UMBILICAL CORD BLOOD NUCLEATED CELLS IN THE TREATMENT OF JUVENILE PATIENTS WITH SEVERE TRAUMATIC BRAIN DISEASE: A PILOT CLINICAL STUDY

Janna Semenova[1], Tolibdjon Akhadov[1], Olga Karaseva[1], Gennady Suschkevich [1], Leonid Roschal[1], Yuri Romanov[2], Vladimir Smirnov[2]

CO-INVESTIGATORS: Svetlana Ryaskina[2], Tamara Dugina[2], Sergey Radaev[2], Anna Darevskaya[2], Sergey Proshkin[2]

[1]Clinical and Research Institute of Emergency Children's Surgery and Trauma; [2]Stem Cell Bank "CryoCenter, Ltd.", Moscow, Russian Federation

INTRODUCTION

Although modern capabilities of neuroreanimation and neurosurgery are characterized by evident progress, specific therapy facilitating the restoration of injured brain tissue is still missing. Even when patient with severe brain injury is rescued, new problems arise due to limited number of technologies capable to restore irreversible changes in the brain structure and function. Under these circumstances the ability of some cells to regenerate central nervous system (CNS) tissue can be proposed as potential instrument for regenerative medicine.

Recently, more and more experimental data appear in literature related to the study of therapeutic potential of many cell types after brain or spinal cord injury in animal models. This worth to mention that the differences between cell types, dosages and techniques of cell application are not that important: positive responses are observed in majority of experiments. As far as clinical data are concerned, cell technologies tested in patients with traumatic CNS disease produce only modest results.

Among numerous cell types suitable for regenerative medicine, umbilical cord blood (UCB) cells are of special value. Serious advantages of UCB cells are biological adolescence, ability to differentiate into various cell types, high synthetic and secretory activity, etc. The practice of UCB cryogenic storage is widely spread in many countries which makes these cells readily available for experimental as well as clinical studies.

AIMS OF INVESTIGATION

The main purpose of clinical investigation organized by Stem cell bank "CryoCenter, Ltd." was the estimation of safety and possible clinical efficiency of UCB cell intravenous administration during treatment and rehabilitation of juvenile patients with severe traumatic brain disease.

The study was initiated after corresponding information came available on safety of intravenous cell infusion to adult patients with various CNS disorders (see Poster # P-RM/TE-01) and corresponding animal studies.

Clinical protocol was approved by Scientific board of Clinical and Research Institute of Emergency Children's Surgery and Trauma, Local and Independent Ethics Committees.

STUDY DESIGN AND METHODS

Patients. This study was limited to 6 children patients (four boys and two girls, 8–16 years old, average age 13.5 years) with severe consequences of brain trauma.

Inclusion criteria. Traumatic genesis of disease; 6–24 months since accident; crude neurological deficiency (Grade III according to Glasgow Outcome Scale); low efficiency of traditional therapy and rehabilitation; absence of indications for surgical intervention by the time of cell infusion.

Exclusion criteria. Deterioration of vital functions; polyorganic failure; autoimmune diseases or severe allergic reactions in anamnesis; acute or chronic infectious or inflammatory processes; viral infections (HIV, hepatitis B and C, CMV, HSV); oncological diseases in anamnesis.

Therapeutic cell preparation. Cord blood was obtained after informed consent during full-term normal deliveries from healthy women at the National Center of Obstetrics and Gynecology (Moscow). RBC-depleted/plasma-reduced nucleated cells were aseptically isolated by sedimentation, re-suspended in autologous plasma with 10% DMSO, aliquoted in 4 ml cryovials and cooled to -90°C using controlled-rate freezer. During quarantinization, all samples were tested for HIV-1/2, hepatitis B and C, HTLV-1/2, HSV-1, CMV, syphilis (seropositive samples were discarded), characterized by AB0/Rh, TNC/CD34^{+}-cell count and sterility. Consequently, cells were stored in liquid nitrogen until use.

For therapeutic application thawed cells were washed from DMSO and re-suspended in physiological saline containing human serum albumin at amount of about 250x10^{6} viable cells.

Cell infusion. After informed consent of parents, patients received two AB0/Rh-identical UCB cell infusions with two weeks interval.

Basic therapy. The effects of cell therapy were estimated at the background of standard therapies for such type of patients and included drugs improving brain metabolism, vascular drugs, as well as physical exercises, massage and physiotherapy.

Duration of study. Total length of trial was 9 months and included two catamnestic examinations (2 and 6 months after cell infusion) and subsequent monitoring for 3 months. Clinical examination involved the estimation of clinical manifestations including cognitive functions and visual disturbances. As objective diagnostic tests neurophysiological methods were used (EEG, induced potentials); MRI and MR-spectroscopy were applied to follow the kinetics of injured areas and metabolic processes.

Investigation cessation. All six patients had medical programs fully completed.

