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Non-Motor Symptoms in Parkinson's Disease and Efficacy of Treatment in a Complex Therapy Using Fetal Stem Cells

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Abstract

Aim: To evaluate the dynamics of non-motor symptoms (NMS) by scores in the patients with Parkinson's disease during complex treatment using fetal stem cells (FSCs) obtained from human fetuses with 5-10 weeks of gestation.

Materials and methods: A comparative study for 63 patients with Parkinson's disease (PD) suffering from NMS with various degree of clinical presentation has been performed to identify the effect of combined treatment on the quality of life, cognitive functions, sleep and extent of depressive disorders in patients. The main group (MG) consisted of 32 patients, who apart from standard therapy underwent treatment using suspensions of FSCs harvested from the fetal liver and brain. The control group (CG) included 31 patients. The patients in both groups have been compared according to their sex and age.

Results: Significant decrease of NMS in patients of the MG was reported 6 and 12 months after treatment. This value was significantly lower compared to the patients in the CG. By means of a detailed evaluation, significant improvement in quality of the objective parameters of sleep, daily activities and decrease of depressive disorders were reported in the MG. The treatment results were significantly higher in the MG if compared to those in the CG over 1 year after therapy.

Conclusion: FSCs therapy proved to induce positive effects on both subjective and objective manifestations of PD by improving the patient's quality of life when included into the standard scheme of treatment for PD patients with developed NMS.

Keywords: Parkinson's disease; Non-motor symptoms; Fetal stem cells

Introduction

Parkinson's disease (PD) – is a rather wide-spread disease, which affects human at the very peak of intellectual activity. Approximately 4 million of individuals all over the world have been suffering from this disease [1,2]. PD is characterized by the loss of dopamine-producing neurons in the compact part of *substantia nigra* – the area of brain responsible for control of motor functions and simultaneously substantial elevation of α-synuclein (αSyn) protein resulting in formation of Lewy bodies – abnormal protein compounds takes place in the residue neurons. αSyn is being accumulated in the neurons and axons, presynaptic terminals and ganglia. Aggregation of αSyn in presynaptic terminals antecedes formation of Lewy bodies and is not constrained to specific dopaminergic nuclei in the brainstem, but involves the numerous zones in central and peripheral nervous systems as well as autonomic nervous system, eye retina, sympathetic and parasympathetic ganglia, nerves, skin, salivary glands and the other organs that stipulates a number of non-motor symptoms in Parkinson's disease (NMS) [3,4].

NMS are demonstrable practically in all patients irrespective to their age, onset and stage of the disease. Numerous NMS appear as early as at the pre-clinical stage of PD and are prior to manifestation of motor symptoms of Parkinsonism. Along with disease progression, some of them (neuropsychiatric, vegetative, gastrointestinal symptoms) attain predominant clinical significance by inducing negative effects on the quality of life of patients and resulting in invalidity and reduced life expectancy.

Nowadays, PD is related to the group of incurable diseases. The fundamental medical drugs for treatment of PD are: preparations of levodopa, dopaminergic receptor antagonists (pramipexole, piribedil, bromocriptinum etc.), monoaminooxidase inhibitors (selegiline, rasagiline), catechol-O-methyl transferase inhibitors (entacapone), norepinephrine-dopamine reuptake inhibitors (amantadine) and some other medicines. The scheme for medicinal treatment is established with consideration of stage and character of disease, age of the patient and comorbidity; with use of monotherapy or treatment by combination of medicines from the above groups. A wide range of side effects is a significant drawback of pharmacotherapy which frequently constrains the use of those drugs and results in gradual adherence to medicines with subsequent treatment ineffectiveness.

Surgery treatment methods are resorted if conservative treatment fails. There are two types of surgery treatment methods used which are destructive operative interventions (thalamotomy and pallidotomy) and neurostimulation circuit. Surgery approach makes it possible to substantially reduce the doses of drugs, however, does not enable a complete withdrawal of pharmacotherapy. A narrow range of surgery treatment indications tends to be a disadvantage for both of these surgery methods and this interferes with their widespread use.

Existing pharmaceutical and surgery methods of treatment are solely targeted at diminishing disease manifestations and cannot ensure

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recovery of the patient. Standard schemes of treatment do not lead to desired results and this encourages searching for the new methods and approaches in treatment of PD.

