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Systemic application of allogeneic umbilical cord blood mononuclear cells in an 8-year old patient having regressive autism: Clinical case report

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Abstract

For the last decades, the interest in autism treatment is increased among both clinical and research societies. Despite significant achievements in understanding of autism etiology and pathogenesis aspects, effective treatment alternatives are yet to be found. Cell therapy was demonstrated to be promising for patients having both neurological and psychiatric disorders. We provide the case report of successful systemic application of allogeneic Human Umbilical Cord Blood Mononuclear Cells (HUCBCs) in 8-year old patient having regressive type of autism resistant for any standard therapy methods. Within 6 and 12 months clinical observation, psychometric and instrumental examinations demonstrated significant improvement followed by development of perception, reduction of somatosensory disorders, restoration of normal emotional status, active socialization, and communication ability. We consider further studies of systemic cell therapy safety and efficiency in patients with autism to be critical for clinicians and researchers.

Keywords

Treatment; Autism; Cell therapy; Psychiatric disorders; HUCBCs.

Background

Autism is a group congenital central nervous system development disorders varying in etiology and pathogenesis. In most cases, social and communicative ability violations and repeated stereotypic behavior are observed. In general, autism is believed to relate to genetic disorders resulting in brain synaptic development interruption. However, the genetics of the disease is complicated [1-3]. Unified protocols of autism drug therapy are lacking due to diversity of potential causes and mechanisms. Most protocols are based on isolated treatment of specific symptoms like aggression, excitation or anxiety, but do not consider other

key disorders (e.g. communication, social skills, sensory disorders, intelligence etc.) [4,5]. The efficiency of such methods is low, and the most of autism patients end up in retirement.

Highly restricted efficiency of the standard therapy dictates an urgent need for new autism treatment approaches. Regenerative technologies and cell therapy is considered to be one of the most promising alternatives in neurology and psychiatry, especially the application of stem cells [6,7]. Moreover, cord blood is a highly available, free of any moral and ethical restrictions and efficient source of stem cells for cell therapy. Early experience of allogenic mononuclear cord blood cell application in patients with various neurologic and psychiatric disorders including cerebral palsy [8, 9], stroke [10], perinatal encephalopathy [11], Alzheimer's disease [12] and schizophrenia [13,14] confirmed high levels of safety and efficiency of such therapy. Potential application of mononuclear cord blood cells in autism patients is even more promising [15-17].

Recently, we performed a Phase I/IIa clinical study of cord blood cell therapy in a group of juvenile autism patients. This case report presents the results of application of mononuclear cord blood cells in one autism patient having significant cognitive disorders. This patient was included into the study due to complete inefficiency of standard drug therapy.

Clinical Case

8 years old patient admitted to the Department of Juvenile Psychiatry with his mother. Mother's complaints included cognitive development delay, hyperactivity, episodes of excitation and aggression, motor stereotypes, significant speech and communicative disorders. Key objective of admittance was focused on therapy and rehabilitation verification.

Anamnesis

Until two years old, the child developed correctly. At this age, an acute respiratory infection accompanied by high fever (>40°C) resulted in significant behavioral changes, aggression, motor overactivity (hyperactivity?), manifestation of speech disorders, development of appeal reaction failure and stereotypic behavior. The first psychiatrist visit was registered in three years old; according to ICD-10 criteria, atypical autism accompanied by cognitive retardation was diagnosed. Magnetic-resonance imaging revealed no significant brain structure disorders. Also, electroencephalography demonstrated no signs of epileptic activity. Beginning with the first admission, patient was treated with several antipsychotic and antidepressant drugs. At the time of the last admission, therapy included 45 mg of Chlorprothixen daily. However, drug therapy was ineffective for five years resulting in persistence of behavioral disorders, emotional instability, and significant cognitive and speech disorders.

Cell samples properties and application procedure

Cord blood samples were collected at least 6 months prior to infusion, quarantined and kept frozen in liquid nitrogen. All samples were provided at no cost by V.I. Kulakov's National Center of Obstetrics, Gynecology and Perinatology, Moscow, Russian Federation. All samples were transported to the processing

laboratory within 4 hours, and cells were collected for future application [18]. Umbilical cord blood mononuclear cells (UCBCs) were resuspended, divided into cryotubes and frozen in liquid nitrogen under –196°C. All mother's and cord blood samples were examined for the general sterility and hemotransmissive infections in an independent lab. All used samples were free from HIV-1/2 (antigen + antibodies), hepatitis B (HBs Ag, anti-HBC-total) and hepatitis C (anti-HCV-total) viruses, T-cell leukosis virus (anti-HTLV-1/2 antibodies), Herpes simplex viruses type 1 and 2 (anti-HSV IgM), cytomegalovirus (anti-CMV IgM), Toxoplasma (anti-Toxo IgM), syphilis pathogen (Syphilis RPR) and multiple bacterial and fungal infections. Also, samples were characterized for AB0/Rh systems and estimated for CD-34$^+$ cells number [19,20].

