes and disrupting complete dystrophin codification. The remaining 20% have small mutations that may cause premature interruption of codons due to reading frame rupture[2]. The frequencies of these

[1]Universidade Federal do Paraná, Hospital de Clínicas, Serviço de Doenças Neuromusculares, Curitiba PR, Brasil.

Lineu Cesar Werneck https://orcid.org/0000-0003-1921-1038; Paulo José Lorenzoni https://orcid.org/0000-0002-4457-7771; Renata Dal-Prá Ducci https://orcid.org/0000-0002-1673-5074; Otto Hernández Fustes https://orcid.org/0000-0003-0778-5376; Cláudia Suemi Kamoi Kay https://orcid.org/0000-0003-0173-0809; Rosana Herminia Scola https://orcid.org/0000-0002-3957-5317

Correspondence: Lineu Cesar Werneck; Rua General Carneiro; 181 / 3° andar; 80060-900 Curitiba PR, Brasil; E-mail: lineu.werneck@hc.ufpr.br.

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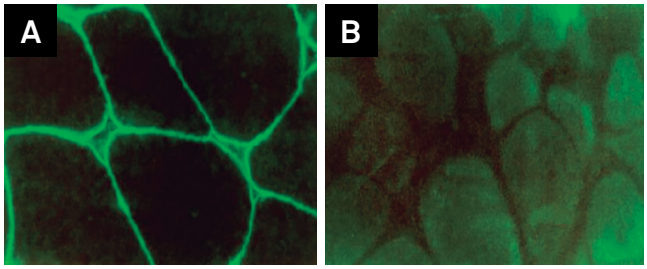

Figure 1. Immunohistochemical stain for dystrophin. A. Normal muscle with dystrophin in the sarcolemma. B. Absence of dystrophin in the sarcolemma of muscle fibers of Duchenne muscular dystrophy.

changes vary depending on the location of the study and are important for the modern therapeutic approach.

In this review, an 'historical analysis' on the published therapies for DMD patients briefly reports the main results of the completed trials and their backgrounds. Experimental research that is ongoing, but not yet applied to DMD patients (e.g. CRISPR-Cas9) has not been included. This review details the few therapies that have had a beneficial response in the DMD patients. In addition, this review may also help physicians understand why some therapies were abandoned while others will be emerging on the horizon in the near future.

METHODS FOR EVALUATING TREATMENT RESULTS

Since the earliest descriptions of DMD, several therapeutic modalities have been employed, some empirically and others based on reports of biochemical or metabolic abnormalities from human and animal studies. Some papers have reported promising results, but the methods of evaluation varied widely and generally did not have a placebo group. This led some researchers to study the natural history of DMD. One of the first parameters used was the age of gait loss resulting from reduction of muscular strength. To obtain a quantitative evaluation of the muscle strength reduction, the Medical Research Council scale (quantitative manual muscle testing force) was used, with small modifications. Several muscles were examined, given a score and then summed[3]. Functional tests have also been developed, where certain activities are requested and the time to execution measured and correlated to the age for that function, or the baseline values compared with the use of the drug being studied. Among the functions evaluated are: time to get up from the floor and stand up; Gowers' maneuver; keeping the arms elevated; chair lift; climbing four steps, going down four steps; time to walk 10 meters; the 6-Minute Walk Test (6MWT – distance walked in six minutes), among others. These tests can be analyzed in isolation or grouped as in the North Star Ambulatory Assessment[4].

TREATMENTS AND THEIR RESULTS

Several treatment modalities have been grouped according to the knowledge of the disease at the time they were evaluated (Table).

Electrostimulation

Since 1861, Duchenne had been applying electrical stimulation in patients with paralysis of any nature but there are no reliable descriptions of the response. The reports of chronic low-frequency electrical stimulation in DMD patients have found some benefit, but only in a few patients and the follow-up was not reported[5]. In 1997, it was experimentally demonstrated that electrical stimulation caused greater accumulation of intracellular calcium, leading to premature degeneration of the fiber by possible activation of the proteases, and could accelerate the degeneration of the muscle fiber. After this discovery, enthusiasm for electrical stimulation was lost and it was no longer studied[6].

Table. Therapies used for Duchenne muscular dystrophy.

