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DUCHENNE MUSCULAR DYSTROPHY: TREATMENT WITH FETAL PROGENITOR CELL TRANSPLANT

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Abstract

Duchenne muscular dystrophy is an inherited X-linked recessive disorder caused by abnormal dystrophin synthesis due to a genetic defect. DMD leads to progressive muscle degeneration. DMD affects about 1 in 4,000 to 6,000 children. On the average, the disease is diagnosed at the age of 3-5 when physical capacity of the affected child is markedly different from that of healthy peers. The authors are reviewing issues arising in the process of treatment of Duchenne muscular dystrophy (DMD).

Goal. Study clinical efficacy of fetal progenitor cell transplantation in DMD treatment.

Materials and methods. The study included 22 patients with DMD aged from 5 to 19 who underwent fetal stem cell transplantation.

The results of treatment with fetal myoblasts for each patient are individual. Laboratory tests before treatment showed a significant increase in the level of CPK in all patients. Many patients also had high levels of ALT, ACT, and LDH.

Stem cells isolated from fetal muscles can differentiate into myocytes, which suggests that stem cell therapy can be effective in Duchenne muscular dystrophy.

The proposed method of fetal progenitor cell administration is an effective and promising therapeutic approach in DMD.

Administration of stem cells directly into the affected muscles results in higher muscle tone, muscle bulk growth stimulation, muscle and general physical power increase, immune boosting, intellectual capacity and psycho-emotional improvement.

The proposed approaches to treatment will result in higher quality of life and longer life span of Duchenne muscular dystrophy patients.

All human studies were conducted in compliance with the rules of the Helsinki Declaration of the World Medical Association "Ethical principles of medical research with human participation as an object of study". Informed consent was obtained from all participants.

Keywords: *Duchenne muscular dystrophy, stem cells, fetal progenitor cells, myoblasts, multi-point intramuscular administration.*

Introduction

Duchenne muscular dystrophy is a hereditary X-linked recessive disorder caused by abnormal dystrophin synthesis due to genetic defect and resulting in progressive muscular degeneration [1].

According to different sources, Duchenne muscular dystrophy affects around 1 in 4, 000 – 6,000 people internationally [2]. On the average, the disease is diagnosed at the age of 3-5 when physical capacity of the affected child is markedly different from that of healthy peers [3]. It is believed that during the first years of Duchenne muscular dystrophy patient life his/her muscle fibers regenerate by means of own stem cells of the muscle differon the reserve of which gradually depletes, which leads to abnormal dystrophin production causing muscle degeneration and fibrosis [4].

In Duchenne muscular dystrophy, muscle weakness is rapidly progressive, and gait problems appear in teenage years. Wheelchair is usually needed by the age of 9-11, but it case is individual. Apart from the progressive muscle weakness, more that 50% of the patients have dystrophin deficiency-induced cardiovascular issues by the age of 15 [5]. In patients aged 20 and older, diaphragm and muscles regulating lung function weaken significantly, therefore they can die from respiratory failure [6]. Gastrointestinal and excretory systems, as well as intellect, are also affected [7].

Over the last years, stem cells are used for Duchenne muscular dystrophy treatment [8].

Methods

Stem cells were isolated in the EmProCell (Mumbai, India) biotechnological laboratory in accordance with international GMP standards.

Stem cells were isolated at the time of organogenesis (beginning stages of muscle system formation) and thoroughly tested for biological safety, aerobic and anaerobic microorganisms, and fungi. Testing also included real time PCR for 12 types of bacteria, karyotyping and gender determination.

In the course of research, treatment method based on application of fetal progenitor cells and fetal tissue extracts aimed at dystrophin production deficit complensation has been developed. The underlying principle of treatment is transfer of unaffected genetic information of the fetal myoblasts cell nucleus into patient's muscle. Implantation of nuclei of fetal progenitor cells with normal genes encoding synthesis of all 79 exons of dystrophin [9] results in restoration of dystrophin production. Inhibition of muscle tissue degeneration gives time for repeated transplantations of fetal stem cells, which, at the end, results in longer life expectancy and higher life quality in most DMD patients.

Stem cells were administered according to our developed method that included transplantation of two types of allogenic fetal progenitor cells from the same fetus: hematopoietic cells of fetal liver for immune tolerance induction thank to which fetal myoblasts (allogenic muscular cells) administered by multiple intramuscular injections at the next stage are treated by the body like its own.

For optimal clinical result, we performed additional subcutaneous administration of fetal myoblasts and fetal placenta extracts containing cytokines stimulating growth and differentiation of both patient's own and transplanted fetal stem cell.

