Effect of Cell Therapy on Metabolite Content in Brain Structures of Children with Consequences of Severe Brain Injury: 1H Magnetic Resonance Spectroscopy Study

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The effect of intravenous injection of human umbilical cord blood cells on the levels of N-acetylaspartate, creatine and phosphocreatine, choline-containing compounds, glutamine and glutamate, and myoinositol in morphologically intact areas of the cortex, white matter, and hippocampus of children with consequences of traumatic brain injury was studied by single voxel 1H magnetic resonance spectroscopy. It was shown that cell therapy increases the content of N-acetylaspartate, a marker of functional integrity of neurons, in the white matter and in the cortex at the boundary between the frontotemporal and parietal lobes and reduces neurological deficit

Key Words: *traumatic brain injury; stem cells; magnetic resonance spectroscopy*

Study of the effects of cell therapy in traumatic brain injury (TBI) was started not far ago. Most data were obtained on experimental animals and the results are primarily positive. For instance, injection of human embryonic neuronal stem cells [4], umbilical cord blood cells [5], and stromal BM cells [6] improved cognitive functions [4], reduced locomotor activity and neurological deficit [5,6] in rats with experimental TBI.

Transplanted cells were primarily accumulated in the brain tissue and express markers of neurons and astrocytes [5,6] and neurotrophic factors [4]. SC get into the injured brain through impaired blood-brain barrier (BBB) and release trophic factors BDNF, NGF, EGF, and insulin-like growth factor [2,9]. Improvement of CNS functions in rats with experimental stroke receiving intravenous injections of umbilical cord blood cells correlated with accumulation of trophic factors in the brain, though no injected cells were found in the brain [1]. These findings attest to the role of trophic factors in the positive effect of cell therapy on CNS functions. Permeability of BBB for neurotrophic factors was demonstrated [8], hence, the effect of cell injection can be also expected under conditions of recovered and undamaged BBB.

The existing hypotheses on the mechanisms of the effect of neurotrophic factors released by SC on the brain (regeneration of damaged neurons under conditions of changed microenvironment [12], replacement of damaged neurons with newly formed cells due to activation of neurogenesis [10]) consider the effect of cell therapy on damaged tissue. At the same time, formation of new neuronal bonds (brain plasticity) is an important compensatory mechanism of neurological disturbances. Neurotrophic factors can affect the formation of new bonds in morphologically intact brain structures, which can increase the number of functionally active neurons and improve the function of CNS.

The method of magnetic resonance spectroscopy (MRS) is a unique possibility of intravital and noninvasive measurement of the concentrations of a number of key metabolites in brain structures. In 1H magnetic

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resonance spectra (1HMR) of the brain, signals of N-acetylaspartate (NAA) are detected; this compound in measurable amounts was found only in neurons. The intensity of NAA signal is proportional to the content of functionally intact neurons in the examined brain areas [7].

The aim of the present study was to detect possible effect of cell therapy on the content of functionally active neurons in morphologically intact brain structures in children with TBI consequences by the method of MRS.

MATERIALS AND METHODS

We examined 6 children (3 boys and 3 girls, age 8-17 years, mean age 12.7 years), patients of Research Institute of Urgent Children Surgery and Traumatology; the patients had severe neurological deficit (group 3 according to Glasgow outcome scale) caused by severe TBI 6-23 months before examination. The patients received two intravenous drop infusions of a suspension of nucleated cells from group- and rhesus-matched umbilical cord/placental blood with a 14-day interval ($5\text{-}10\times10^6$ cells per 1 kg body weight for each infusion). The cell suspension was prepared from cryopreserved concentrate of human umbilical cord/placental blood SC (mononuclear fraction). The concentrate was prepared according to appropriately registered medical technology. The main characteristics of SC concentrate of umbilical cord/placental blood are presented in Table 1. The concentrate was stored at -196°C. The concentrate was aseptically defrosted before use, washed from the cryoprotector with sterile physiological or plasma-substituting solution, and cell viability was evaluated by trypan blue exclusion test. The time from defrosting to infusion did not exceed 4 h.

