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Complex Treatment of the Patients with Alzheimer's Disease Using Stem Cell Preparations Including Extracted Fetal Stem Cells

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Abstract

Objective: The principal aim of this study was to evaluate effectiveness of combined therapy method with inclusion of the standard protocol of treatment using medicines along with application of separated fetal stem cells (FSCs). Higher efficacy of treatment of the patients with Alzheimer's Disease (AD) is defined as a technical value of our complex therapy.

Material and Methods: We performed a comparative study of the groups of patients including women and men aged from 50 to 85 years who were presented with diagnostic criteria of Alzheimer's type dementia by DSM- IV-TR, NINCDS-ADRDA as well as study of the patients with confirmed diagnosis of AD in accordance with ICD-10-CM suffering from mild and moderate-to-severe stages of dementia by CDR and MMSE scales. Along with standard treatment by use of stable doses of medicines our patients were administered FSCs suspensions containing stem cells extracted from the tissues of fetal liver and brain (human cadaveric fetuses of 7-12 weeks gestation) which were acquired as a result of medical abortion due to social and family planning reasons.

Results: In a process of the comparative study the results we received emphasize treatment significance and advantages of complex therapy method compared to standard treatment used as isolated therapy for AD patients suffering from mild-to-moderate dementia. The suggested method of complex treatment is safe and contributes to improved cognitive functions as well as increased daily activities among such a group of AD patients ($p<0.05$).

Conclusion: Use of preparations with extracted FSCs in complex treatment of the AD patients with mild and moderate grades of dementia has been proven to be safe and effective method of therapy which contributed to better cognitive functions and increased everyday activity among patients with AD.

Keywords: Alzheimer's disease; Dementia; Fetal stem cells; Cognitive functions; Complex treatment

Abbreviations: AD: Alzheimer's Disease; APP: Amyloid Precursor Protein; c-jun: c-Jun N-Terminal Kinase; NMDA: N-Methyl-D-Aspartate Receptors; APOE: Apolipoprotein E; ChEI: Cholinesterase Inhibitors; tau phosphorylation: Phosphoprotein with 79 Potential Serine (Ser) and Threonine (Thr) Phosphorylation; CD: Cognitive Deficit; FSCs: Fetal Stem Cells; MSCs: Mesenchymal Stem Cells, DSM-IV-TR: Diagnostic and Statistical Manual of Mental Disorders IV; NINCDS-ADRDA Alzheimer's Criteria: National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association; ICD-10-CM: International Classification of Diseases; 10th Revision, Clinical Modification; CDR scale: Clinical Dementia Rating Scale; MMSE: Mini-Mental State Examination scale; CNS: Central Nervous System; HIV: Human Immunodeficiency Virus; DMSO: Dimethyl Sulphoxide; ADAS-cog: Alzheimer Disease Assessment Scale-Cognitive; PSMS: Physical Self-Maintenace Scale; ADLs: Activities of Daily Living; CGI-I: Clinical Global Impression-Improvement Scale

Introduction

First time Alzheimer's disease (AD) was described in 1907 by German psychiatrist Alois Alzheimer. It was a clinical case in 56-year-old woman with major memory impairment, gradual affection of speech and visual-spatial disturbances [1]. Thus, at present days, in accordance with the International classification of diseases, AD (GT30/F00) is designated as a primary degenerative disease of the brain of unknown etiology which is characterized by neuropathological and neurochemical manifestations commonly presented with a slowly progressive disease course for several years.

There are over 47.5 million of individuals suffering from dementia worldwide and annually 7.7 million among them are regarded as new disease incidence cases. AD is the most common cause of dementia and 60-70% of all dementia clinical cases refer to this disease. In conformity with epidemiology studies about 25 million of individuals have been suffering from AD throughout the world; on top of that, this figure is ultimately being increased; and, according to the prognosis, the number of them may reach as much as 114 million of patients until 2050 [2].