Prior to clinical application, UCBC samples were aseptically defrosted, thawed from cryoprotector (DMSO), estimated for cell viability using trypan blue test and transferred to infusion medium. Ready-to-use samples are stored in sealed polymer containers and consist of 20 ml of pale pink opalescent liquid and 250 ± 50 millions of cord blood mononuclear cells supplemented with rheopolyglukin and human serum albumin. All prepared samples were transported to the clinic in thermal containers at +1 – +4°C. The time range between samples defrosting and infusion never exceeded 2 hours. All patients obtained intravenous infusions of group- and rhesus-compatible cell samples containing 250 ± 20 millions of cells per one infusion. Each patient received 4 infusions with a 14-days interval.

Cognitive function was evaluated using Wechsler Intelligence Scale for Children (WISC) several subtests prior to therapy initiation (baseline) and in 6 and 12 months after UCBC infusions (dynamics). The dynamics of autistic disorders was estimated using specialized questionnaires CASD (Checklist for autism spectrum disorders) and ATEC (Autism treatment evaluation checklist) at baseline and in 6 months after UCBC infusions. Also, Auditory Brainstem Evoked Potentials (ABEP) were examined both at baseline and in 6 months after UCBC infusions. ABEP method was used to assess the severity of brain-ear connection disorders and to examine functions of pontomedullary and pontomesencephalic brain structures. EEG was also performed in each case at baseline and in 6 months after UCBC infusions.

Therapy tolerance

Systemic application of 250 ± 20 millions of UCBCs was well tolerated by all patients including the reported. No adverse events were registered in all cases during observation.

Results

At baseline, severe communicative, emotional, and behavioral disorders of the patient failed the standard WISC examination. To solve this, we have chosen several separate subtests focused on specific cognitive functions. "Digit span" subtest assessed the operative memory and active attention, while the "Picture completion" subtest evaluated patient's perceptional abilities, observation, and concentration. Other subtests included "Block design" test for analytical and synthetic abilities and "Coding" subtest for attention structure and visual/motor coordination. Tests results are provided in Table 1.

Table 1: Results of individual WISC subtests at baseline and in 6 and 12 months following cell therapy.

Subtest type	At baseline	6 months	12 months
Digit span (attention and memory)	1 (attention – 0, didn't understand the guide; memory – 0 points, normal value is 5)	**2** (attention – 0, didn't understand the guide, memory – 2 points, normal value is 5)	1 (attention – 0, didn't understand the guide, memory – 2 points, normal value is 5)
Picture completion (perception, concentration)	0	0 (didn't understand the guide)	**5**
Block design test (analysis/ synthesis under visual template)	4	**5**	**8**
Coding (visual/motor coordination, speed of new skill formation)	2	**4**	**9**

Outcome's analysis revealed significant increase of values in "Picture completion", "Block design" and "Coding" subtests. Interestingly, Wechsler's method was successfully and fully applied in this patient in 12 months after UCBC cell therapy. Verbal intelligence indicator in formal numerical term reached 42 points, while nonverbal reached 79 points. We couldn't evaluate the total intelligence indicator using Wechsler's test due to significant range of parameter values. We registered significant improvement of contact orientation, attention and emotional/volitional and behavioral control resulting in successful application of WISC at the end of observation.

CASD and ATEC scales assessment also revealed significant improvement (Tables 2 and 3). ATEC total score decreased from 16 points at baseline to 6 points in 6 months after cell therapy. ATEC total score also decreased from 80 to 16 points. Specifically, ATEC scale included several parameters demonstrating significant improvement: "Sociability" parameter (decreased from 27 to 3 points), "Health/physical development/behavior" parameter (decreased from 23 to 7 points), "Speech/language/communication" parameter (decreased from 18 to 5 points), "Sensory/cognitive awareness" parameter (decreased from 12 to 1 points) (Table 3). In CASD scale, the most significant improvement was observed in two sections, including "Perseverations" (decreased from 4 to 0 points) and "Mood disorders" (decreased from 2 to 0 points) (Table 2).

Table 2: Results of CASD questionnaire application at baseline and in 6 months after cell therapy.

CASD questionnaire sections	At baseline	6 months
Problems with social interaction	1	**1**
Perseverations	4	0
Somatosensory disorders	2	**1**
Atypical communication and development	5	**4**
Mood disorders	2	**0**
Problems with attention and safety	2	**0**
Total score	16	**6**

Table 2: Results of ATEC questionnaire application at baseline and in 6 months after cell therapy.