The authors developed method of multi-point administration of fetal myoblasts – intramuscular administration of the cell formulation in many points all over the body in accordance with the scheme. In comparison with regular intravenous or subcutaneous administration of stem cells, the effects of multi-point intramuscular administration are much more demonstrative.

Our method resulted in positive results in Becker's Emery-Dreifuss, Duchenne and myotonic muscular dystrophy as well as in myositis and motor neuron disease.

As a part of the study that lasted for 5 years, many DMD patients were treated with this method aimed at dystrophin production deficit compensation [11-13].

Results

For optimal clinical effect in DMD, combination of stem cells and fetal tissues extracts is selected individually for each case of DMD and its complications. This treatment results in the following:

- inhibition of the disease progression (longer period of independent ambulation etc.)

- preservation of muscle and physical power
- gait quality improvement (in walking patients)
- improvement or restoration of some skills (climbing stairs, combing, getting up from the floor or sitting position)
- reduction of pseudohypertrophy or muscle straining
- decreased values of ALT, ACT, CPK and LDH signifying subsidence of inflammation in the muscle tissue
- prevention or subsidence of the symptoms of myopathy complications
- intellect and psycho-emotional state improvement, higher self-esteem
- immune boosting
- life quality improvement

The results of treatment with fetal myoblasts depend on the patient. Laboratory tests performed before the treatment demonstrated marked CPK elevation in all the patients. Many patients also had elevated ALT, ACT and LDH. Functional condition was evaluated on Muscular Dystrophy Functional Rating Scale (MDFRS) (Table 1) [10].

After stem cell therapy in accordance with our method, clinical presentation has significantly changed in all the patients. Improvements of general functional status on MDFRS and principal blood parameters were reported in 80% of patients. In 20% of patients, principle blood parameters in DMD either improved insignificantly or remained practically unchanged. Such patients are recommended to repeat stem cell therapy. Repeated administration of fetal myoblasts results, at the least, in CPK decrease, which is regarded as a positive effect in DMD.

Clinical case. 17-year old patient was diagnosed with DMD at the age of 3 when high CPK was detected. Diagnosis was confirmed by the genetic test (deletion of exons 48-50). History of present illness: born naturally, full term, birth weight – 2,7 kg. Pregnancy was uneventful. The boy suffered asphyxia at birth and was transferred to the intensive care for the newborns where he stayed for 7 days. Family history is negative for neuromuscular disorders.

Psychomotor development: started walking at the age of 3, fine motor skills are well-developed for the given age. Speech underdevelopment until the age of 4 when he started talking. At present, speech is absolutely normal.

The patient was ambulant until the age of 14. Three years ago, he started feeling weakness in the lower extremities, and walking was becoming more difficult month after month. One year ago, he started feeling weakness in the right arm.

In accordance with the developed method, the patient underwent intravenous administration of fetal liver hematopoietic stem cells followed by multi-point intramuscular administration of fetal myoblasts into the muscles of pelvis, hips, ankle, feel, shoulder girdle, shoulders, forearms and wrists. In total, there were 77 injections 0,2 ml each. The next stage was subcutaneous administration of fetal myoblasts and fetal placenta extracts into the frontal abdomen. All stem cell suspensions and fetal tissue extracts were made from the cells of one male (XY) fetus.

MDFRS Scale data both before the treatment and 3, 6, 9, 12 and 15 months after it are provided in the Fig. 1.

It is obvious that the patient has improvements in mobility, basic activities of daily life, functional impairment and significant improvement in arm functions.

There also was stabilization of the principal blood parameters as early as three months after the first administration of stem cells: first of all, CPK level decreased from 7946 to 1170 U/l, LDH – from 750 to 510 U/l, ALT and ACT also decreased.

After second administration of stem cells, CPK level decreased to 807 U/l, which is close to the upper reference value for this parameter, which means that it was possible to achieve remission of the disease and gain time for stabilization and body preparation for repeated administration of stem cells and fetal tissue extracts.

The patient reported power increase in the lower extremities and more confident walking distances that were impossible to manage before the treatment. He had better use of the right arm, reported improved sleep, more active lifestyle and much higher energy level.

The patient is followed-up on regular basis and is following medical recommendations.

The above clinical case proves that multi-point

administration of stem cells in accordance with the developed method to DMD patients results in muscle activity improvement. The results can be explained by the fact that stem cells isolated from fetal muscles are a source of myocytes restoring impaired or lost functions.

Exact mechanisms of stem cell effects in DMD are not yet fully studied, but even nowadays we have a very powerful biological weapon inhibiting the progression of the disease and restoring muscle tissue, which gives big hope for longer life expectancy of the patients suffering from it.

