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Therapeutic approach with Ataluren in Duchenne symptomatic carriers with nonsense mutations in dystrophin gene. Results of a 9-month follow-up in a case report

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Duchenne muscular Dystrophy (DMD) is a X-linked degenerative disorder affecting skeletal muscles and myocardium caused by mutations in the dystrophin gene, mainly deletions and duplications. Point-mutations account for 13% and stop codon mutations are even more unfrequent. A drug treatment for patients with DMD caused by stop codon gene mutations and still ambulant, has become recently available, based on the clear demonstration of its efficacy in slowing the course of the disease. The drug is able to read through the stop codon; furthermore it has the advantage of an oral administration and a better patient's compliance. We report a case of a still ambulant 27 year-old DMD symptomatic carrier with a stop-codon mutation in exon 53 (c.7792C > T; p.Gln2598Stop), who started the treatment with Ataluren at a dosage of 2,250 mg/die, reporting a prompt subjective improvement in muscle strength. Unfortunately two months after, the patient discontinued taking the drug for a traumatic femur fracture requiring surgical repair and prolonged rehabilitation. With the resumption of the drug intake in February 2018, the patient reported almost immediately an improvement in motor skills, including the possibility of recovering walking, first with support and then unsupported. These results seem even more encouraging, as Duchenne patients hardly recover the ability to walk following a fracture at this age and extend the possibility to treat with ataluren also the symptomatic Duchenne carriers who have nonsense dystrophin gene mutations. Furthermore the case here reported supports the concept that symptomatic DMD female carriers must enjoy the same therapeutic opportunities offered to males.

Key words: Duchenne dystrophy, symptomatic DMD carriers, Ataluren

Introduction

Duchenne muscular dystrophy (DMD) is the most frequent muscle disorder in childhood, characterized by progressive muscle wasting and weakness, leading to the loss of ambulation usually about the age of 12 years. It is caused by mutations in the dystrophin gene, encoding the protein dystrophin. The most part of mutations are deletions (75%) followed by duplications (15-20%) and point mutations (5-10%). Among the latter the nonsense mutations are even rarer. Dystrophyn plays a critical role in maintaining the sarcolemmal stability during muscle contraction.

Treatment options for DMD have been widely explored over the past 30 years. Steroids are considered standard care for DMD patients and have demonstrated evident benefits to patients by increasing muscle strength, reducing muscle fibrosis and inflammation (1). However several side-effects have been reported in the literature, whose severity often depends on the type of steroid used (prednisone *vs* deflazacort) (2). Several approaches aimed at restoring dystrophin expression have been recently reported with promising results. They include gene replacement through the use of viral and nonviral approaches (3, 4), overexpression of utrophin that was proposed to act as a surrogate to compensate for the lack of dystrophin (5) with promising results also in humans (6), and strategies to ameliorate symptoms by increasing muscle strength, reducing muscle fibrosis, and decreasing inflammation. Although promising, these strategies can only improve the quality of life of patients and delay the disease progression.

In recent years, great emphasis has been placed on the discovery of pharmacological approaches able to restore normal, full-length dystrophin and potentially

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reverse the course of the disease. Read-through (RT) of nonsense mutations, thank to its ability to bypass the premature stop codon and to act on virtually any region of the dystrophin gene, independently of the location in which the mutation resides, is one of these approaches.

The ability of certain antibiotics to suppress PTCs in eukaryotic cells has been known since the early 1990s (7).

In 2003 our work group reported the results (8) with gentamicine treatment in 4 Duchenne patients, with point mutations resulting in premature stop codons, still ambulant or in wheelchair stage for less than 4 months. Skeletal muscle changes were monitored by dynamic tests and Creatine Kinase (CK) values; at the beginning and end of treatment, cardiac and respiratory status were evaluated by electrocardiography, echocardiography, acoustic densitometry and vital capacity. Three out of four patients, who had the most permissive UGA as stop codon, showed positive results; in one patient – the youngest among them – there was a dramatic re-expression of dystrophin by both immuno-histochemistry and Western blot.

Recently a new drug, derivative of aminoglycosides has been developed by PTC Therapeutics. It is *Ataluren*, a novel, orally administered small-molecule compound approved within the European Union, Iceland, Liechtenstein, Norway, Israel and South Korea under the trade name Translarna™ to treat patients with DMD still ambulant, aged 5 years and older. Ataluren interacts with the ribosome, which is the component of the cell that decodes the mRNA molecule and manufactures proteins, enabling it to read through premature nonsense stop signals on mRNA and allow the cell to produce a full-length, functional protein.