ATEC questionnaire sections	At baseline	6 months
Speech/Language/Communication	18	**5**
Sociability	27	3
Sensory /Cognitive awareness	12	**1**
Health/Physical development/Behavior	23	**7**
Total score	80	**16**
Problems with attention and safety	2	**0**
Total score	16	**6**

Electrophysiological analysis of ABEP revealed improvement of acoustic signal transmission between acoustic nerve and olivary complex (interval I – III was 2,57 sec at baseline and 2,35 sec in 6 months) at the end of observation.

EEG analysis was performed to assess brain general functional status, level of bioelectrical activity maturity, severity of functional disorders and to localize pathological focuses. It demonstrated significant improvement of EEG patterns in 6 months following UCBC therapy (Figure s1,2).

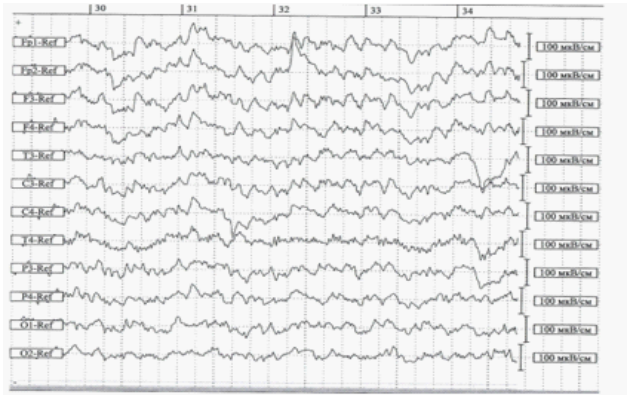

Figure 1: EEG at baseline.

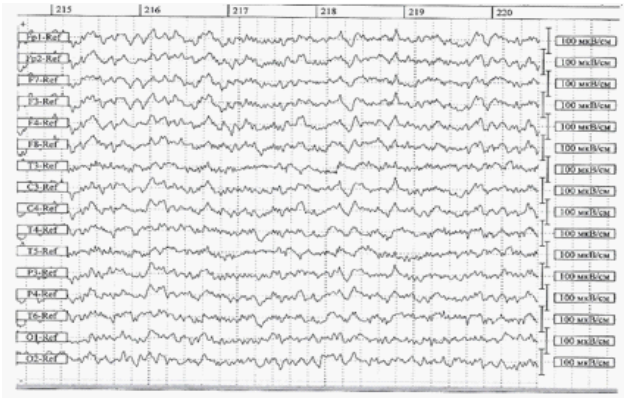

Figure 2: EEG in 6 months after UCBC therapy.

A follow-up EEG performed in 6 months after UCBC therapy demonstrated the increase of alpha waves number, decrease of background EEG amplitude, reduction of polyphasic potentials in bilateral occipital leads, and decrease of slow-wave activity (theta waves and sporadic delta waves). Also, we observed significant reduction of general amplitude characteristics and clear zone formation resulting in gradual amplitude increase from frontal cortex to occipital lobes; this pattern was not registered at baseline. Quantitative EEG analysis with closed eyes demonstrated significant increase of alpha wave amplitude and reduction of theta wave amplitude in bilateral parietal and occipital leads. Such reorganization of EEG-pattern suggests the improvement of brain structure functional status and reduction of functional immaturity of brain structures following cell therapy.

In several months after UCBC therapy, we observed significant vocabulary increase, formation of simple speech and active development of communicative skills. However, the peak improvement occurred in emotional life and behavior: patient became considerably calmer, aggression and affective outbursts

were overcome resulting in complete cancellation of antipsychotic therapy. Moreover, rapid cognitive development affected child's art skills (Figures 3,4). At baseline, drawings were repetitive and objectless. After UCBC therapy, significant improvement of attention, perception and fine motor skills was observed resulting in crucial changes in drawing skills, and development of reading and writing skills.

