УДК 616-073+616.83 *DOI http://dx.doi.org/10.5281/zenodo.1434222*

CLINICAL EFFICACY OF FETAL PROGENITOR CELL TRANSPLANTATION IN DUCHENNE MUSCULAR DYSTROPHY

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КЛИНИЧЕСКАЯ ЭФФЕКТИВНОСТЬ ТРАНСПЛАНТАЦИИ ФЕТАЛЬНЫХ ПРОГЕНИТОРНЫХ КЛЕТОК ПРИ ЛЕЧЕНИИ МЫШЕЧНОЙ ДИСТРОФИИ ДЮШЕННА

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КЛІНІЧНА ЕФЕКТИВНІСТЬ ТРАНСПЛАНТАЦІЇ ФЕТАЛЬНИЙ ПРОГЕНІТОРНИХ КЛІТИН ПРИ ЛІКУВАННІ М'ЯЗОВОЇ ДИСТРОФІЇ ДЮШЕННА

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Резюме (Summary)

The authors are reviewing issues arising in the process of treatment of Duchenne muscular dystrophy (DMD). DMD is a hereditary X-linked recessive disorder caused by abnormal dystrophin synthesis due to genetic defect and resulting in progressive muscular degeneration. DMD affects about 1 child in 4,000 — 6,000. 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.

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

Materials and methods. The study included 37 patients with DMD aged from 5 to 19 who underwent fetal stem cell transplantation.

The results of treatment with fetal myoblasts differ for each case. Laboratory tests before the treatment were remarkable for significant elevation of CPK in all the patients. Many patients also had high levels of ALT, ACT and LDH.

The proposed method of fetal progenitor cell administration is an effective and promising method of DMD treatment. Administration of stem cells directly into the affected muscles (intramuscularly) results in higher muscle tone, muscle bulk growth stimulation, muscle power and physical capacity increase, immune boosting, improved cognitive and intellectual skills and psycho-emotional state in general.

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

Автор розглядає проблеми, що виникають в процесі лікування м'язової дистрофії Дюшенна (МДД). МДД - це рецессивное, зчеплене з Х-хромосомою спадкове захворювання, пов'язане з порушенням продукції білка дистрофина внаслідок дефекту генів, відповідальних за його синтез, яке викликає розвиток прогресуючої м'язової дегенерації. МДД вражає приблизно 1-го людини з 4-6 тисяч. В середньому хворобу діагностують у віці 3-5 років, коли фізичні можливості хворої дитини починають помітно відрізняться від можливостей здорових ровесників.

Мета роботи — вивчення клінічної ефективності трансплантації фетальних прогеніторних клітин при лікуванні МДД після використання СК за розробленою методикою.

Матеріали та методи. У дослідження взяли участь 37 пацієнтів з МДД у віці від 5-ти до 19-ти років після введення фетальних стовбурових клітин.

Результати від лікування фетальними миобластами для кожного пацієнта індивідуальні. Клініко-лабораторні дослідження перед початком лікування показали у всіх пацієнтів значне підвищення рівня КФК. У багатьох пацієнтів також були підвищені рівні АЛТ, АСТ і ЛДГ.

Запропонована методика введення фетальних прогеніторних клітин є ефективним і перспективним методом лікування МДД.

Введення стовбурових клітин безпосередньо в уражені м'язи пацієнта внутрішньом'язово супроводжується підвищенням м'язового тонусу, стимуляцією росту м'язової маси, збільшенням м'язової сили і фізичної витривалості, зміцненням імунітету, розвитком розумових здібностей і психоемоційного стану в загальному.

Ключові слова: *м'язова дистрофія Дюшенна, стовбурові клітини, фетальні прогеніторні клітини, міобласти, багатоточкове внутрішньом'язове введення*

Автор рассматривает проблемы, возникающие в процессе лечения мышечной дистрофии Дюшенна (МДД). МДД – это рецессивное, сцепленное с Х-хромосомой наследственное заболевание, связанное с нарушением продукции белка дистрофина вследствие дефекта генов, ответственных за его синтез, которое вызывает развитие прогрессирующей мышечной дегенерации. МДД поражает примерно 1-го человека из 4-6 тысяч. В среднем болезнь диагностируют у возрасте 3-5 лет, когда физические возможности больного ребенка начинают заметно отличатся от возможностей здоровых ровесников.

Цель работы — изучение клинической эффективности трансплантации фетальных прогениторных клеток при лечении МДД после использования СК по разработанной методике.

***Материалы и методы*.** В исследования приняли участие 37 пациентов с МДД в возрасте от 5-ти до 19-ти лет после введения фетальних стволовых клеток.