For the recent years attention of scientists has been drawn to the study of clinical application of stem cells. A number of clinical trials on transplantation of stem cells extracted from fetal mesencephalon were conducted for the patients with PD which proved the principle evidence that cells replacement is able to restore the affected brain functions in PD [5].

Precursors of neural tissue can be extracted from the fetal material. Results obtained after investigations prove their potential to differentiation into the dopaminergic neurons inside of the affected nervous system [4,6-8]. Fetal stem cells (FSCs) derived from neural tissues were implanted into the striatum of the patient with PD, which has become the first breakthrough within a strategy of stem cells use in treatment of CNS diseases. Consequently FSCs therapy has become the alternative source for such replacement strategies [9].

Fetal dopamine-producing cells have been proven to survive after transplantation and could be well engrafted into the brain of the "host" which furtherly induce favorable clinical effects in the patients [5,10-12]. Diagnostics by means of positron-emission tomography demonstrated an increased uptake of fluoro-dihydroxyphenylalanine inside of the integuments [9,13], which is an evidence of complete engraftment of the cells administered [11,14].

A clinical study was performed on the scientific base of University of Colorado – where the surgery transplantation of dopaminergic neurons deriving from the human fetus parietal brain after 7-8 weeks of gestation was made directly into cerebral tissues of the patients with severe PD. The results proved that among young patients (aged 60 years and below), standardized tests for PD shown significant improvement after transplantation if compared to the patients in the CG. During improvement over the first year, relapse of dystonia and dyskinesia was present in 15 % of patients [15].

Materials and Methods

63 patients with PD suffering from NMS of various degrees were investigated in Cell Therapy Center EmCell. The patients were assigned to 2 groups which were compared according to the sex, age, NMS and stage of the disease. Thus, the MG included 32 patients with the mean age 47.2 ± 6.7 years, 68.75% were men among them and 31.25% – women. The CG consisted of 31 patients and their mean age was 49.3 ± 5.8 years including 61.29% of men and 38.71% – women. No one among the patients of the MG and CG suffered from infectious, malignant or psychic diseases. All patients were undergoing routine therapy (dopamine receptor agonists, central anticholinergic drugs, monoaminooxidase inhibitors type B, and medicines containing L-dihydroxyphenylalanine in different combinations). The patients of the MG as supplementary to standard treatment were administered FSCs suspensions harvested from the cadaveric tissues of fetal liver and brain with 5-10 weeks of gestation which were medically aborted pursuant to family planning purposes with absence of development pathology and infections in human fetus. All donors were practically healthy women and had negative test results for hemic infections.

All patients who intended to start treatment signed the informed consent. Diagnosis of PD was confirmed based on the clinical presentation (hypokinesia, muscular stiffness, resting tremor and postural instability), data of disease history, magnetic-resonance tomography (MRI), electroencephalography investigations and laboratory results. Evaluation for the NMS and extent of their influence on the quality of life was performed with the help of NMS scale [3], Modified Schwab and England ADL Scale [16]. For identification of cognitive disturbances mini–mental state examination (MMSE) was applied [17]. Sleep assessment was conducted in accordance with the modified Parkinson's Disease Sleep Scale 2 (PDSS-2) [18]. Beck's depression inventory (BDI) was used to evaluate the degree of depressive disorders in the patients [8].

Analysis of all indices was made before treatment, 6 and 12 months after it. Biotechnology process of suspension preparing included stem cells extraction from different growth zones in human fetus (liver, brain, heart and soft tissues of the embryo); assessment of cells viability and programmed cryopreservation; testing for bacterial and viral infections. Cryopreservation was made by use of 5% dimethyl sulfoxide (DMSO) as cryoprotectant to pursue the 3-stage program for freezing at the initially adjusted temperature of 1°C/min and crystal forming initiation.

Defrost of cryopreserved suspension was made by water bath thawing of cryopreserved suspension at 37.5°C immediately before to administer FSCs and cells viability was controlled. Pre-administration cell suspension viability was tested by trypan blue staining and cells were counted using 2 methods in parallel: Goryaev chamber and Automated Cell Counter NC-100 (Nucleo Counter Type 900-004 Chemo Metec, Denmark 2010). Cells viability before freezing made up 83.0 ± 3.0%. Right after cryopreservation in low temperature (t -196°C) cryobank and water bath thawing of cryopreserved suspension at 37.5 ± 0.1°C cells viability composed not less than 74.8 ± 1.0%.

