ORIGINAL ARTICLES

Drug treatment of Duchenne muscular dystrophy: available evidence and perspectives

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Duchenne muscular dystrophy (DMD) is a disease linked to the X-chromosome which affects 1 in 3,600-6,000 newborn males. It is manifested by the absence of the dystrophin protein in muscle fibres, which causes progressive damage leading to death in the third decade of life. The only medication so far shown to be effective in delaying the progression of this illness are corticosteroids, which have been shown to increase muscle strength in randomised controlled studies; long-term studies have demonstrated that they prolong walking time and retard the progression of respiratory dysfunction, dilated cardiomyopathy and scoliosis. Several potential drugs are now being investigated. Genetic therapy, involving the insertion of a dystrophin gene through a vector, has proven effective in animals but not humans. Currently under clinical study is Ataluren, a molecule that binds with ribosomes and may allow the insertion of an aminoacid in the premature termination codon, and exon-skipping, which binds with RNA and excludes specific sites of RNA splicing, producing a dystrophin that is smaller but functional. There are also studies attempting to modulate other muscular proteins, such as myostatin and utrophin, to reduce symptoms. This paper does not address cardiomyopathy treatment in DMD patients.

Key words: Duchenne muscular dystrophy, drug treatment, clinical trials

Introduction

Duchenne muscular dystrophy (DMD) is a disease linked to the X chromosome affecting 1 in 3,600-6,000 newborn males. It is characterised by weakness in the proximal muscles, expressed through a positive Gowers' sign upon getting up, abnormal gait, hyphertrophy in the calf muscles and elevated creatinekinase. Most patients are diagnosed at the age of 5, when the symptoms become more evident. The disorder has a progressive course of muscle weakness also affecting the cardiac muscles and respiratory system. Affected boys usually stop walking at the age of 13, and if untreated die before their twenties from cardiac difficulties or respiratory infections (1, 2).

DMD is caused by mutations in the dystrophin gene, resulting in the severe reduction or complete absence of the dystrophin protein, which is found in the sarcolemma of muscle fibres and is composed of four parts: the C-terminal domain which joins other proteins in the membrane called dystroglycan complex, the rod domain, the cysteine-rich domain and the N-terminal domain, which binds with the actin (3). The C-terminal domain therefore interacts as a bridge between the sarcolemma and the extracelullar matrix, and communicates with other membrane proteins through the dystrophin-glycoprotein complex. It is postulated that dystrophin is essential to transduce the strength of the contractile apparatus to the extracellular matrix and protects muscle fibre from any damage caused by muscle contraction, which could lead to necrosis (3, 4). The absence of dystrophin leads to mechanical damage of the sarcolemma, loss of calcium homeostasis, and progressive degeneration of muscle fibres.

The dystrophin gene is the largest gene found in humans and accounts for approximately 0.1% of the total human genome. It is located on the short arm of the X-chromosome and is composed of 79 exons and 7 promoter regions (4). The reported frequency of different mutations leading to DMD varies widely. According to the Leiden database (www.dmd.nl), duplications of one or several exons correspond to 7% of the mutations, point mutations account for 20%, while deletions are observed in 72% of the patients (4, 5). Most deletions occur between the exons 44 and 55, corresponding to the dystrophin's rod domain (6). If these mutations alter the reading frame of dystrophin (out of frame-mutation), protein formation is truncated, no dystrophin is produced and the patient de-

velops DMD. If the mutation is "in frame", the dystrophin is smaller in size but still functional, in which case the patient is diagnosed with Becker muscular dystrophy (7).

Although the molecular origins of DMD have been known for several years, there is still no curative treatment for the disease. To this date, the only treatment shown to be effective in slowing the progression of the illness are corticosteroids. They have changed the natural history of DMD. However, their exact mechanism of action is not completely understood (2).