Main mechanism	Therapy
Electrostimulation	Electricity
Cholinesterase Inhibitors	Galantamine
Drugs acting on biochemical, metabolic and oxidative stress	Laevadosin, Allopurinol, vitamin D and Calcium, Creatine and Glutamine, Coenzyme Q10, Idebenone
Drugs acting on growth, height and muscular function	Mazindol, Growth Hormone, Isaxonine, Anabolics (Sinestrol and Oxandrolone), Albuterol
Drugs producing changes in sarcolemma and calcium aggregate	Verapamil, Flunarizine, Nifedipine, Diltiazem
Drugs interfering in the muscle blood flow	Methysergide, Tadalafil
Conjunctive tissue proliferation	Penicillamine, Pentoxifylline
Inflammatory reaction	Azathioprine, Cyclosporine
Corticosteroids	Prednisone, Prednisolone, Deflazacort
Restoration of premature termination codon	Gentamicin, Ataluren
Exon Skipping	Drisapersen, Eteplirsen

CHOLINESTERASE INHIBITORS

Galantamine

Galantamine is an anticholinesterase drug with action in the central and peripheral nervous systems. It prolongs the action of the acetylcholine in the receptor located in the myoneural junction. An attempt to use galantamine in DMD patients was made, with no apparent response[7].

ANTIOXIDANT DRUGS ACTING IN BIOCHEMICAL AND METABOLIC PATHWAYS

Muscle injury in muscular dystrophies of animals and humans induces several metabolic abnormalities, with increases in free radical levels, and subsequent oxidative stress and changes in others muscle fiber components. This raised the possibility that antioxidant therapy or the replacement of abnormally low substances found in DMD may have some effect on the disease progression.

Laevadosin

Laevadosin is a mixture of nucleotides and nucleosides that supposedly could have some effect on DMD patients, as reported by Thomson and Guest in 1963[8]. However, a study with 20 DMD patients, of whom 10 were controls, over a period of 42 days, did not show any benefit with respect to muscle strength compared with placebo, although it reduced creatine kinase in some patients[9].

Allopurinol

Considering the hypothesis of a defect in purine metabolism, allopurinol was tried to facilitate the transport of purines. The first study, by Thomson and Smith in 1978[10] had 16 DMD patients who used allopurinol for 18 weeks. Some patients improved strength for more than six months compared with placebo. These data have subsequently not been confirmed[11].

Vitamin D and calcium

The role of calcium in the physiology and function of muscle fiber has been known for long time. It was found that DMD patients had a vitamin D deficiency compared with controls. This deficiency may be the cause of osteopenia and a higher incidence of fractures found in patients with DMD, and which is aggravated by the use of corticosteroids. A correlation was found between muscle strength and reduction of bone mass, as well as reduction of bone mass and the cumulative dose of corticosteroids. Administration of vitamin D with or without alendronate has shown an increase in bone mass but the effect on fracture reduction was uncertain and there was no change in the functional progression of the disease[12].

Creatine and glutamine

After the demonstration of creatine reduction in DMD muscle and the possible beneficial effect, supplementation with creatine 0.10 g/kg/day was tested in a double-blind study with 30 DMD patients over a period of four months, where half of the patients were using corticosteroids. There was an increase in grip strength ($p < 0.05$) in the dominant hand and strength in general ($p = 0.056$), with no improvement in activities of daily living[13]. Another attempt with creatine was made in 50 DMD patients (16 on placebo, 15 taking creatine 5 g/day and 19 taking glutamine 0.3 mg/kg/day). The creatine and glutamine groups had minor deterioration in manual muscle strength tests. In the functional stair-climbing test, the group that used creatine had a better performance ($p = 0.015$). These drugs were safe and well tolerated in the prescribed doses, but the impact on long term survival was not studied[14].

Coenzyme Q10

Based on studies in animals, a therapeutic approach was taken by Folkers and Simonsen in 1995[15], using coenzyme Q10 in one DMD patient, and other neuromuscular disorders. The patients become more active and could walk and run better. After this initial study, coenzyme Q10 was used in 13 DMD patients using corticosteroids, with an initial dose of 400 mg, with escalations of 100 mg/day. Improvements on several muscular and functional tests during the study period (six months) were observed[16]. These data were corroborated in patients using corticosteroids and various vitamin and dietary supplements, through multivariate analysis. In these patients, the duration of walking increased in 155 patients with coenzyme Q10 ($p = 0.007$) and in 246 patients who used vitamin D ($p = 0.004$)[17]. The long-term survival was not established.

Idebenone

Idebenone is a synthetic analogue of coenzyme Q10 with similar effects and could be useful in respiratory function. It was studied over 52 weeks in 31 DMD patients compared with 33 participants on placebo, aged between 10 and 18 years, in a dose of 900 mg/day, testing several parameters of respiratory function. The patients who used the idebenone improved their peak inspiratory flow (L/min), forced expiratory volume, forced expiratory volume in one second, and had a smaller decline in peak inspiratory flow (2.5% vs 6.27%, $p = 0.031$). These data suggest some preservation of pulmonary muscle function in the long term[18].

DRUGS ACTING ON GROWTH, HEIGHT AND MUSCULAR FUNCTION

In 1981, Zatz et al. described a DMD patient with slower progression of the disease associated with growth hormone deficiency[19]. This opened up the possibility of using growth hormone inhibitors as a therapeutic option.