Nowadays AD is referred to as a heterogenic disease. The main hypothesis of the disease onset is a pathological amyloid protein formation which is accumulated both on the walls of cerebral vessels and within a parenchyma of the brain [3-7]. Such deposits have specific features being named "senile plaques". Appearance of them is

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contributed by intercellular space oxidation and reduced activity of lysosomal hydrolases which result in worsened amyloid resorption. Amyloid deposits lead to death of neurons adjacent to the plaque both because of their proximate toxicity and due to gene expression of apoptosis inductors (c-jun). In particular, effects on amyloid and Amyloid Precursor protein (APP); activation of N-Methyl-D-Aspartate (NMDA) receptors and initiation of free-radical oxidation are remarkable. Within this cascade of changes one cannot exclude immune response boost as well. Thus, one of the clue places in a process of pathogenesis is referred to isoform of Apolipoprotein E (APOE) which exerts influence on transition of APP to amyloid and it has its proper negative impact on the processes of regeneration of synaptic structures [8,9].

In addition, so-termed processes of secondary pathogenesis can develop which are characterized by inflammatory changes, oxidative stress, violation of energy substances production by the cell, decreased vasoreactivity and excitotoxicity, etc. [10].

Abnormalities of cholinergic, glutamatergic and catecholaminergic systems which are tightly associated with cognitive and mnestic processes have a supreme significance in pathogenesis of AD [11].

Nowadays, no one of existing treatment methods can give a chance to stop degeneration and death of the group of cells which are susceptible to the above pathology process. In this relevance, the basic purpose of modern therapy of AD is a symptomatic treatment within the shortest possible time. Available treatment methods are directed at inhibiting progression of cognitive deficit and compensation of behavioral and psychotic disturbances along with promoting fewer burdens for the care-givers of AD patients.

Standard treatment of AD consists in use of 2 main groups of medicines: Cholinesterase Inhibitors (ChEI) and modulators of glutamate NMDA receptors. Degenerative processes which take place in the nuclei basalis of Meynert result in hypocholinergic syndrome which is presented as impairments of concentration and excitation, cognitive and psychotic dysfunctions [11]. Glutamate participates in a pathology cascade of β-amyloid accumulation in the brain. Activation of NMDA receptors contributes to pathological tau phosphorylation [12].

In order to reduce the grade of Cognitive Deficit (CD) and to reach a continuous compensation of AD modern medicine offers treatment by use of donepezilum, rivastigmin, galantaminum (ChEI) and memantinum which are usually prescribed for the patients with AD.

All ChEI mentioned above are widely used in clinical practice for such patients. Despite of different mechanisms of action and certain characteristics remarkable for each drug of this group, meta-analyse of all existing data shows that their efficacy and safety for the patients with AD are in general the same [2]. Add of memantinum to the therapy based on ChEI increases its effectiveness [13,14]. Memantinum belongs to the group of selective noncompetitive antagonists of NMDA receptors which can prevent excitotoxic activation of receptors. Metaanalyse of the results of studies revealed that combination of ChEI and memantinum leads to improved cognitive, functional and behavioral disturbances; induces a favorable general clinical impression only in the patients with moderate and severe AD severity [15]. Effectiveness of combination of ChEI and memantinum was put into question after studies in accordance with DOMINO-AD protocol [16]. Not enough efficacy of treatment as well as appearance of multiple side effects could be outlined among the drawbacks of existing treatment methods for AD patients.

One more direction in treatment of AD is use of neurotrophic preparations. Within a complex of low-molecular-weight neuromodulators cerebrolysin is the most effective in treatment of AD and that is likely to pass through blood-brain barrier [16]. In particular, such a combination with cerebrolysin allowed doctors to reduce adverse effects and contributed to better tolerance of ChEI by AD patients. However, there is a restriction for prescribing this combination, namely in cases of its use by the patients with marked psychotic abnormalities.

No one from the above mentioned treatment methods is 100% effective in view of reduced CD and its safety in therapy for AD patients. This fact encourages researchers all over the world to search for principally new, accessible and effective methods of therapy for the patients with AD.

A new direction in therapy for AD patients could be a complex treatment using suspensions which contain extracted Fetal Stem Cells (FSCs). Numerous studies were devoted to embryonic stem cells use and acquired from adult donors cells which had been successfully transplanted into the intact brain of mice or rats. Inside of the affected brain transplanted stem cells intentionally migrated to the affected sites in the brain where they began proliferation into the functional neurons. Stem cells precursors of neural cells can be administered intravenously and despite of this route of transplantation they migrate to the affected foci of the brain and promote functional restoration in them.