The levels of proton-containing metabolites in brain structures was measured 45 and 15 days before and 15, 45, and 150 days after transplantation by single-voxel MRS. A 3-Tesla medical tomograph (Phillips Achieva 3.0 T) equipped with ViewForum software was used. Free induction decay signal was detected on a standard head coil of the tomograph. During MRS, the following images were obtained: axial T2 weighed and T2-FLAIR, saggital T2-FLAIR, coronary T1-FLAIR, and native angiographic images in three projections to exclude the presence of vessels in the studied volume during MRS study. The volume of interest (3 cm^3) was positioned with a pulse sequence PRESS with echo time TE=35 msec and time between pulse sequences TR=2000 msec. Water signal was suppressed using a presaturation pulse. Spectroscopic voxel was successively aimed at non-damaged areas (according to MRS data): white matter and cortex at the boundary between the frontotemporal and parietal lobes, white matter of the frontal lobe, cortex of the temporal lobe, and hippocampus. For obtaining optimal signal/noise ratio at minimum time of recording the number of signal accumulations was 32.

Free induction decay signal was processed using SpectroView software. Signals of metabolites were approximated by Gaussian lines; their amplitudes were calculated and standardized to the resonance amplitude of non-suppressed water. Water content in unchanged tissue (MRS data) is constant and used as an internal concentration standard [3].

The spectral data were processed statistically using Statistica 6.0 software. Significance of differences

TABLE 1. Parameters of SC Concentrate from Umbilical Cord/Placental Blood

Parameter	Mean±SD
Number of samples included in statistical analysis	500
Sample volume without anticoagulant, ml	69.8±28.0
Total leukocyte count before isolation, $\times10^9$	1.05±0.61
Yield of nucleated cells after isolation, % of initial	50.5±12.2
Erythrocyte depletion level, % (by hemoglobin content)	97.82±0.19
Cell viability after isolation, %	99.9
Ratio of cell population in SC concentrate (flow cytofluorometry data, %)	
lymphocytes	51.3±10.9
monocytes	13.8±3.8
granulocytes	25.1±11.0
Hemopoietic precursors ($CD34^+/CD45^+$, ISHAGE protocol), %	0.46±0.26
Cell viability after test freezing-defrosting, %	90.8±10.1

between the means were determined using Student *t* test at $p \leq 0.05$ significance level.

RESULTS

Figure 1 shows typical 1HMR spectra of intact areas according to diagnostic MRS: frontal lobe, white matter and cortex at the boundary between the frontotemporal and parietal lobes, and hippocampus recorded 45 days before cell transplantation.

Apart from NNA, the spectra included signals of lipids not observed in normal spectra, integral signal of phosphocreatine (Cr) involved in energy metabolism, signal from intermediates of lipid metabolism, choline-containing compounds (Cho), resonance of astrocyte marker myoinositol (mI), and superposition of resonances of glutamatergic system components glutamine and glutamate (Glx) [11].

The results of statistical analysis of spectra recorded before cell transplantation and in dynamics after it are presented in Fig. 2. Two main conclusions can be made from these data: 1) metabolic status of all examined brain structures was stable and the content of each metabolite remained constant before cell transplantation; 2) SC modulate the level of NAA in the cortex and white matter at the boundary between the frontotemporal and parietal lobes, while in the frontal lobe and hippocampus it remained unchanged. Temporal dependence of NAA content in the cortex and white matter at the boundary between the frontotemporal and parietal lobes during recovery (Fig. 2) suggests that this parameter significantly increases in the cortex after 45 days and remains above the initial level 150 days after cell transplantation. The content of NAA in the white matter also significantly increased. The increase in NAA content in the cortex and white matter was 22 and 31%, respectively. No dynamics of the content of other metabolites was observed.