Mazindol

Mazindol is an inhibitor of growth hormone. In 1988, Coakley et al., used mazindol in DMD patients[20]. They did not find any significant changes in muscle force or functional tests. This study was interrupted due to the side effects. Another study with 83 DMD patients (3 mg/day for 12 months against placebo) was done and no benefit was observed in relation to muscle strength, contractures or functional tests[21].

Growth hormone

Over the years, it has been found that one of the side effects of corticosteroids was the reduction of the growth of patients with DMD. Growth hormone was administered for one year in patients using corticosteroids. This increased the rate of growth, improving some motor functions without side effects or change in cardiac function[22]. However, as the patient grows, the muscle mass and weight increases, without improvement of muscle strength. This may impair the gait in some patients.

Isaxonine

Isaxonine is a synthetic compound that, in animals, accelerates nerve regeneration and increases the percentage of plasma membrane proteins in muscle fibers. A double-blind study was performed over a period of two years, using 20 patients with ambulatory DMD, who were aged 5.5 to 10 years. There was no significant difference in disease progression compared with placebo[23].

Sinestrol

The fact that anabolic steroids accelerate muscle growth led a Russian author to try sinestrol in 15 DMD patients, compared with 14 controls over three weeks. Six months after withdrawal of sinestrol, they found that the disease continued to progress, although less so in the treated group[24].

Oxandrolone

Oxandrolone is an anabolic steroid that can promote growth with minimal toxicity. It facilitates weight gain in chronic debilitation and promotes growth in boys with constitutional delay and may increase muscle protein synthesis. Oxandrolone was tested in DMD patients in a double-blind, six-month study, at a dose of 0.1 mg/kg/day in 26 patients, and 25 controls receiving placebo before the use of corticosteroids. Those who used oxandrolone had a better performance, but this was not statistically significant ($p = 0.13$). When only strength in the upper limbs was analyzed, the treated group showed stabilization ($p = 0.005$). In this study, the authors thought it may be useful before starting corticosteroids[25].

Albuterol

Albuterol is a beta-2a adrenergic receptor agonist that, theoretically, would maintain the protein structure and indirectly act as an anabolic effect. Albuterol was used in 14 DMD patients (seven albuterol *vs* seven placebo) at a dose of 12 mg/day for 12 weeks. For comparison, muscle strength was tested manually and using several functional tests. In the short term, the patients who used albuterol increased lean body mass, and improved running time ($p = 0.025$), but there was no change in muscle strength observed[26]. This small impact on the long-term survival of DMD patients was also responsible for there being no current use of this drug in DMD.

DRUGS PRODUCING CHANGES IN SARCOLEMMA AND CALCIUM ACCUMULATION

In 1975, Mokri and Engel described wedge-shape lesions with failure in the plasma membrane of muscle fibers in patients with DMD[27]. This disruption of the membrane allowed the output or the ingress of material, creating abnormalities in the underlying cellular organelles and myofibrils. One of these substances accumulated in large-dark fibers was calcium, which can activate the proteolytic enzymes leading to necrosis[28] (Figure 2). This finding led to the investigation of calcium antagonists in DMD.

Verapamil

Verapamil was used in DMD patients (three patients on verapamil *vs* five on placebo) using electronic ergometers as a method of evaluation. The three patients who used verapamil improved in strength, but it was not a "dramatic" effect. Over time, the DMD patients increased their PR interval on an electrocardiogram and, as a precaution, the drug was discontinued[29].

Flunarizine

The effect of flunarizine on DMD patients was evaluated in a double-blind controlled study of 27 patients (13 on medication) for one year. No difference was found between the treated patients when compared with the placebo group. Some patients in the flunarizine group had a faster functional decline in their lower limbs[30].

Nifedipine

Nifedipine was administered in 105 DMD patients, at a dosage of 0.75-1.0 mg/kg/day for the first six months and then 1.5-2.0 mg/kg/day for another 12 months, which was then compared with a placebo group. No statistically significant difference was found in any of the analyzed parameters[31].