Conclusions

Stem cells isolated from fetal muscles can differentiate into myocytes, which suggests that stem cell therapy can be effective in Duchenne muscular dystrophy.

The proposed method of fetal progenitor cell administration is an effective and promising therapeutic approach in DMD.

Administration of stem cells directly into the affected muscles results in higher muscle tone, muscle bulk growth stimulation, muscle and general physical power increase, immune boosting, intellectual capacity and psycho-emotional improvement.

The proposed approaches to treatment will result in higher quality of life and longer life span of Duchenne muscular dystrophy patients.

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Table 1. Muscular Dystrophy Functional Rating Scale

Domains			
Mobility	**Basic activities of daily life**	**Arm function**	**Functional Impairment**
1	**2**	**3**	**4**
1 Stair climbing	1 Feeding	1 Managing objects over head	1 Severity of upper limb joint contracture
2 Outdoor mobility	2 Combing hair	2 Carrying objects	2 Severity of lower limb joint contractures
3 Indoor mobility	3 Brushing teeth	3 Cleaning table	3 Number of contracted joints in the upper limbs
4 Transfers from bed to chair	4 Dressing upper/lower parts of body	4 Writing	4 Number of contracted joints in the lower limbs
5 Wheelchair manipulation	5 Toileting	5 Turning books	5 Severity of neck contracture
6 Standing from sitting	6 Bathing	6 Picking up small objects	6 Strength of the neck
7 Sitting from lying		7 Managing objects over head	7 Strength of the trunk
8 Rolling			8 Scoliosis
9 Changing body position in bed			9 Orthopnea
			10 Sputum clearance
			11 Ventilator assisted
Total for Mobility =	**Total for Basic activities of daily life =**	**Total for Arm function =**	**Total for Impairment =**
Total Score =			

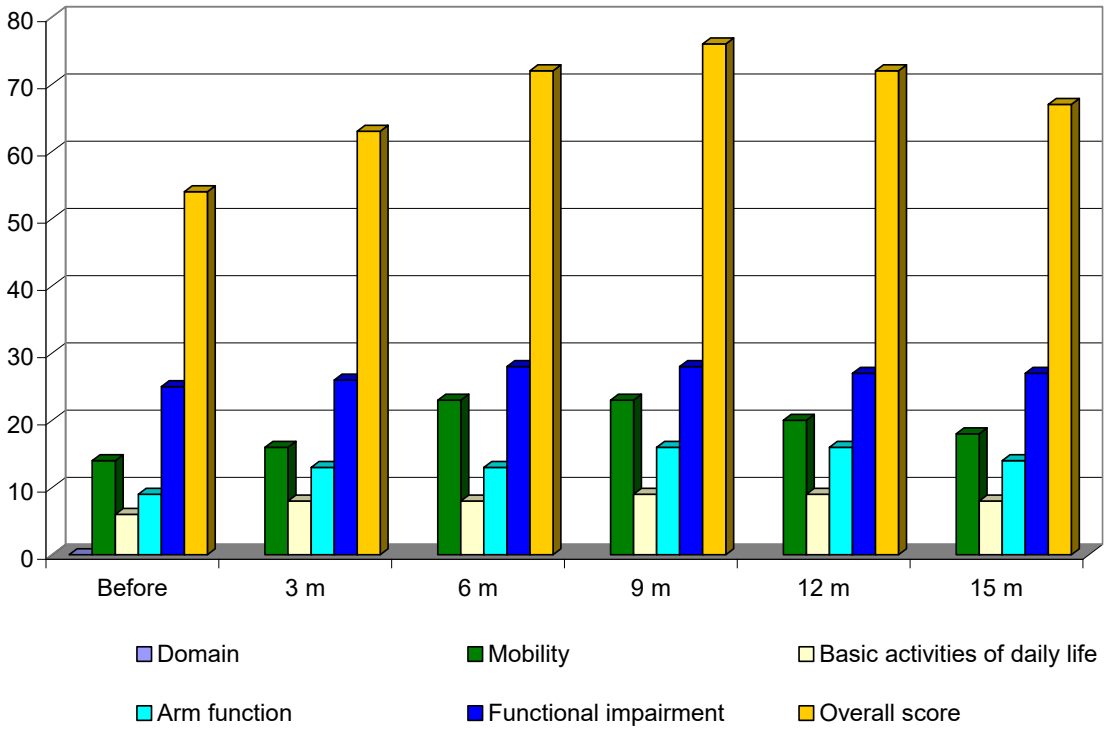

Figure 1. Patient's Results on MDFRS Scale