Cell Therapy of Comatose States

V. I. Seledtsov, S. S. Rabinovich*, O. V. Parlyuk, O. V. Poveshchenko, S. V. Astrakov**, D. M. Samarin, G. V. Seledtsova, V. V. Senyukov, V. Ya. Taraban, and V. A. Kozlov**

Translated from *Kletochnye Tekhnologii v Biologii i Medicine*, No. 3, pp. 146-149, August, 2006
Original article submitted March 6, 2006

We demonstrated that liquor from adult humans can maintain proliferative activity of cells of immature nervous tissue *in vitro*. The paper presents the results of a retrospective clinical study of the efficiency of cell therapy in the treatment of II-III degree comatose patients with severe brain injury. Cell suspension consisting of cells derived from immature nervous and hemopoietic tissues was injected into the recipient subarachnoidal space through a cerebrospinal puncture. The mortality in the study group was 8% *vs.* 56% in the control group. The 1.5-year follow-up demonstrated significantly better quality of life in patients receiving cell therapy in comparison with patients of the control group. Cell therapy proved to be ineffective for patients in a comatose state caused by hypoxic encephalopathy. The study demonstrated the efficiency of cell therapy in patients with severe brain injury during the acute period of the disease.

Key Words: *cell therapy; comatose state; brain injury*

Severe brain injury (BI) is associated with high mortality reaching 70-85%. The survivors often remain disabled [1]. Coma is a critical state associated with brain injury, when a series of secondary life-threatening pathogenetic processes are triggered [3]. It is obvious that the treatment of BI during the acute period is of priority importance for the disease outcome. Unfortunately, combined drug therapy of patients in a comatose state, including water-salt balance regulators, neuroprotectors, hormones, vitamins, and drugs improving cerebral blood supply, cannot crucially improve the consequences of BI [3,7]. On the other hand, experimental data indicate the possibility of effective use of cell technologies in the treatment of patients with severe neurological disorders. It was shown, for example, that allogenic donor cells transplanted into the CNS survive and function in accordance with their own developmental program [4,5]. These cells can regulate activities of various compartments of the brain by producing soluble factors [4,6]. Donor cells derived from immature brain tissue can participate in the formation of new neural contacts and promote the recovery of nerve communications, impaired as a result of pathological processes [4,5]. We showed in this study that liquor from adult humans can maintain proliferative activity of cells of the immature nervous tissue and compared the results of cell therapy in 25 patients with BI in a state of II-III degree coma.

MATERIALS AND METHODS

Clinical studies were carried out in accordance with the protocol approved by the Academic Council and Ethic Committee of Institute of Clinical Immunology. Close relatives of the patients gave informed consent to their participation in the study.

The tissues were collected from human fetuses (16-22-week gestation) after spontaneous or prostaglandin-induced abortions. Cell suspension was

Institute of Clinical Immunology, Siberian Division of Russian Academy of Medical Sciences; *Novosibirsk State Medical Academy; **Municipal Clinical Hospital No. 34, Novosibirsk. ***Address for correspondence:*** vs@online.nsk.su. V. I. Seledtsov

0007-4888/06/1421-0129

prepared as described previously [8]. The cells were cryopreserved by the standard method in FCS with 10% dimethylsulfoxide and stored in liquid nitrogen vapor. Cell suspension was defrosted on the day of transplantation at 37°C. Cell viability was evaluated by the standard method using trypan blue staining.

The effect of the liquor on cell proliferation was evaluated on samples of nervous tissue cultured in a 96-well round-bottom plates (BDSL) in a concentration of 2×10^5 cell/well for 48 h at 37°C and 5% CO_2 in RPMI 1640 with 10 mM Hepes, 4 mM L-glutamine, 5×10^{-5} M mercaptoethanol, antibiotics (all reagents from Sigma), and the liquor. The intensity of proliferation was evaluated by incorporation of ^{3}H-thymidine added (0.75 μCi) into each well 6 h before the end of culturing.

Cell suspension for one transplantation included 2×10^8 viable cells (nervous:hemopoietic hepatic tissue cell ratio was 10:1). Cell suspension was injected into the recipient subarachnoidal space through a cerebrospinal puncture.

The main group consisted of 25 patients (8 women and 17 men) aged 18-63 years, hospitalized with severe BI in coma of II-III degree. Their status was evaluated by 3-5 points according to the Glasgow coma scale. Diffuse axonal injury (DAI) of the brain was diagnosed in 15 (60%) cases, in 11 cases it was combined with compression of the brain with hematoma. Ten patients (40%) presented with severe contusion of the brain, combined in 9 cases with compression of the brain, which was urgently eliminated. Intensive care measures stabilized cardiovascular activity and respiration, but consciousness was not restored during 5-8 weeks. The probability of the development of a long-term vegetative status was high for all patients. Significant objective changes detected by magnetic resonance imaging, EEG, and transcranial Doppler ultrasonography, together with clinical manifestations of brain dysfunction and obvious failure of traditional therapy, were indications for cell therapy. Magnetic imaging showed diffuse atrophic changes in the white and gray matter of the brain in the majority of cases. Atrophy of frontal lobes in DAI was diagnosed 2-3 weeks after admission to the hospital. EEG showed decreased functional activity of the brain with disappearance of α-rhythm; transcranial Doppler ultrasonography indicated decreased linear velocity of the cerebral bloodflow.