Diltiazem

In 1988, Pernice et al. studied the therapy with diltiazem at a dose of 5 mg/kg in DMD patients (13 patients *vs* 13 controls)[32]. The initial study lasted one year and no difference was found in all analyzed parameters, but the diltiazem group

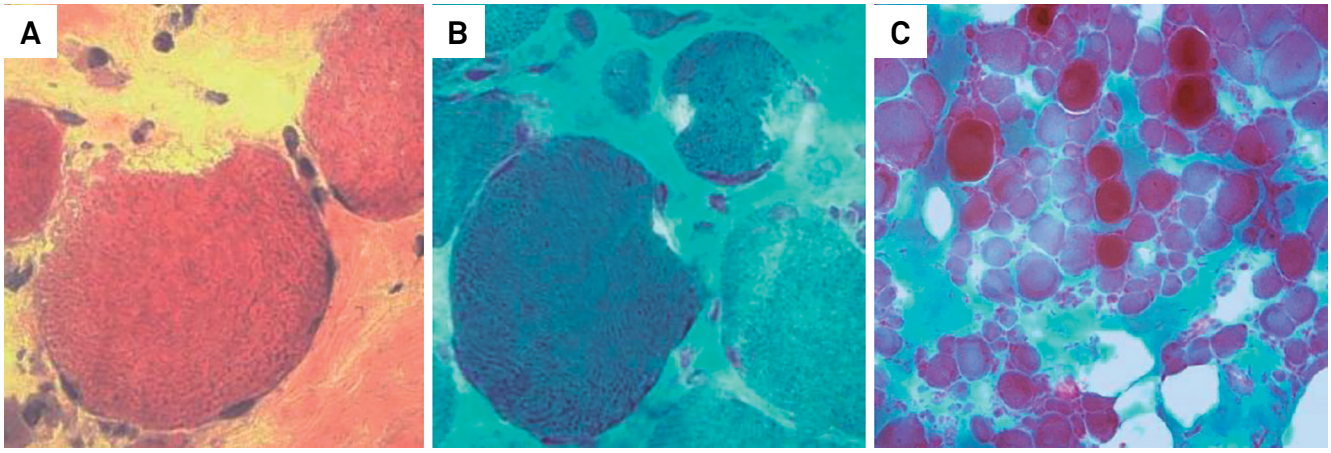

Figure 2. Duchenne muscular dystrophy. A, B: Wedge-shape lesions in the sarcolemma. C: Large-dark fibers. (A: hematoxylin and eosin stain; B, C: Modified Gomori trichrome stain).

had a lower percentage of calcium-positive muscle fibers than the control biopsies. The study was extended to three years with 46 patients and compared with placebo but no beneficial response was found. A similar study was done with a dose of 8 mg/kg/day in 11 DMD patients, and 11 controls. The muscle strength continued to decrease. In the group that used diltiazem, this reduction was smaller, but without statistical significance[33].

A meta-analysis with calcium channel antagonists, covering five studies showed no benefit in DMD patients. However, this meta-analysis covered small number patients. It was concluded that calcium-channel antagonists were of no use in DMD[34].

DRUGS INTERVENING IN MUSCLE BLOOD FLOW

In the 1970s, animal experiments using monoamines (serotonin and nor-epinephrine) associated with imipramine, produced histological changes similar to that seen in DMD patients. Parker et al. raised the hypothesis of myotoxicity or muscular ischemia in the pathogenesis of DMD[35]. Several researchers have turned to drugs that could intervene in muscle blood flow by increasing circulation or inhibiting substances with a possible myotoxic effect.

Methysergide

A trial with methysergide 8 mg/day improved scores on the time to perform the Gowers' maneuver, gait time, and grip strength compared with placebo. However, these were not statistically significant in the short term[36].

Tadalafil

Associated with the plasma membrane of the muscle fiber, there is a variant of neuronal nitric oxide synthase, which acts on the formation of nitric oxide and is involved in the regulation of muscle blood flow. Nitric oxide modulates adrenergic vasoconstriction (functional sympatholysis) during muscle exercise to improve perfusion. Due to the dystrophin deficiency in the sarcolemma of DMD patients, this could hypothetically create disruption of the enzymatic system of the neuronal nitric oxide synthase with a consequent reduction in the blood flow. Tadalafil was used in 331 DMD patients, one group receiving 0.3 mg/kg/day and another group 0.6 mg/kg/day, compared with a placebo group. In functional tests over a 48-week period, tadalafil did not prevent progressive decline in gait or any of the other parameters[37].

ANTIFIBROTIC DRUGS AGAINST CONJUNCTIVE TISSUE PROLIFERATION

Necrosis and phagocytosis induce endomysial connective tissue proliferation with muscle fibrosis and shrinkage and progressive muscle retraction (Figure 3). To try to reduce fibrosis and the proliferation of connective tissue, and perhaps influence the evolution of the disease, several drugs have been used[38,39].

Penicillamine

Based on experimental data from chickens, DMD patients were treated with penicillamine (15 patients *vs* 15 controls), but after 11 months there was no significant difference in the degree of contracture or muscle strength[38]. Another study with 97 DMD patients, for whom penicillamine was combined with vitamin E, showed no difference between the treated patients and placebo[39,40].

Pentoxifylline

Pentoxifylline is a phosphodiester with an anti-inflammatory effect that inhibits the formation of tumor necrosis factor alpha, and reduces fibrosis by interfering in the metabolism of metalloproteinases and collagen, influencing the transformation of the growth factor β that is "upregulated" in DMD.