The main route of MSCs use is intravenous infusion to the recipient [17-20]. In case MSCs are administered systemically, the stem cells are trapped into capillary beds of different tissues, especially the lungs [17,21-23]. Researchers assessed intra-arterial injection of MSCs proving that delivery of MSCs through the internal carotid artery significantly improves stem cells migration and homing in the injured brain compared to injection via the femoral vein [24]. In a similar manner, for the patients with subacute Spinal Cord Injury (SCI), administration of MSCs via the artery vertebralis leads to a greater functional improvement than when cells were administered through the intravenous route [25]. Studies reveal that administration of cells into the artery may lead to "microvascular occlusions" [24]. Whereas in treatment of Myocardial Infraction (MI), delivery of bone marrow cells or MSCs directly into the heart or adjacent sites of damage increases the number of cells found in the Peri-Infarct Region [26].

Much evidence in literature also exists concerning the explanation of the mechanisms of MSCs migration towards the target tissues and significance of cell surface receptors and molecules contributing to such a migration. The role of activated endothelial cells in migration of MSCs has also been broadly investigated by the scientists. In this respect, the factors promoting migration of MSCs and their homing in target tissues were also described.

Homing effect significantly depends on the chemokine receptor, CXCR4, and its binding partner that was previously characterized in Hematopoietic Stem Cells (HSCs) homing, that is, stromal derived factor-1 CXCL12 [26-30]. Wynn et al. demonstrated that CXCR4 is resent on a subpopulation of MSCs, which aid in CXCL12-dependent migration and homing [31].

Likely migration and homing requires that cells can attach to and migrate between endothelial cells in order to reach the target tissues.

According to the data of literature, observation on the animal models gave evidence that transplanted stem cells or cells precursors of neurons which preserve viability can migrate being further differentiated into cholinergic neurons, astrocytes and oligodendrocytes

contributing to recovery of cognitive deficit. Besides replacement of the lost or affected cells, stem cells can stimulate endogenic precursors of neurons, intensify structural neuroplasticity and favor decreasing of proinflammatory cytokines that cause inhibition of neuronal apoptosis [32]. Cerebral transplantation of MSCs can not only reduce amyloid load and pathological tau phosphorylation in the brain, but also prevents decrease of cognitive functions and memory impairment related to AD-like pathologies in mice with APP/PS1 mutation [33]. Clinical studies prove safety, effectiveness and translation potential of complex therapy by use of stem cells [34].

Materials and Methods

Patients

Comparative study was performed for the groups of patients (women and men) being allocated with age ranges from 50 to 85 years including those who matched a diagnostic criteria of Alzheimer's type dementia by DS-IV-TR and NINCDS-ADRDA Alzheimer's Criteria [35,36]. The same study was made for the patients with confirmed diagnosis of AD in accordance with ICD-10-CM [37,38] suffering from mild and moderate-to-severe stage of dementia by CDR scale along with those having 14-25 scores by MMSE scale. Written informed consent was acquired from the patient and/or the caregiver prior to be admitted to complex treatment and observation. Our study was approved by the local ethics committee on the base of Kyiv City Clinical Emergency Hospital being located at the address: 3 Bratyslavska str., Kyiv City, Ukraine.

Neurology diseases (Parkinson's disease, stroke, history of traumatic brain injuries, toxic and metabolic encephalopathies, epilepsy, demielinising diseases and inherited degenerative abnormalities of CNS), neoplastic processes of any localization, narcotic drugs use (including alcohol abuse and toxic mania), systemic and endocrine diseases in the stage of decompensation were among the inclusion criteria for our patients the same as severe organic decompensation, infectious diseases including HIV and viral hepatitis infections; grade 3 arterial hypertension.

Patients included into an observational study were randomly allocated into 2 groups.

Comparative assessment of the groups with allocated patients is presented in the Table 1.

Procedure

According to the method of complex treatment for the patients with reported AD we have treated 35 patients of the Main Group (MG). Against the background of conventional treatment using the stable doses of medicines [39] the patients were administered FSCs suspensions containing stem cells extracted from the tissues of fetal liver and brain (human cadaveric fetuses of 7-12 weeks gestation) which were acquired as a result of medical abortion due to social and family planning reasons.