The increase in NAA content after transplantation of umbilical cord blood cells suggests that cell therapy increases the level of functionally active neurons in morphologically intact cortex and white matter at

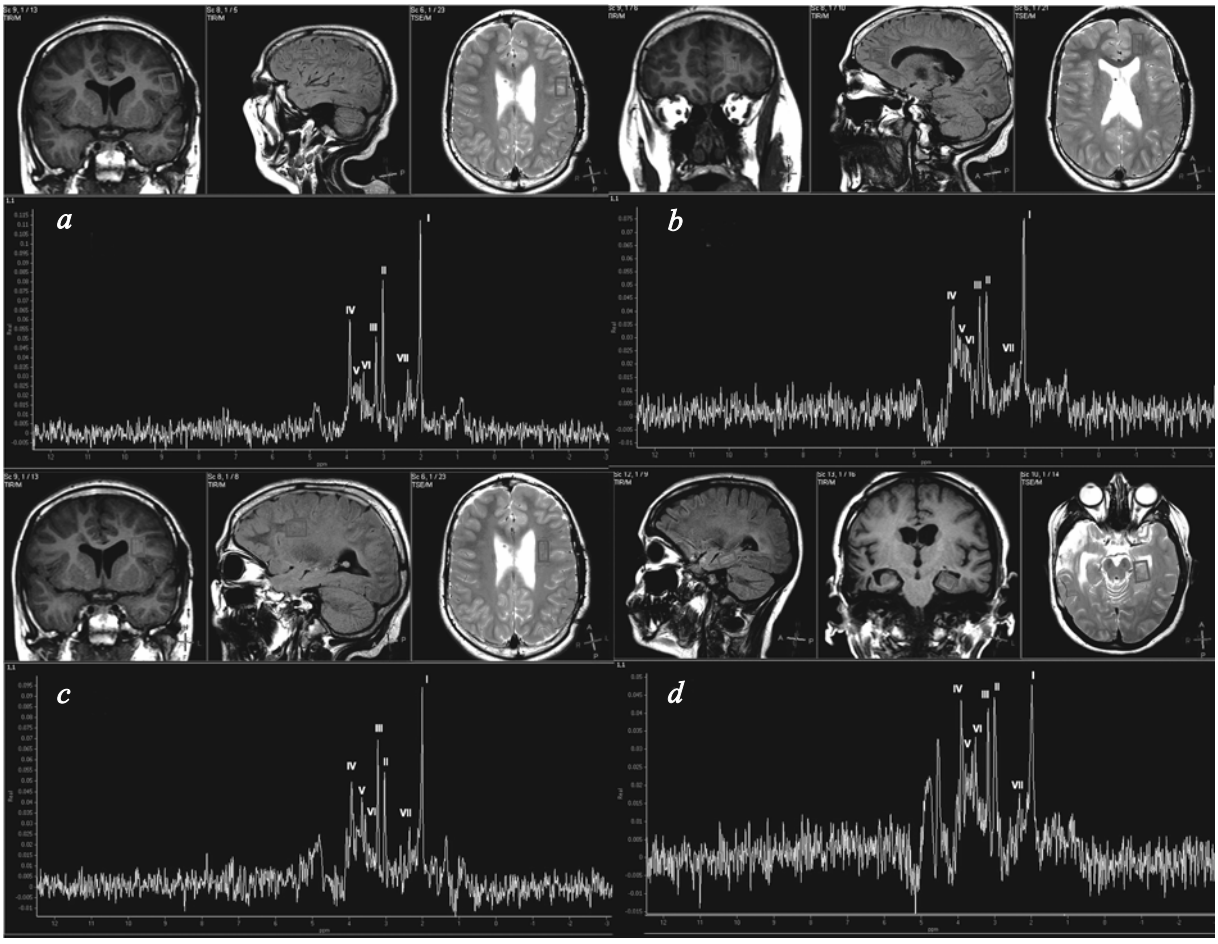

Fig. 1. 1HMR spectra of morphologically normal brain areas (MRI data) in children with chronic TBI. *a*) cortex at the boundary between the frontotemporal and parietal lobes, *b*) frontal lobe (cortex); *c*) white matter at the boundary between the frontotemporal and parietal lobes; *d*) hippocampus. Main metabolites: I: NAA (N-acetylaspartate); V and VII: Gl*1 and Gl*2 (signals of glutamate/glutamine); II and IV: Cr1 and Cr2 (signals of creatine+phosphocreatine); III: Cho (integral signal of choline-containing compounds); VI; mI (signal of myoinositol).

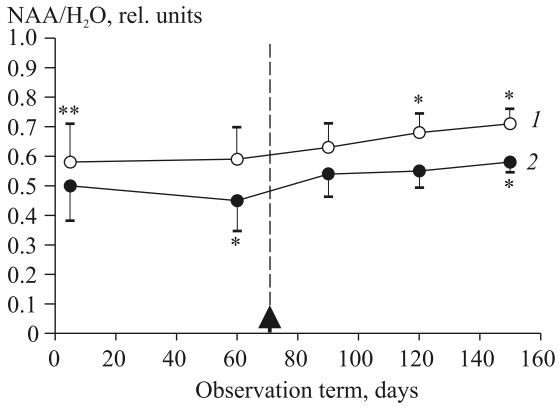

Fig. 2. Mean changes in NAA level in the cortex (*1*) and white matter (*2*) at the boundary between the frontotemporal and parietal lobes in children with severe chronic TBI at the beginning (day 0) of the study and in dynamics before and after transplantation of umbilical cord/placental blood cells. **p*=0.01, ***p*≤0.05 compared to the level before cell transplantation. Here and in Fig. 3: arrow and vertical line marks the moment of SC injection.

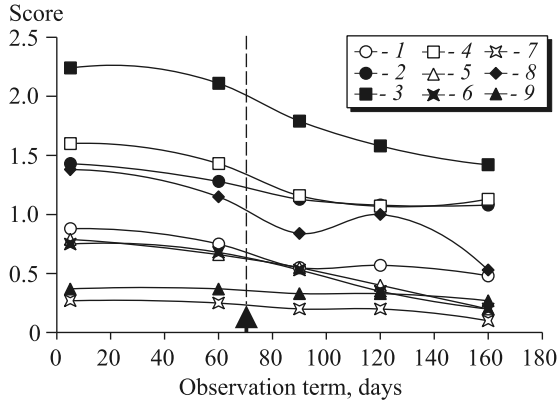