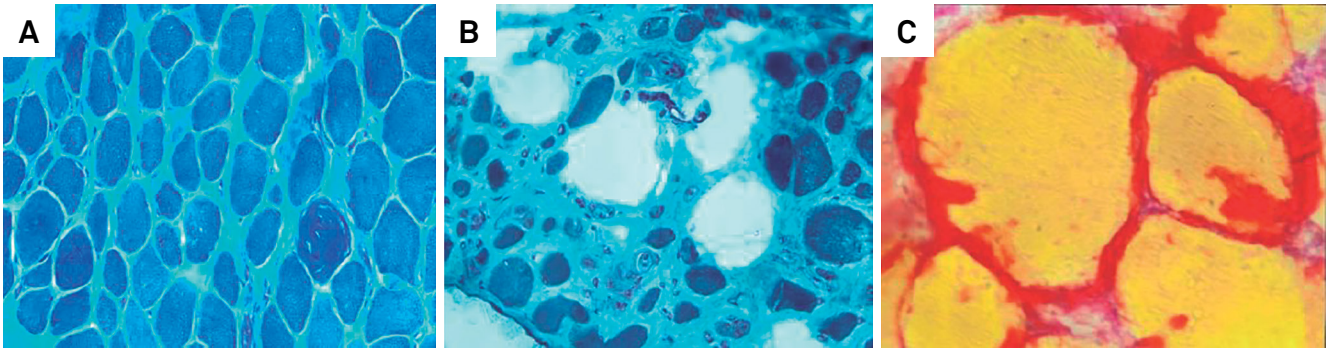

Figure 3. Duchenne muscular dystrophy. Increased endomysial connective tissue. A, B: Modified Gomori trichrome stain. C: Sirius red stain.

Pentoxifylline was administered to 62 DMD patients and compared with placebo, with doses ranging from 400 to 1200 mg/day. All patients were using corticosteroids. No significant change was found among the 30 analyzed parameters[40].

ANTI-INFLAMMATORY DRUGS

The DMD patients, at some stage of the disease, have a mild-to-moderate inflammatory reaction (Figure 4). The action of corticosteroids when modifying the natural history of DMD has raised hypotheses of their mechanism of action, and among them was the possible anti-inflammatory effect. In order to obtain the same anti-inflammatory effect as corticosteroids and to use smaller doses, some immunosuppressive drugs have been tested[41,42,43].

Azathioprine

In 1993, a randomized controlled trial of prednisone was tested, in addition to azathioprine 2-2.5 mg/kg/day for 18 months in 99 DMD patients. The addition of azathioprine did not show any benefit and the conclusion was that the effect of corticosteroids was not due to immunosuppression[41]. Azathioprine reduced inflammatory infiltrate and cell response in muscle biopsies at the same intensity as corticosteroids, suggesting that the corticoid effect does not have its effect due to the reduction of the inflammatory reaction[42].

Cyclosporine

In 1993, at the same time as the azathioprine study, cyclosporine was tested by Sharma et al., in 15 DMD patients at a dose of 5 mg/kg/day for eight weeks and the strength of the anterior tibial muscle was assessed by maximal voluntary contraction and stimulation[43]. The force increased while the patients were using cyclosporine but the progression of weakness continued when the drug was stopped. A double-blind study was done with 77 DMD patients, who received cyclosporine at the dose of 3.5-4.0 mg/kg/day, and paired with a group of 76 DMD patients using placebo, for three months. In this study, another group received concomitant prednisone 0.75 mg/kg every other day for 12 months. Cyclosporine alone or in combination with prednisone did not improve muscle strength or the functional capacity of mobility[44].

CORTICOSTEROIDS

In 1974, Drachman et al., in view of the few controversial reports of corticosteroid use in DMD patients, empirically used prednisone in 14 patients at an initial dose of 2 mg/kg/day and then reduced the dose to 1/3 of the original. They noticed an improvement in mobility, gait, and agility, and a creatine kinase serum reduction in 13 patients. They considered the response good in seven patients and moderate in six, over a period of three and 28 months. They