FSCs administration was performed during 2 days: fetal liver stem cells were infused via intravenous drip-feed using 0.9% sodium chloride solution on treatment day 1; fetal brain suspensions were administered subcutaneously into the area of anterior abdominal wall during day 2. Therapeutic dose calculation was individually established for each administration containing not less than 2.2 ± 0.75 ml of cells in the suspension with the nucleated cell count of 2.73×10^6/mL per transplantation and cell precursors of CD34+ ranged from 0.3 to 2.54×10^6/mL per one injection. The amount of vital cells in the suspension corresponded to 70.0 ± 10.0%.

Statistical results processing was fulfilled using software Statistica v.6.0 (StatSoft Inc., Tulsa, USA) with calculation for the mean values and standard deviation. Significant differences between mean values were assessed by use of the Student's t-test (for parametric statistics).

Results and Discussion

Significant decrease of presented NMS in the patients of the MG (according to the NMS scale) was observed 6 months after FSCs treatment with concurrent routine therapy from 99.78 ± 1.86 scores to 95.53 ± 1.82 scores ($p<0.05$). In patients of the CG this value accounted for 100.74 ± 1.86 scores over 6 months after treatment and remained practically unchanged in comparison with the baseline 100.80 ± 1.88 scores ($p<0.05$). Significant decrease in the mean scores according to the NMS scale was identified 12 months after treatment in patients of the MG (93.81 ± 1.87) in comparison with the baseline (99.78 ± 1.86), whereas over 6 months after treatment this parameter was (95.53 ± 1.82), $p<0.05$, respectively (Table 1). Thus, significant decrease of NMS was reported in the patients of the MG over 6 and 12 months after treatment in accordance with the NMS scale.

The indices of sleep quality among the patients of the MG improved after FSCs therapy use in combination with the standard treatment over

Scale / Scores	Before Treatment		6 Months after Treatment		12 Months after Treatment	
	MG	CG	MG	CG	MG	CG
	Cognitive disturbances					
MMSE, M ± m	26.53 ± 0.24	26.90 ± 0.16	26.97 ± 0.20	27.00 ± 0.14	27.18 ± 0.15	26.87 ± 0.10
	Depressive disorders					
BDI M ± m	15.16 ± 1.06	15.48 ± 0.76	12.22 ± 0.71	14.42 ± 0.71	11.41 ± 0.64	14.16 ± 0.68
	Sleep disturbances					
PDSS-2, M ± m	23.66 ± 0.44	23.51 ± 0.44	21.13 ± 0.42	22.70 ± 0.43	20.88 ± 0.41	22.03 ± 0.40
	Non-Motor Symptoms and Overall Activity					
Schwab and England ADL Scale, M ± m	73.44 ± 1.24	72.25 ± 1.37	78.75 ± 1.25	74.52 ± 1.02	79.06 ± 1.22	74.19 ± 1.01
NMS scale, M ± m	99.78 ± 1.86	100.80 ± 1.88	95.53 ± 1.82	100.74 ± 1.86	93.81 ± 1.87	101.03 ± 1.88

Table 1: Dynamics of NMS in patients of the MG and CG.

6 and 12 months. After evaluation of the sleep quality index among the patients of the MG and analysis of testing results according to the PDSS-2 scale, over 6 months after treatment this value composed 21.13 ± 0.42 scores, which is significantly better therapy result if compared to the data baseline 23.66 ± 0.44 and the same evaluation data in patients of the CG – 22.70 ± 0.43 scores, respectively ($p<0.05$). Among the patients of the MG tendency to reduction in the parameter of sleep quality was observed over 12 months after initial treatment and ranged 20.88 ± 0.41 scores, however, the rate of significance was not reached in comparison with the baseline 23.66 ± 0.44 scores ($p>0.05$). The patients of the CG tend to demonstrate improvement in the mean parameters of sleep over 6 and 12 months from the start of observation, however, those rates were not significant ($p>0.05$) (Table 1).

The next stage of our study was identification of changes in cognitive deficiency among the patients with PD according to the MMSE scale. The values of cognitive disturbances in PD patients with NMS in the MG revealed a positive dynamics both over 6 months (26.97 ± 0.20 scores), and within 12 months after treatment – (27.18 ± 0.15 scores) respectively, when compared to the baseline (26.53 ± 0.24 scores), $p<0.05$.