RESULTS

No side effects were registered in any patient during the infusion procedures (breath and hemodynamic monitoring, skin conditions, etc.) or subsequent control examinations.

Summarizing results, one can conclude that qualitative transition from one group to another (Glasgow Outcome Scale) was observer only in one patient by the time of study ending the girl clinical characteristics were equal to group II (Moderate disability, "Disabled but independent"). Important to note that it is in this clinical observation neurological deficiency was lowest and associated with negative changes in psycho-emotional status and cognitive disturbances. In other patients group-to-group qualitative transition was not noticed.

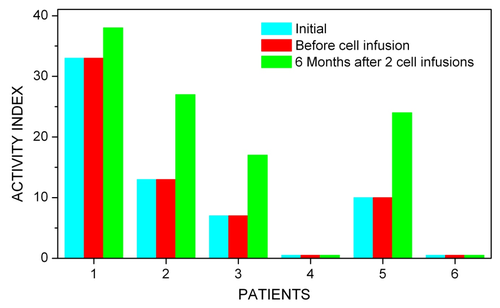

Figure 1. The changes of patients' physical activity after UCB cell infusion.

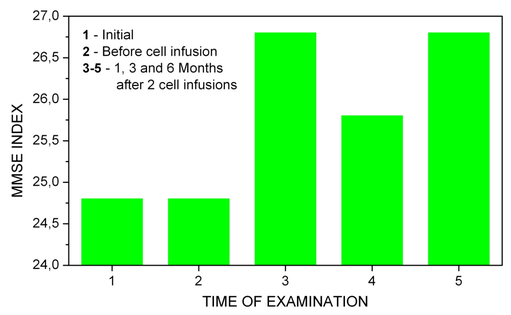

Figure 2. The dynamics of neural function restoration according to MMSE scale after UCB cell infusion.

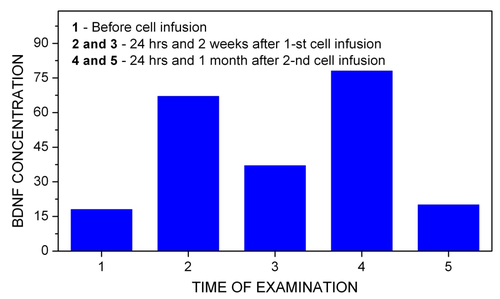

Figure 3. Changes of BDNF serum concentration in response to UCB cell infusions.

Nevertheless, one has to emphasize certain positive tendencies. The dynamic estimation of the treatment results demonstrated evident positive changes of motor and cognitive functions in the majority of patients during three months after cell administration (*Figures 1 and 2*).

Although all patients by the time of inclusion to the study corresponded to group III (Severe disability, "Conscious but disabled"), the manifestation of neurological deficiency was variable. As a result of treatment, 4 of 6 patients demonstrated stable positive dynamics in the restoration of motor functions as well as restitution of cognitive functions out of which the most pronounced changes were observed for the parameter "neurodynamics of mental processes".

In two patients having severe neurological deficiency reliable changes in the restoration of motor and cognitive functions were not detected. However, even in these cases one can see the tendency to positive dynamics, namely, elevation of patients' abilities, positive changes in visual and hearing functions. Thus, in 5 of 6 patients (83 %) positive effects on visual functions (the enlargement of field of view and visual acuity) was observed one month after cell infusion.

Summarizing data of EEG-monitoring one should emphasize following positive moments: appearance of rhythmic activity in caudal areas in 4 children which did not have these rhythms earlier. In 50 % of patients interhemispheral asymmetry appeared which is considered as the sign of decrease in diffuse changes, larger activation of one hemisphere related to the character and side of brain injury.

Dynamic analysis of brain evoked potentials demonstrated the absence of the deterioration in the state of sensory paths. In the half of patients these parameters normalized; the improvement of cortex component of acoustic response was also observed in 50 % of patients.

MRI analysis did not show any additional pathological changes in the brain tissue. According to MR-spectroscopy data no significant dynamics in metabolic status was observed after cell infusions. Posttraumatic changes of the brain tissue were also absent which indicates the absence of positive dynamics in cell replacement.

As indirect indicator of mechanisms which may be triggered by UCB cell infusion, serum levels of BDNF (Brain-Derived Neurotrophic Factor) were assayed (*Figure 3*). Thus, clinical improvement observed can be explained to a significant degree by trophic influence of infused UCB cells on injured brain.

CONCLUSIONS

- **Intravenous infusion of allogenic, AB0/Rh-identical, RBC-depleted UCB nucleated cells to patients of young age at late stages of severe traumatic brain disease is technically easy and safe. No side effects were registered during the whole period of observation.**
- **Cord blood cells do not cause any changes in metabolic status and posttraumatic changes of the injured brain according to MRI examination.**
- **Results obtained suggest that UCB cell infusion has neuroprotective effect and exerts trophic support on brain tissues resulting in positive clinical dynamics.**

CRYOCENTER
Stem Cell Bank