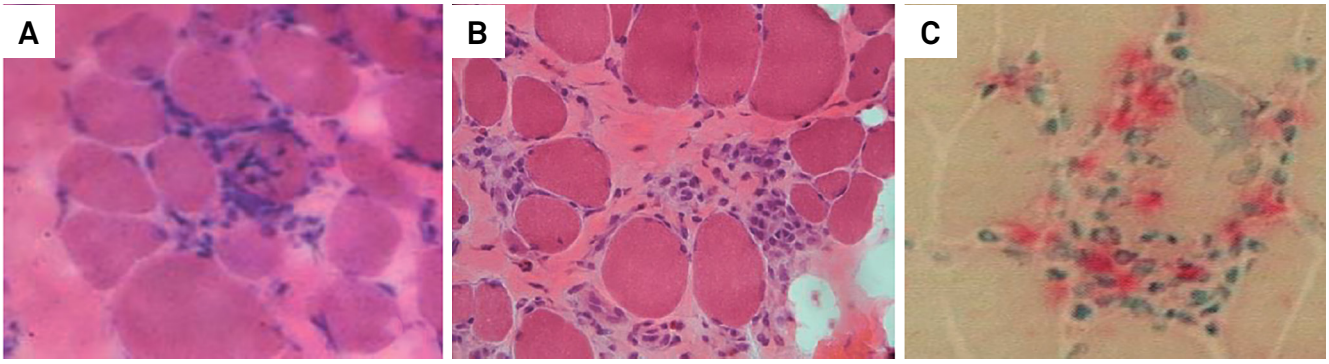

Figure 4. Duchenne muscular dystrophy. Inflammatory reaction. A, B: hematoxylin and eosin stain. C: Acid phosphatase reaction.

concluded that prednisone prolonged the period of ambulation and improved the patients' quality of life[45].

Even without controlled studies, the prescription of prednisone in DMD patients was diffused, corroborated by the clinical practice of apparent improvement. In 1989, a placebo-controlled trial of 103 patients using prednisone 0.75 and 1.5 mg/kg/day for six months, showed improvement in strength and functional activities in DMD patients[46]. Since then, prednisone has routinely been prescribed because the benefits are greater than the side effects.

Cooperative studies with a greater number of patients (440) confirmed that corticosteroid administration prolongs muscle function in the upper and lower limbs, maintaining the strength for longer, helping in day-to-day functions, walking for a longer time, improving quality of life and survival. In addition, it has beneficial effects on pulmonary ventilation and cardiomyopathy[47].

The largest study on the action of corticosteroids enrolled 5,345 DMD patients, 2,658 of whom were continuously using corticosteroids (49.7%), 2,015 had never used (37.7%) and 522 had used corticosteroids in the past (9.8%). Those who used corticosteroids continuously walked for a longer time (three years) when compared with those who did not. The use of corticosteroids significantly reduced the number of scoliosis surgeries, delayed the use of assisted ventilation, but did not influence the incidence of myocardiopathy after 20 years. The single deletion of exon 45 treated with corticosteroids delayed the loss of gait and increased the survival time compared with the other types of deletions[1].

Benefits vs adverse effects

Corticosteroid therapy improves muscle strength by prolonging gait time, preserves upper limb function, helps prevent scoliosis, reduces cardiomyopathy progression, and delays the need for invasive ventilation, with some patients surviving longer than 30 years. However, chronic use of corticosteroids causes delayed growth and puberty, weight gain, skin changes, a Cushingoid appearance, behavioral disorders, adrenal suppression, reduction of bone mineralization, cataracts and metabolic changes[48]. Each of these complications should be monitored and, when they arise, they should be appropriately treated.

Deflazacort versus Prednisone

Although there are several corticosteroid drugs, only prednisone and deflazacort have trials in DMD patients that have been published.

Deflazacort is a synthetic heterocyclic corticosteroid obtained by the fusion of methyloxazoline in the prednisone structure with great effectiveness and good tolerability. It causes less retention of sodium, has a strong anti-inflammatory action with immunosuppressive activity, low interference in carbohydrate metabolism and in the metabolism of calcium and phosphorus[49].

Deflazacort may replace prednisone, and a study has shown that the time to loss of the gait in DMD patients was slightly longer, but with increased side effects such as a Cushingoid appearance, erythema, hirsutism, increased weight, nasopharyngitis, delayed puberty, cataracts, and vertebral fractures. Even with more side effects, it seems that the deflazacort group had a better functional result, but this warrants a head-to-head comparison study[50].

Despite these studies of side effects, both corticosteroid drugs (prednisone and deflazacort) remain the most important therapy for DMD patients, extending the duration of gait and delaying the appearance of scoliosis when the patients become wheelchair bound.

RESTORATION OF DYSTROPHIN

Transcription through nonsense mutations

Gentamicin

In 1979, Singh et al., found that the addition of aminoglycoside antibiotics enabled the researchers to ignore some nonsense mutations in fungal and yeast cultures[51]. In the following years, it was demonstrated that gentamicin addition promoted the codons of premature termination readthrough in the *mdx* mouse with a point mutation (nonsense) in the *DMD* gene, which led to the production of dystrophin and reduction of the serum creatine kinase levels in this animal model[52].