Though the changes identified match with subjective evaluation in the patients, their significance is not substantiated. In the patients of the CG almost vanishing dynamics was demonstrable both in accordance with the testing scores by MMSE scale and after the subjective evaluation of the patient (Table 1).

During the period of observation in the patients, alteration in everyday activity was evaluated by use of testing scores corresponding to the Schwab and England ADL Scale accordingly. The results achieved prove the significant increase in a day time activity among the patients of the MG over 6 months after the treatment – 78.75 ± 1.25 scores both in comparison with the baseline values – 73.44 ± 1.24 scores, and according to the initial values in the CG – 74.52 ± 1.02 scores respectively, ($p<0.05$). By analysis of the parameters in the MG within 12 months after treatment (79.06 ± 1.22 scores) minor positive dynamic changes were characteristic if compared to the same values in the MG over 6 months after treatment which is significant in comparison with baseline and the parameters after assessment of patients in the CG – 74.19 ± 1.01 scores over 12 months after treatment ($p<0.05$).

In comparative study of the depressive disorders by use of Beck's depression inventory scale for the patients both in the MG and the CG (15.16 ± 1.06 and 15.48 ± 0.76 scores, respectively) mean values identified were almost the same, which account for the sub-depressive disorders among the patients. As it is shown in the Table 2, the patients are allocated in accordance with the degree of presented depressive disorders at different stages of observation.

The analysis of depressive disorders according to Beck's depression inventory scale in the patients of the MG over 6 months after treatment indicates a significant improvement ($p<0.05$) – 12.22 ± 0.71 scores in comparison with the baseline and the same values among the patients of the CG – 14.42 ± 0.71 scores. This tendency is also preserved at the assessment of values over 12 months after beginning of observation among the patients. The parameters of presented depressive disturbances are significantly lower in the patients of the MG if compared to the CG – 11.41 ± 0.64 scores and 14.16 ± 0.68 scores, respectively ($p<0.05$).

During a detailed analysis on distribution of the patients according to the degree of severity of presented depressive disorders against the background of treatment, the growing number of cases without depressive disorders among the patients of the MG is clearly demonstrable, whereas the same score in the CG patients remains unchanged. The patients who presented more severe category of depressive disorders had tendency for downgrading to less severe disorders which was also demonstrable among the other categories of disorders (both moderate and expressed disorders on the Beck's depression inventory scale) in patients of the MG (Table 2).

Thus, among the patients with PD, who administered FSCs along with the standard therapy, decrease in the presented NMS was observed both in accordance with objective and subjective evaluation. Although, we realize that the informative base for observations is not enough for the widespread clinical application of this method. Even though our clinical study had rather short-time duration (12 months) after administration of FSCs, we did not attest any adverse effects which might influence the functions of the brain and cardiovascular system in the patients and also no allergy reaction was clinically attested. Therefore, one can speak about safety of all further long-term observations.

Degree of Disorder by BDI scale	Before Treatment		6 Months after Treatment		12 Months after Treatment	
	MG, n=	CG, n=	MG, n=	CG, n=	MG, n=	CG, n=
Disorders are absent	5	4	7	4	9	4
Sub-depressive disorders	11	11	17	15	17	14
Moderate depression	9	12	5	8	4	11
Expressed depression	7	4	3	4	2	2
Severe depression	0	0	0	0	0	0

Table 2: Distribution of patients by the degree of presented depressive disorders according to Beck's depression inventory scale within a period of observation.

Conclusions

1. FSCs administration is a safe and effective treatment method which could be used in complex therapy along with medicinal preparations applicable for the patients suffering from PD with the NMS; however, there is a demand to pursue all subsequent clinical studies.

2. Complex treatment of the patients with PD and presented NMS by application of fetal stem cells and combined use of standard therapy over 6 and 12 months of observation resulted in significant decrease in number of NMS manifested on the NMS scale, ($p<0.05$), in comparison with the CG.

3. Combined treatment of PD patients with NMS using FSCs along with standard therapy over 6 and 12 months revealed significant decrease in sleep disturbances among the patients of the MG on PDSS-2 scale ($p<0.05$), if compared to the same manifestations in the CG patients.

4. Complex therapy for the PD patients with manifested NMS using fetal stem cells in combination with the standard treatment over 6 and 12 months after the study leads to significantly decreased depressive disturbances evaluated according to the Beck's depression inventory scale among the patients, ($p<0.05$), compared to the CG.

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