Value		Main group (MG)	Control group (CG)	Notice#
Number of patients		35	32	
Mean age, Years		76.9 ± 8.2	78.2 ± 6.6	p<0.05
Persistence of AD*, in years		2.4 ± 1.1	3.0 ± 0.9	p<0.05
Sex	men	9	4	
	women	26	28	
Dementia severity by CDR scale	mild	13	9	
	moderate-to-severe	22	23	
Score by MMSE scale		17.4 ± 2.8	16.9 ± 2.8	p<0.05
*Persistence of the AD which refers to the duration of a disease after the onset of primary manifestation.				
#Significant difference compared to assessment before beginning of complex therapy for the patients.				

Table 1: Comparison of the patients allocated in the MG and CG during the study.

Administration of FSCs suspensions was performed in conformity with therapy program during 2 days. The patients of the MG received fetal liver stem cells in drip-feed infusions for the first treatment day during this study. The above transplantation was made along with premedication infusion of 0.9% sodium chloride. For the second day all patients of this group were administered suspensions containing stem cells of fetal brain which were injected subcutaneously into the anterior abdominal wall. Characteristic features of all suspensions for each transplantation were individually selected, however, the volume of each injection was not less than 2.80 mL of stem cell suspension including the nucleated cells which made up in the least $3.7 \pm 2.6 \times 10^6$/mL and cells precursors of $CD34^+$ in ranges from 0.41 up to 2.44×10^6/mL per 1 infusion with the range of viable fetal stem cells not less than 70.0% ± 10.0% per suspension.

Control group (CG) included 32 persons who were administered conventional therapy by use of the standard doses of medicines for AD treatment.

The medicines of different groups were included into the conventional treatment scheme with the next daily drugs doses recommended: Donepezilum-10 mg, Galantaminum-24 mg, Rivastigminum-12 mg, Memantinum- 20 mg and extract of Ginkgo Biloba-240 mg. All drugs above were recommended for the patients in combinations or as a monotherapy. Combination of medicines was individually defined; the drugs dose might not even reach the recommended therapeutic target doses.

The patients were under observation of the doctors after administration of the suspensions containing stem cells extracted from fetal liver and brain immediately after preparations defrost and water bath thawing following stem cells cryopreservation. Over 6 and 12 months after treatment doctors began studying the signs of pathological process in accordance with clinical, laboratory and neuropsychology parameters.

Technology process

Preparation of the suspension consists in separation of cells from different growth zones of liver and brain of the fetus. Simultaneously, bacteriology and virology studies are performed along with evaluation of stem cells viability following extraction. Cells viability before cryopreservation composes not less than 83.0% ± 3.0%. Programmed cryopreservation is conducted pursuant to the parameters asserted. A process of cryopreservation is performed with the help of IceCube 14 S, SY-LAB, Austria 2012 and by means of 3-stage freezing program under condition with initiation of crystals formation and start velocity of 1°C/min., using 2 cryoprotectant solutions simultaneously: dimethyl sulphoxide (DMSO) and dextran-40. Storage of a suspension in the low temperature cryobank is performed at a generally defined t°=-196°C.

Directly before administration FSCs suspensions are exposed to water bath thawing at t°+37.5 ± 0.1°C immediately after defrost of the cryopreserved preparation. Then viability of extracted stem cells is controlled by use of a cytoscopy analysis. During this study a quantitative calculation was performed by means of 2 methods: using

cytoscopy (Goryaev chamber) and automatic cell analyzer NC-100 Nucleo Counter Type 900-004 Chemo Metec, Denmark 2010. Stem cells viability made up not less than 74.8% ± 1.0% in the time span after cells water bath thawing.

Assessment

Our study was performed in accordance with the specially established protocol. Assessment of Cognitive Deficit (CD) was made with the help of modified Alzheimer Disease Assessment Scale-Cognitive (ADAS-cog) [40]. Assessment for activities of daily living in the patients was controlled by means of Physical Self-Maintenace Scale (PSMS or ADLs) [41]. We used Clinical Global Impression-Improvement scale (CGI-I) in order to assess all acquired therapeutic effects [42].

Analysis of the values above was performed prior to treatment, over 6 months and 12 months after therapy. Statistic processing of the established results was made by Statistika v.12.0 with the help of calculation of the mean values and standard deviation scores. Statistical significance was evaluated using the Student t-test.

Results

Early post-transplantation effects

All patients of the MG revealed a syndrome of early post-transplantation improvement: general condition became better; they felt much strength and energy in the body. At the very beginning of treatment there was no single case with similar advantages which might be reported by the patients of the CG.