Результаты от лечения фетальными миобластами для каждого пациента индивидуальны. Клинико-лабораторные исследования перед началом лечения показали у всех пациентов значительное повышение уровня КФК. У многих пациентов также были повышены уровни АЛТ, АСТ и ЛДГ.

Предложенная методика введения фетальных прогениторных клеток являет-

ся эффективным и перспективным методом лечения МДД.

Введение стволовых клеток непосредственно в пораженные мышцы пациента внутримышечно сопровождается повышением мышечного тонуса, стимуляцией роста мышечной массы, увеличением мышечной силы и физической выносливости, укреплением иммунитета, развитием умственных способностей и психоэмоционального состояния в общем.

Ключевые слова: мышечная дистрофии Дюшенна, стволовые клетки, фетальные прогениторные клетки, миобласты, многоточечное внутримышечное введение

Rationale

DMD 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, DMD 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 DMD 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 DMD, 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 DMD treatment [8]. In our opinion, stem cells isolated from fetal muscles can differentiate into myocytes, which suggests that stem cell therapy can be effective in DMD.

The authors are of the opinion that the proposed approaches to treatment will result in higher quality of life and longer life span of DMD patients.

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 accord-

ing 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.

Results and Discussion

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 (Table 2). 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.

The data presented in the table cannot be regarded as ultimate results, but demonstrate improvements after treatment with fetal myoblasts.

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

Table 1

Muscular Dystrophy Functional Rating Scale

Domains			
Mobility	**Basic activities of daily life**	**Arm function**	**Functional Impairment**
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 =			

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 Table 3. The first administration of stem cells was performed on December 9, 2016, repeated — on September 12, 2017.

It is obvious that the patient has improvements in mobility, basic activities of

Table 2

Examination results in DMD Patients: Main Blood Parameters and MDFRS Scale (n = 22)

Patients	Before the treatment				3 months				6 months			
	ALT,IU/l 8-40	LDH, IU/l < 470	CPK, IU/l 20-190	MDFRS Scale Total Score	ALT, IU/l	LDH, IU/l	CPK, IU/l	MDFRS Scale	ALT,IU/l	LDH,IU/l	CPK,IU/l	MDFRS Scale
1.	123	979	14072	39	55	510	1560	63	52	496	1648	62
2.	138	696	7991	46	47	490	1450	59	45	475	1420	64
3.	101	750	7946	54	51	510	1170	63	49	467	1608	72
4.	98	735	6450	46	45	620	1670	49	42	570	1620	56
5.	111	820	7670	42	90	538	1920	49	60	520	1650	54
6.	95	656	5380	47	48	420	1490	59	40	410	1480	62
7.	148	840	8920	42	95	760	6100	44	87	720	5930	46
8.	80	760	7480	47	58	520	1680	52	70	630	1720	50
9.	95	820	8690	48	56	540	1730	54	48	510	1670	58
10.	100	890	8840	45	63	580	1740	49	40	463	1690	56
11.	162	920	12120	39	88	580	1890	46	75	545	1760	49
12.	76	780	6360	46	50	662	1760	48	42	512	1670	52
13.	110	878	7560	44	68	694	5778	47	55	560	5657	49
14.	89	789	6472	43	58	582	3738	46	53	547	3626	50
15.	78	839	8620	42	51	622	1787	45	48	490	1524	52
16.	108	828	7575	42	64	585	1715	46	50	514	1568	50
17.	86	785	5638	45	58	528	1595	48	45	478	1547	54
18.	132	950	10820	42	97	760	10110	44	105	786	9150	45
19.	83	675	4430	48	38	445	980	54	42	460	920	58
20.	95	746	4547	46	65	548	1670	48	50	576	1690	55
21.	86	739	5675	44	68	587	1758	46	57	592	1740	48
22.	118	873	6739	42	73	756	1879	45	54	568	1560	48

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

Table 3

Patient's Results on MDFRS Scale

Domain	Before treatment	3 m 10.03.17	6 m 09.06.17	9 m 07.09.17	12 m 15.12.17	15 m 16.03.18
Mobility	14	16	23	23	20	18
Basic activities of daily life	6	8	8	9	9	8
Arm function	9	13	13	16	16	14
Functional impairment	25	26	28	28	27	27
Общий балл	54	63	72	76	72	67

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

1. The proposed method of fetal progenitor cell administration is an effective and promising therapeutic approach in DMD.
2. 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.

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Впервые поступила в редакцию 02.09.2018 г.
Рекомендована к печати на заседании редакционной коллегии после рецензирования