Drugs

Corticosteroids: prednisone and deflazacort

Glucocorticoids, more precisely prednisone and deflazacort, are the main drug treatment for DMD. They have been used for over two decades and the benefits are well known now. They are the only medication that has been shown to increase muscular strength. Early studies have proved that their use prolonged ambulation and improved their functionality in everyday activities. Long-term studies have shown that they also reduce the need for scoliosis surgery, enhance lung function, and help maintain cardiac function (8, 9).

In 2005, the American Academy of Neurology and, in 2008, a Cochrane review, evaluated all randomised controlled trials about the use of corticosteroids and concluded that prednisone administered at doses of 0.75 mg/kg/day showed an increase in muscular strength and improved results in standardised functional tests in the short-term (10, 11). The increase in muscle strength occurs during the first six months of treatment, followed by a stabilisation period of two years, with a subsequent decline that is slower than in the untreated patients (12).

Five recently-published, long-term controlled nonrandomised trials (extending beyond 3 years) with prednisone or deflazacort reported that given one of these drug, patients can ambulate 2 to 5 years longer than those not receiving corticosteroids, the need for spinal stabilisation surgery was reduced, and the need for non-invasive ventilation delayed (8). Two of these studies evaluated cardiac function and demonstrated that the treated patients' left ventricular ejection fraction was significantly better preserved compared with untreated patients (8). Another study revealed that, 93% of patients treated with prednisone presented no ventricular dysfunction at the age of 12 compared to 53% of untreated patients (13). Studies also show an increase in life expectancy (14). Eagle mentions that in 1960, the average life expectancy for DMD patients was 14.4 years, which by 1990 had risen to 19.3 years with the use of corticosteroids, antibiotic therapy and intensive care treatment. Nowadays it has risen to 24.5 years, probably due to the use of non-invasive ventilation (8, 14).

In summary, there is substantial evidence to recommend the use of corticosteroids to all DMD patients with the objective of preserving walking time as long as possible and reducing lung, heart and orthopaedic complications (2). Naturally, the adverse effects of the corticosteroid treatment must also be considered. The most frequent adverse effect in long-term treatment is a reduction in the patient's height. Weight gain is the second most frequent one, but the main reason for discontinuing treatment (8). Weight gain in DMD-patients on steroid treatment is a multifactorial issue. It is not just a side effect of the corticosteroids – it is also a result of their reduced mobility, as weight gain generally is more pronounced in non-ambulatory patients. Deflazacort caused less weight gain but more cataracts than prednisone. Vertebral fractures varied in frequency by 5-32% in treated patients. About 80% of these fractures were discovered during routine scoliosis radiograph studies and not accompanied by clinical symptoms (8, 15). Other adverse effects include cushingoid facies, acne, hirsutism, arterial hypertension, behaviour disorder, delayed puberty, vertebral fractures, immunosuppression and gastrointestinal problems. Although these side effects are less frequent when used within the indicated doses, they are nevertheless conditions that must be monitored.

The indicated dose of prednisone is 0.75 mg/kg daily. Doses of less than 0.3 mg/kg are less effective, and daily administration seems to be more effective than on alternating days (10).

Intermittent regimens are postulated to have a better safety profile in terms of adverse effects, but the data from the only randomised controlled trial of intermittent prednisone were unavailable (which would have allowed statistical analysis and comparison with another regimens). One open cohort trial with intermittent deflazacort reported prolongation of ambulation (12). Therefore, randomised controlled studies are required to establish the optimal treatment schedule over the long-term.

The current recommendation for initiating treatment is when the patient is in the "plateau phase". This generally occurs between the ages of 4 to 6, when the boy stops making motor progress. Currently, many physicians keep up the treatment, even after the DMD patient has lost the ability to walk, with the objectives of preserving the function of the upper extremities, reducing the progression rate of scoliosis, and slowing the impairment of respiratory and cardiac function (2). Further studies are still needed to determine if non-ambulatory patients continue to benefit from this treatment.