Adverse reactions and safety

Treated patients in the MG had no evidence of complications related to "graft vs. host" reaction after stem cells administration. The scheduled course of therapy was completed for all of the patients. Over the 24th day after treatment 1 patient of the MG reported appearance of vascular and autonomic crises which later recurred in this patient. One more MG patient presented the elements of aggressive behavior over the day since therapy had been started. Condition of both patients mentioned above improved after correction of the standard therapy scheme. In the same way 2 patients of the CG revealed aggressiveness in behavior for the 14th and 17th days after treatment beginning respectively; the first of them had a day-time drowsiness and marked vegetative disturbances for the 5th day after therapy. State of these patients improved after correction of their conventional treatment.

AD patients revealed a normal susceptibility to intravenous and subcutaneous administration of stem cell suspensions extracted from fetal liver and fetal brain. No single case of allergy reactions or episodes of psychomotor excitement among the patients of the MG were recorded by our doctors.

Main Effects

Assessment of cognitive functions: As it is demonstrable from the Figure 1, positive changes in values of cognitive functions by ADAS-cog scale were remarkable showing 4.55 scores from 24.56 ± 2.18 over 6 months after complex treatment with inclusion of fetal stem cell suspensions and after initial observation the scores made up to 20.01 ± 2.92. Such a positive dynamics was also observed over 12 months (20.07 ± 2.67) after treatment beginning ($p<0.05$ for all). Simultaneously, the patients of the CG demonstrated a statistically significant positive changes in the values of cognitive functions by ADAS-cog scale which recorded 1.7 scores from 23.81 ± 2.26 after primary assessment up to 22.11 ± 2.64 over 6 months after therapy. Over 12 months the above score did not change its statistical significance, nevertheless, it alternates the vector by way of worsening of the parameters above and constitutes up to 23.09 ± 2.81 ($p>0.05$ for all).

Assessment of physical self-maintenance: After comparison of physical self-maintenance over 6 months after treatment among the patients of the MG we noticed a statistically significant improvement of values by ADLs scale which constituted up to 2.14 ± 0.47 (see Table 2). Positive dynamics in comparison with the baseline score 2.53 ± 0.61 was observed after the study over 12 months 2.04 ± 0.58 ($p>0.05$ for all). In the meantime, the patients of the CG reported a statistically significant improvement of the scores by ADLs scale which shown up to 2.14 ± 0.47, both over 6 and 12 months after beginning of treatment ($p>0.05$ for all).

In the below diagrams (Figures 2 and 3) we can observe percent (%) of distribution of values after assessment of the patients in accordance with the physical self-maintenance scale over 6 and 12 months after beginning of treatment. As one can notice the best percentage of improvements in toilet and bathing as well as feeding has already become remarkable for the 6th month since treatment started. There is no negative dynamics among each of the sub-categories of patients. Simultaneously, patients of the CG reported a negative dynamics of the results with dressing.

Over 12 months after beginning of observation decrease in a percentage difference from the baseline values in the patients of the CG can be demonstrable, whereas we recorded similar percent values in the patients of the MG which were preserved showing almost stable scores.

When therapeutic effects are compared according to the scale of Clinical Global Impression-Improvement scale (CGI-I) among the patients of both groups over 6 months from beginning of treatment we observed statistically significant advantage of the suggested method of therapy in the MG patients compared to those who had been following the conservative scheme of therapy. The same advantage was also reported while comparing the scores over 12 months after FSCs administration ($p>0.05$ for all) (see Table 3).

Consequently, among the patients with AD who were administered FSCs along with standard therapy decrease of cognitive deficit and

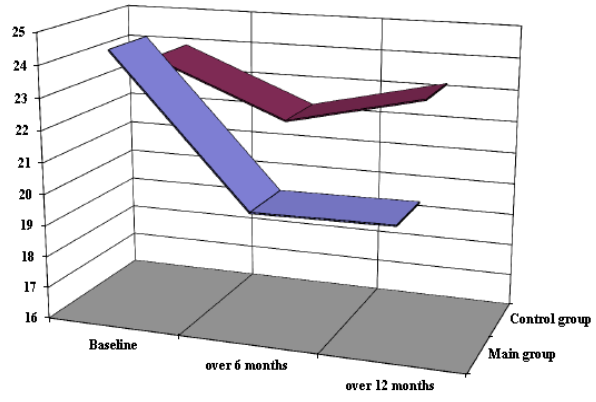

Figure 1: Comparative diagram on dynamics of cognitive functions among the patients of the MG and CG (mean scores of the groups by Alzheimer disease assessment scale-cognitive (ADAS-og).