Gentamicin was initially tested in two DMD patients and two patients with Becker muscular dystrophy. This study resulted in production of an incomplete truncated dystrophin in these patients, with no change in muscle strength. However, these patients reduced their serum creatine kinase levels and had no side effects[53]. Gentamicin 7.5 mg/kg/day was given intravenously for 14 days to 10 patients with stop codon in the *DMD* gene (DMD group) and 10 patients with other frameshift abnormalities in the *DMD* gene (control group). The stop codon group had a significant reduction of serum creatine kinase up to day 28. After six months, they showed stabilization of the force, functional tests without alteration, discrete increase in forced vital capacity ($p = 0.06$), reduction of serum creatine kinase level ($p = 0.04$) and an increase of 15% in the dystrophin levels in muscle ($p < 0.001$)[54].

In the following years, with the results of aminoglycosides studies achieving transcription of nonsense mutations in DMD, motivated trials to develop biological molecules with the same properties (e.g. ataluren), to overcome a premature termination codon[55].

Ataluren

Ataluren is a biological molecule that allows different premature termination codons readthrough, such as UAG, UAA, and UGA during mRNA transcription. This drug promotes the incorporation of specific amino acid units in premature

termination codons (Glc, Lys, Tyr in the codons UAA and UAG; Trp, Arg and Cys in the UGA codon)[55].

One study evaluated therapy with ataluren for 48 weeks in 174 DMD patients. In this trial, DMD patients were divided into three groups: 57 patients in the group using ataluren at a dose of 40 mg/kg/day, 60 patients in the group using ataluren at a dose of 80 mg/kg/day and 57 patients in the placebo group. Most of the DMD patients were using corticosteroids (71%). The main parameter of evaluation was the 6MWT. The 40 mg/kg/day group reduced the distance by 12.8 meters and the placebo group by 44.1 meters (p = 0.056). The difference in the 80 mg/kg/day group was negligible. However, in the 40 mg/kg/day group, there was a subgroup of patients (less than nine years old, receiving corticosteroids and with a 6MWT baseline less than 350 meters in the beginning) in whom the difference in distance covered (68.2 meters longer than the placebo group) was statistically significant (p = 0.0053). Previous studies had found that DMD patients who walked more than 350 meters in the six minutes did not change significantly in 48 weeks. In the functional tests (running or walking 10 meters, climbing four stairs, descending four stairs, and supine-to-stand), the group that used ataluren 40 mg/kg/day was better than placebo, although not statistically significant[56].

Another study evaluated therapy with ataluren in 230 DMD patients using corticosteroids for longer than six months (115 received ataluren 40 mg/kg/day *vs* 115 receiving placebo). In this trial, the DMD patients were divided into groups (younger and older than nine years of age) that were analyzed using functional tests and the North Star Ambulatory Assessment (NSAA). In the 6MWT, no group showed a significant difference. In the subgroup with a baseline greater than 300 meters and less than 400 meters, DMD patients had a significant improvement after 32 weeks, which increased up to 48 weeks (p = 0.007). In the groups with a baseline walk less than 300 meters and greater than 400 meters, the results were not significant. The beneficial effect of ataluren in relation to gait occurred in 50% (47/114) of the patients. In the NSAA, although not significant, the ataluren group had a better performance. In addition, in the subgroup with a baseline walk between 300 and 400 meters, the proportion was 14.3% *vs* 25.3% in the placebo group (p = 0.010). There was also no difference in quality of life between groups[57].

Exon skipping

Antisense nucleotides (ASO) are nucleic acid chains of 8-50 bases (oligos). These oligos have a corresponding base in the pre-mRNA and mRNA sequence modulating their function and are additionally ligated (antisense). Antisense nucleotides bind at specific mRNA sites mimicking DNA-RNA pairing. The sequences skipping the exons induce the inclusion of spliced exons. Administration of an ASO that antagonizes the "natural antisense transcripts" in the mRNA allows the translation of the corresponding protein[58].

In 2003, ASO use, in *mdx* mice with a mutation in exon 23 of the dystrophin gene, induced persistent production of this protein in a large number of muscle fibers, which resulted in the functional improvement of the animals without inducing immune responses[59]. This, and other experiments, were the basis for therapeutic attempts to restore the reading frame of the dystrophin interrupted by the premature stop of the translation. The ASOs intend to replace the deleted exons, promoting the exon skipping and restoring the reading frame of the mRNA. This can produce a functional dystrophin, even internally deleted, resulting in a more benign phenotype[2].

Drisapersen

Drisapersen is an ASO (2'-O-methyl-phosphorothioate) that induces exon skipping of exon 51 from the dystrophin, restoring the interruption of the reading frame and allowing continuity of mRNA reading. A phase 2 study was undertaken, comparing three groups of DMD patients with exon 50 deletion, over 24 weeks and followed up for 48 weeks: 17 patients received doses of 3 mg/kg/week, 18 patients received 6 mg/kg/week and 16 patients received a placebo. The 6 mg/kg/week group showed a discrete benefit in the 6MWT test at 24 weeks (p = 0.051), which persisted at 48 weeks (p = 0.154). However, no difference was found in the muscle force and functional tests[60].