In 2017, a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial (9) was carried out at 54 sites in 18 countries located in North America, Europe, the Asia-Pacific region, and Latin America. Boys aged 7-16 years with nonsense mutation DMD (nmDMD) and a baseline 6-minute walk distance (6MWD) of 150m or more and 80% or less of the predicted normal value for age and height were randomly assigned (1:1), to receive ataluren orally three times daily (40 mg/kg per day) or matching placebo. Randomisation was stratified by age (< 9 years *vs* ≥ 9 years), duration of previous corticosteroid use (6 months to < 12 months *vs* ≥ 12 months), and baseline 6MWD (< 350 m *vs* ≥ 350 m). The primary endpoint was change in 6MWD from baseline to week 48.

The results showed that 6MWD values did not differ significantly between patients in the ataluren group compared to the placebo group. However, a significant effect of ataluren was observed in the subgroup of patients with a baseline 6MWD between 300 and 400 m. Furthermore patients in the ataluren group had a less decline in physical function compared with patients in the placebo group, as measured by the timed function tests after 48 weeks of treatment, though only the four-stair descend was statistically significant.

These results encouraged clinicians to extend the treatment with Ataluren also in DMD non ambulant patients. Ebrahimi-Fakhari et al. (10) reported their experience in 4 non-ambulatory nmDMD patients, routinely investigated by cardiac function, pulmonary function tests and muscle strength. Mean age at loss of ambulation was 10.1 ± 0.5 years, mean age when initiating Ataluren treatment 14.1 ± 1.4 years. They compared changes in left ventricular fractional shortening, forced volume vital capacity and BMI from two defined time periods (18-26-month period prior to and after Ataluren start). They concluded that serial echocardiography, pulmonary lung function tests, and assessment of muscle strength indicated mild attenuation of disease progression after initiation of Ataluren treatment in all DMD patients.

DMD female carriers are usually asymptomatic. However, 2.5-7.8% of them may present muscle symptoms and/or cardiomyopathy, due to the reduced synthesis of dystrophin. Several pathogenic mechanisms have been suggested to explain the onset of symptoms in female carriers, the most frequent among them is a skewed X- chromosome inactivation with percentages of silencing the X-chromosome carrying the wild allele from 85 to 100%.

We decided to assess the response to the treatment with Ataluren in a symptomatic DMD female carrier sharing a non sense mutation in dystrophin gene.

Case report

We report the case of a DMD manifesting carrier aged 26 years and still ambulant, who received ataluren in the last 9 months. She came at our observation when she was 12 years old. The mother reported a first medical evaluation when she was 18 months old for delay in motor milestones and very high CK levels (11.000 U/L *vs* 195 U/L). Muscle biopsy revealed a mosaic pattern of dystrophin with marked reduction/absence of dystrophin in most fibers, alternating with others with normal protein expression. PCR molecular analysis did not reveal any deletion/duplication, confirmed by MLPA testing. The X-chromosome inactivation analysis was not informative. The patient was treated with deflazacort and anti-oxidant drugs (Vitamin C, Vitamine E, Ubiquinone) since the age of 12, with a slight deterioration in muscle strength; at the age of 25 year she was still able to walk, but she had lost the ability to to get up from the floor at the age of 10 years. In 2016 the NGS analysis identified the causative mutation of the disease in a stop-codon (p.Gln2598Stop) at exon 53 of the dystrophin gene, so in October 2017, at

Table 1. Results of dynamic test pre and post-treatment with Ataluren.

NSAA	6MWT	PUL
Baseline	100 meters	55/80
Baseline (after fracture)	Unable to walk	52/80
12 weeks of treatment	76 meters (with support)	55/80
24 weeks of treatment	52 meters (without support)	56/80
36 weeks of treatment	55 meters (without support)	56/80

the age of 25 years 6 months, she was elected for treatment with Ataluren, at a dosage of 2250 mg/die, soon reporting a subjective well-being and a strength improvement. In December 2017 the patient, due a traumatic femur breaking surgically corrected, was forced to discontinue the drug for 2 months. With the resumption of the drug intake in February 2018, the patient reported almost immediately an improvement in motor skills, including the possibility of recovering walking, first with support and then unsupported. The results of the dynamic tests – North Star (NSAA), 6 Minute Walking Test (6MWT) and Power – Upper-Limbs (PUL), performed before treatment and at three-month intervals are shown in Table 1. No change in cardiac function and respiratory tests was appreciated between baseline and 9 month evaluation.

Discussion

Data here reported – though preliminary and limited to only one patient – suggest that treatment with Ataluren can be of benefit in older patients with DMD and should be extended to symptomatic DMD female carriers to. In our case, the results are even more encouraging as Duchenne patients hardly recover the ability to walk after a fracture at this age. In fact, after 36 weeks of treatment, our patient recovered the motor skill before the accident, and refers a greater autonomy in daily life, confirmed by the INQoL test. These results also extend the possibility to treat with ataluren the symptomatic Duchenne female carriers, presenting nonsense dystrophin gene mutations.

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