Value	MG, n=34			CG, n=34		
	baseline	over 6 months	over 12 months	baseline	over 6 months	over 12 months
Total score by physical self-maintenance scale (ADLs)	17.73 ± 1.61	15.74 ± 1.47 p<0.05#	15.91 ± 1.51 p<0.05#	17.30 ± 1.51	16.90 ± 1.47 p<0.05#	17.09 ± 1.45 p<0.05#
#Significant difference in comparison with assessment before complex treatment.						

Table 2: Comparison of dynamics of physical self-maintenance among the patients of MG and CG.

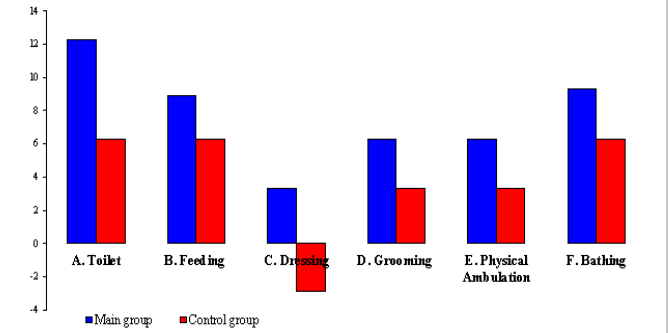

Figure 2: Comparative diagram of dynamics of hygiene skills and physical self-maintenance among the patients of the MG and CG by physical self-maintenance scale over 6 months (percentage (%) in accordance with the scores baseline).

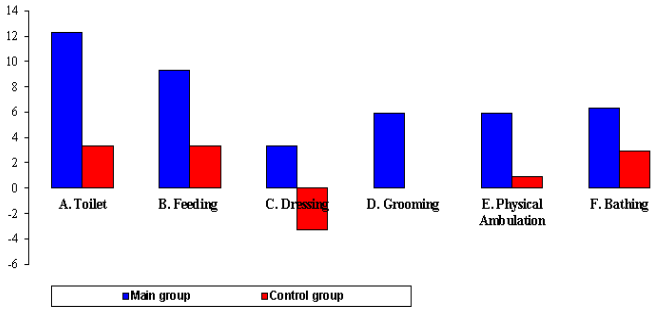

Figure 3: Comparative diagram of dynamics of hygiene skills and physical self-maintenance among the patients of the MG and CG by physical self-maintenance scale over 12 months (percentage (%) in accordance with the scores baseline).

Value	MG, n=34		CG, n=30	
	Over 6 months	Over 9 months	Over 6 months	Over 9 months
Total Score by global impression-improvement scale (CGI-I)	3.76 ± 0.21	3.89 ± 0.24	4.04 ± 0.24	4.11 ± 0.29

Table 3: Comparison of therapeutic effect among the patients of MG and CG.

improved self-maintenance characteristics were remarkable according to both objective and subjective assessment.

In addition, analysis of data revealed significantly higher efficacy of therapy by use of our suggested method of treatment compared to the conventional scheme for management of the patients suffering from AD. Despite of this evidence we are aware of likely informative base as not quite sufficient for extensive application of this treatment method. Our clinical study persisted relatively short time period (12 months) after administration of FSCs during which we did not observe any side effects that might have influence on function of the brain and cardiovascular system; no evidence of allergy reaction was clinically reported by the patients either. Therefore, one can speak about reliability of all further long-term observations in this direction.

Conclusions

1. The data obtained after a comparative study of the results emphasize significant advantages in the respect of dementia with mild and moderate severity among the patients with AD who were administered complex treatment using stem cell preparations containing extracted FSCs compared to those patients who solely followed their traditional therapy approach.

2. Combined treatment using the stable doses of medicines in AD patients along with suspensions containing FSCs is a safe method of complex treatment.

3. Use of preparations containing extracted FSCs in complex treatment of the AD patients with mild and moderate grades of dementia contributes to improvement of cognitive functions and advantages associated with everyday activity in such patients with AD.

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