Deflazacort is an oxazoline derivate of prednisone and at a dose of 0.9 mg/kg/day, is as effective as prednisone in treating DMD. The choice depends on the local availability of deflazacort and prednisone, on its costs, formula-

tions and patient preferences. One small, randomised trial suggests higher incidence and severity of weight gain with prednisone than with deflazacort (13). Deflazacort is thus preferred by some patients. However, the risk of development of cataracts is elevated in comparison with prednisone: in non-randomised studies, 10-30% of cataracts were observed at an average of 3.2 years of treatment with deflazacort. These patients should therefore be monitored yearly by an ophthalmologist (2, 12).

Other immunosuppressants

It has been postulated that the benefit of corticosteroid treatment might be attributed to the immunosuppressive effect. Thus, further studies with other immunosuppressants were carried out. However, a randomized, controlled trial of 99 boys with DMD given azathiprine alone or in combination with prednisone demonstrated that azathioprine had no beneficial effect (16). A placebo-controlled, double-blind study of 146 ambulant patients with DMD who received cyclosporine-A or placebo alone and in combination with prednisone found no difference of muscle strength and functional abilities between the treatment groups (17).

Genetic therapy

Research has recently been carried out on the potential of genetic therapy whereby the dystrophin gene is inserted. However, several obstacles have been met along the way. The size of the dystrophin gene makes it difficult to work with in gene therapy. Thus smaller genes, micro or mini-dystrophin, have been developed, which can be inserted into a vector. The most suitable vector found so far is a virus associated with the adenovirus, a non-pathogenic parvovirus, but it has been shown to cause an immunological response. In order to assess the response, *mdx mice* dys-/dys- have been created, and there is evidence that when the gene is injected, the dystrophin is partially expressed and muscular strength is improved. However, in preliminary studies on humans, 90 days after treatment initiation this gene expression was not observed. Results suggest that cellular immunity inhibits the success of this therapy (18, 19).

Exon skipping

In Becker's muscular dystrophy, the dystrophin protein is smaller and partially functional, resulting in less severe illness. In DMD patients, as mentioned earlier, the mutated gene manifests deletions, duplications and point mutations which interrupt the genetic information's reading frame. At present, researchers are seeking to inject a molecule capable of interfering with the RNA splicing signals to omit an additional adjacent exon, thus restoring the reading frame and allowing for the expression of a protein which is smaller but partially functional, as with patients who have Becker muscular dystrophy. This synthetic, modified RNA molecule is referred to as an antisense oligonucleotide (AO) and is capable of binding with specific pre-RNA sites, masking and excluding this exon from the splicing (5). This therapy would be specific for each mutation. Current exon skipping studies are addressing the skipping of exon 51, whose "skipping" is applicable to the largest group of patients, comprising 13% of all patients with DMD, followed by mutations in exon 45. Two AO are currently under study:

- *2'-O-methyl-phosphorotioates (2OMP)*: studies were originally carried out on *mdx mice* with 2OMP on exon 23. They showed the presence of dystrophin in many skeletal muscle fibres, but not in the heart (20). A clinical study was then performed on four patients with DMD with PRO051/GSK2402964 (2OMP targeting exon 51), and the investigators observed that 64-97% of the muscle fibres expressed the dystrophin protein at an amount of 17-35%. No side effects were observed (21). A multicentric study is currently taking place (22, 23).

- *Phophorodiamidate morpholino oligomer (PMOs)*: studies with *mdx mice* have shown the presence of dystrophin not only in muscle fibres, but also in the heart after administration of high doses of the drug. Long-term repetitive treatments even demonstrated improve motor function (24). In humans, a study targeting the exon 51, in seven DMD patients (AVI-4658) showed that those receiving high doses (0.9 mg) produced the dystrophin at 22-32% levels of normal in 44-79% of their muscle fibres. In this study, no signs of toxicity were observed (25), while a previous one, performed on non-human primates, had shown tubular degeneration in the kidneys (26). A disadvantage of this drug is that the effect is only transitory and limited to the time in which the AO remains in the tissue. Furthermore it produces levels of dystrophin below 30-60% of normal, which is the amount postulated to suffice for compensation of muscular dysfunction (27).