In the follow-up phase 3 study, 186 DMD patients (125 receiving 6 mg/kg/week and 61 receiving placebo) were tested for 48 weeks using the same criteria as the phase 2 study. The 6MWT test showed better performance but was not statistically significant (p = 0.415). There was no statistical difference in the NSAA (p = 0.757), in the speed of climbing four steps (p = 0.718) and descending four steps (p = 0.513) or the velocity of running 10 meters (p = 0.881). In a later analysis, when separating the patients who covered between 300 and 400 meters in the 6MWT at basal evaluation, drisapersen was found to be favorable. In the overall evaluation of all parameters from the patients who improved and worsened, the clinical impression was significant (p = 0.002) and drisapersen may be useful in less-affected patients[61]. Despite potential promising results, the study with drisapersen was terminated by the sponsors and has not been marketed.

Eteplirsen

Eteplirsen is a morpholino phosphorodiamidate oligomer formed from 30 nucleotides that induces exon 51 skipping in the pre-mRNA of DMD. It was tested in 12 patients randomized among 186 DMD patients eligible for exon 51 skipping (deletions of exons 45-50; 48-50; 49-50; 50; 52) who were older than seven years of age, and using corticosteroids. They were compared with 13 controls with the same criteria of the randomization. The historical controls who did not received eteplirsen had a distance in the 6MWD 151 meters less (p < 0.01) than the treated group during the

study period (36 months). Pulmonary function was stabilized in the treated patients, when compared with the natural history over that period[62].

Eteplirsen increased muscle dystrophin after 180 weeks of use in 11 treated DMD patients, compared with 13 controls. Dystrophin increased 100% when measured by real-time PCR techniques, 11.6 times by Western blot techniques ($p < 0.007$) and 7.4 to 15.5 times by immunohistochemistry ($p < 0.001$)[63].

Despite the results obtained with drugs that allow transcription of premature termination codons and exon skipping, there are doubts about the real benefit of their clinical application and the modification of the natural history of the disease. Eteplirsen was approved, based on the study of only 12 DMD patients, where the main objective was the distance covered in six minutes in a subgroup of DMD patients. A joint analysis of the patients using eteplirsen and drisapersen found that the latter showed a favorable response in the 6MWT, but neither drug was statistically significant in the NSAA. The change in the amount of dystrophin was very small and the long-term significance is unknown[64].

In addition, a major problem at this time is whether the high cost of these medications (eteplirsen $300,000/year and ataluren $385,440) is worth the small benefit observed[65].

FINAL COMMENTS

Despite the great effort of several researchers, most drugs, except the corticosteroids, did not substantially change the course of DMD and most of them showed disappointing or controversial results.

Research for new therapeutic alternatives continues and there are several clinical studies in progress, and some in phase 2 or 3 have been registered in clinical trials (https://clinicaltrials.gov/). These trials are retesting old medications as well new drugs, exploring different metabolic pathways to minimize the collateral effects of the available therapies: 1). Antioxidants for prevention of muscle fiber injury (idebenone, metformin, L-arginine, L-citrulline, creatine with and without glutamine). 2) Antifibrotic (Givinostat, FG-3019, pamrevlumab). 3) Antimyostatin (BM5-986089, MYO-029, domagrozumab). 4) Utrophin modulator (ezutromid). 5) Granulocyte stimulating factor (filgrastim analog). 6) Anti-inflammatory (doxycycline, edasalonexent, vamorolone, HT-100, MA-0211). 7) Cell therapy (cardiac stem cells). 8) Exon 53 skipping (golodirsen). 9) Dystrophin substitution (viral-microdystrophin vectors)[66].

CONCLUSION

The only medications that altered the natural history of DMD were the corticosteroids, associated with physiotherapy, orthoses, controlled exercises, surgical orthopedic measures, nutrition, family counseling, psychological family aid when necessary, pulmonary and cardiologic support. Coenzyme Q10, idebenone and creatine may possibly have a small impact on the long-term survival of DMD patients and their use is warranted due to lack of collateral effects. All these measures have increased the survival of DMD patients to around 27 years of age and some could survive up to 40 years[67,68].

The newer drugs that have some action on dystrophin production have had a very small impact (ataluren and eteplirsen) and may be used in a selective group of patients to improve some functions but so far have not shown any change in evolution or quality of life.

Possibly in the near future, one or more medications in the early stages of research, added to those actually in use, may provide better control and perhaps a cure for Duchenne muscular dystrophy.

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