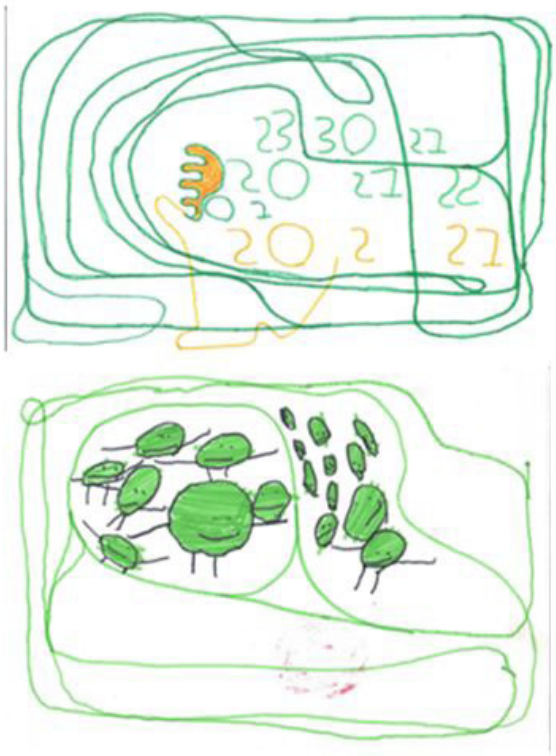

Figure 3: Drawings at baseline.

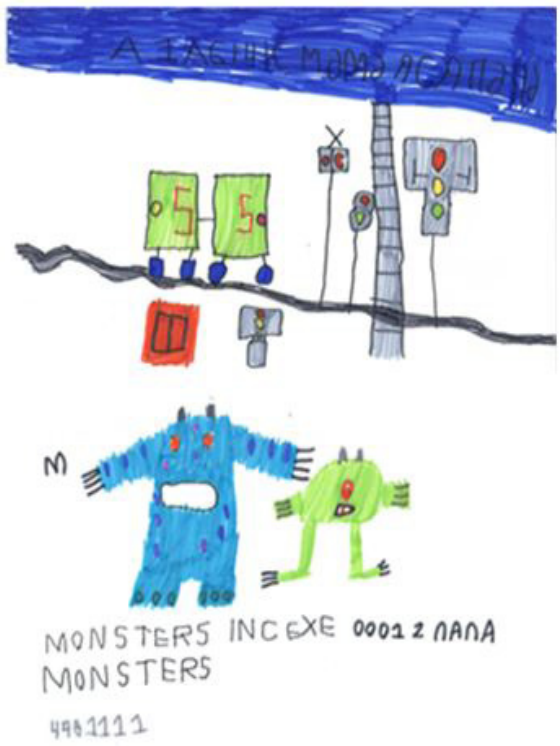

Figure 4: Drawings in 6 to 9 months after UCBC therapy.

Discussion

In recent decades, a great interest has arisen for stem cell application in neurological and psychiatric patients. Multiple studies report individual specific clinical cases demonstrating various outcomes of stem cell therapy, some are a true breakthrough [21-25]. We believe this to be the one. In many patients, stem cell therapy results in significant functional improvement, but therapy non-responders are also observed. This can result from pathogenetic variability of autism types. Some autism patients demonstrate immunological disorders resulting in active proinflammatory cytokine release, blood-brain barrier failure and subacute brain tissue inflammation. Proinflammatory cytokines penetrate blood-brain barrier and activate microglia and astrocytes resulting in pruning and synaptic transmission disorders. Histological analysis demonstrates the shortening of dendrites, mostly in frontal, temporal and motor areas, increase of cortical columns number and decrease of its size, and blurring of grey and white matter border [26,27]. Proinflammatory immune status of such patients results in abnormal immune reactions for multiple provoking factors (e.g. infections, immunization) and formation of autoantibodies for various own tissues. Eventually, this leads to regression of previously developed skills and progression of autistic symptoms [28,29]. In support to this theory, there are reports of anti-inflammatory therapy successful application in patients with regressive

autism [30,31]. Multiple studies demonstrate that stem cells have immunomodulatory effects resulting in suppression of proprietary immune system factors (e.g. dendrite cells, natural killers and complement system) and cytotoxic T-lymphocyte and T-helper activity. Moreover, stem cells translate their functions to other cell types, like regulatory T-lymphocytes (T-regs) resulting in extended therapy efficiency even after infused cells are being lysed [32-34].

Reported patient manifested regression and disease progression at the age of 2 following infection process suggesting the regressive type of autism. Immune disorders prevail in this type of autism. This fact might explain the observed significant improvement and UCBC therapy high efficiency in a pharmacological non-responder juvenile patient.

Conclusion

Umbilical cord blood cell therapy is well tolerated and free of any serious adverse events or immunological reactions. UCBC application in a juvenile patient having regressive type of autism resulted in significant cognitive improvement and regression of major autistic symptoms. The range of the improved functions included perception development, emotional status, socialization, and communicative skills. Moreover, antipsychotics were completely cancelled after cell therapy. This case clinical outcome suggests that UCBC application is effective, at least in patients with certain types of autism. In further studies, biological markers must be determined to differentiate various endophenotypes of autism and to develop pathogenetic treatment alternatives.

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