Premature stop-codon read-through: aminoglycosides and ataluren

This form of treatment applies only to boys with mutations resulting in premature termination codons, estimated to occur in approximately 13-15% of the DMD population. Stop codon read-through has been shown to be capable of suppressing termination codons by creat-

ing RNA misreading, thereby allowing the insertion of alternative amino acids at the site of the mutated premature termination codon. The result is generation of a full-length dystrophin protein with only one amino acid substitution.

Aminoglycosides

In cell cultures, gentamicin interacts with the 40s ribosomal subunit in the transcription of RNA, suppressing the termination codons and inserting in its place another amino acid which replaces it. In studies on *mdx mice* and in humans, gentamicin was capable of producing dystrophin expression in muscle fibres at 20% of normal levels (5). However, studies on DMD patients remain controversial. In fact one of them showed a beneficial effect of the muscle strength and a re-expression of dystrophin in muscles (28), while in another study on 12 DMD patients performed over a six-month period, dystrophin expression was detected in only 6 of the 12 patients, and no clinical benefits were observed (29-30).

Ataluren (PTC124)

Ataluren is an orally administered drug, which, in theory, would have the same effect as gentamicin, but which is bound to the 60s ribosomal subunit. Its efficacy in *mdx mice* is similar to gentamicin, producing dystrophin expression in 20-25% of muscle fibres (5).

With these results, a double-blind, randomised, multicentric study was carried out on 174 patients. After 48 weeks of taking low doses of ataluren, the patients showed some improvement in the 6-minute walk test but final results have not been published.

Myostatin

Myostatin is a protein forming part of the transforming growth factor-B family, which regulates muscle size. As muscular hypertrophy has been observed in knockout mice of the myostatin gene (*mys-/mys-*), a block of myostatin could serve as a potential treatment of DMD. Several ways of blocking myostatin have been proposed: follastatin, myostatin receptor blocker, and destructive exon-skipping for the myostatin gene (5).

A myostatin antibody was injected into mdx mice, producing muscle hypertrophy and greater strength. Only one clinical study has been carried out in humans with an antibody against myostatin (MYO-029) in adult patients with muscular dystrophy, which was well tolerated but did not increase muscle strength (32).

Utrophin

Utrophin shares 80% of the dystrophin sequence and is overexpressed in the muscle of patients with DMD. It has been suggested that its upregulation could delay the progression of the illness. The injection of heregulin, L-arginine or nitric oxide in mdx mice increases the expression of utrophin in histological preparations (33) and the intraperitoneal injection of TAT-utrophin protein increases muscle strength in mdx rats. A study was carried out in healthy individuals using the drug developed by Summit PLC (C110/BM195), which proved to be well tolerated, although the pharmacokinetics of the drug did not allow the continuation of the treatment (5).

Vitamin D supplement

Patients with DMD have an increased risk of developing pathological fractures of the long bones and the spine: First, bone mineral density decreases as a consequence of reduced mobility. Second, the risk of osteoporosis is increased with a well-known side effect of long-term corticosteroid treatment. Third, they tend to have low levels of vitamin D, probably due to decreased sunlight exposition. Therefore, physical exercise and sufficient calcium and vitamin D in the diet are recommended. Vitamin D supplementation is advised in patients with proven Vitamin D deficiency (serum 25 hydroxyvitamin D < 50 mg/ml) (2, 35).

There is no evidence that bisphosphates should be used prophylactically in children receiving steroid treatment, however administration of this drug is recommended when pathological fractures occur (7, 35, 36).

Conclusion

Although the molecular basis of Duchenne muscular dystrophy has been known for several decades, there is still no treatment that cures this illness. Active research on this topic is currently underway. Until now, the only medication shown to be effective in slowing the progression of this disease are corticosteroids.

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