Fig. 3. Severity (mean score) of psychopathological disturbances (3 corresponds to maximum disturbances) in children with severe TBI at the beginning (day 0) of the study and in dynamics before and after transplantation of umbilical cord/placental blood cells. *1*) contact and emotional sphere; *2*) programming and control; *3*) neurodynamics of mental activity; *4*) memory; *5*) speech; *6*) praxis; *7*) gnosis; *8*) visual and spatial functions; *9*) thinking.

the boundary between the frontotemporal and parietal lobes. Since we studied a chronic state with BBB impermeable for SC, the observed increase in the number of functionally active neurons can be attributed to the action of neurotrophic factors released by cells and crossing BBB.

The mechanism underlying the increase in the content of functionally active neurons cannot be determined from results obtained in this study. The observed effect probably reflects brain plasticity, because in the effect of neurogenesis in the analyzed morphologically intact structures is minimum.

It can be expected that the increase in the count of functionally active neurons improves the functions of CNS. Indeed, a positive dynamics in the recovery of cognitive functions was observed in patients receiving transplantation of umbilical cord blood cells (Fig. 3 shows averaged characteristics of neuropsychological status for the group of patients). However, the qualitative leap in the improvement of the functional status of CNS, transition from group 3 to group 2 according to Glasgow outcome scale, was noted in only one patient.

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