Control group was formed to match the main group: a control patient with similar clinical characteristics was restrospectively selected at random for comparison with each patient of the main group.

In addition, cell therapy was carried out in 5 female patients (35-55 years) in a state of II degree coma, which developed as a result of acute cerebral hypoxia associated with syncope conditions.

The results of treatment including cell therapy were evaluated by Glasgow outcome scale and Karnovsky scale of functional activity. The data were processed statistically using Mann—Whitney nonparametric test.

RESULTS

The liquor can produce a negative impact on viability of transplanted allogenic cells [2], and therefore we studied the effect of liquor from BI patients on viability and proliferation of cells of immature nervous tissue. The presence of the liquor had virtually no effect on cell viability evaluated by trypan blue staining. In addition, the liquor maintained (in a dose-dependent manner) proliferative activity of cells evaluated *in vitro* in culture (Fig. 1). We found that nerve cells cultured for a long time (1 month and longer) generated axonal growth and formed communicative relationships with each other. Presumably, the liquor microenvironmental factors promoted the growth and realization of the functional potential of immature donor cells. On the whole, these data are in line with the results of experimental studies indicating the capacity of stem cells to survive and produce functionally active descendants under conditions of allogenic cerebral microenvironment [4,5].

Good and satisfactory results of transplantation were recorded in 20 (80%) cases. The awakening syndrome developed on days 3-5 after cell transplantation: the patients opened their eyes and followed some instructions. After 7-12 days they started communicating with the relatives and staff. Recovery of the main mental functions was observed by days 15-20 after transplantation. By this period

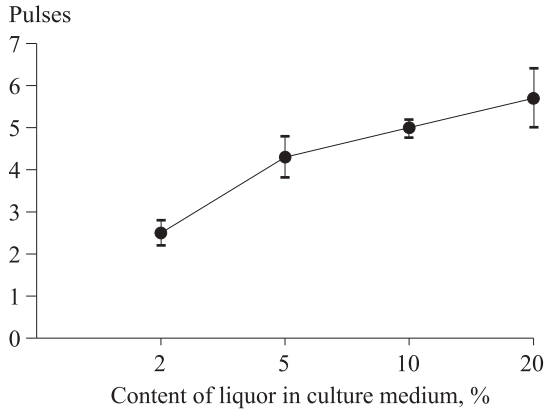

Fig. 1. Effect of liquor on proliferative activity of cultured immature nervous tissue cells *in vitro*. Ordinate: ^{3}H-thymidine incorporation into cells ($\times10^3$).

TABLE 1. Brainstem Symptoms in Patients before and 12-15 Days after Transplantation (*n*=25)

Symptom		Before therapy		After therapy	
		abs.	%	abs.	%
Disorders in respiratory rate and rhythm		19	76	0	0
Absence of pharyngeal reflex		25	100	0	0
Extrapyramidal tetrasyndrome		23	92	2	8
Reduction or absence of corneal reflexes		25	100	0	0
Reduction or absence of pupil reaction to light		19	76	2	8
Looking up paresis		23	92	3	12
Oculocephalic reflex	none	5	20	0	0
	incomplete	16	64	2	8

TABLE 2. Disease Outcomes in Patients with BI

Outcome	Control group (*n*=25)		Main group (*n*=25)	
	abs.	%	abs.	%
Lethal	14	56	2	8
Unsatisfactory	6	24	3	12
Satisfactory	5	20	9	36
Good	0	0	11	44

α-rhythm appeared and the cerebral bloodflow reached the lower threshold normal level. Positive changes in the stem symptoms corresponded to these shifts (Table 1).

Magnetic resonance tomography showed no appreciable changes in the brain matter during the early posttraumatic period. However, after 1-1.5 years atrophic changes virtually completely regressed in all patients with good and satisfactory results.

In 3 (12%) patients the awakening syndrome was also recorded on days 3-5 after transplantation, but it was not followed by complete recovery of mental activity. Pronounced mnestic disorders persisted and the patients were in need of care. Cell transplantation was repeated and resulted in a significant improvement of mental status.

The positive effect of treatment (awakening syndrome) was noted in 2 more (8%) patients, but they died from extracranial complications.

Control group consisted of 25 patients aged 19-60 years (the severity of BI and prognosis of the disease course were comparable to those in the main group). Patients of both groups received standard treatment during about the same period.

Cell transplantation sharply reduced mortality from BI and increased significantly the number of patients with good or satisfactory outcome of the disease (Table 2). The awakening effect observed during the first week seemed to be due to distant effect of donor cells on various brain compartments, rather that their direct involvement in the formation of new neural communications. Complex, balanced, lasting, and incessant action is an important constituent of the therapeutic effect of transplanted cells. Consciousness disorders in BI are caused by the cortex-stem dysregulation. We hypothesized that transmitters produced by transplanted cells stimulated primarily the brain stem compartments. Stimulated stem activity, in turn, caused the awakening syndrome.

Transplantation therapy promoted functional rehabilitation of patients. The quality of life according to Karnovsky scale evaluated 1.5 years after treatment significantly increased (80 *vs.* 55% in the control). The patients were observed for 4-6 years. One year after injury the mental status was completely restored in 20 patients. Psychologically they consider themselves completely rehabilitated. Eleven patients resumed working at different periods after injury, seven changed their occupation for reasons not associated with the trauma, and two elderly housewives performed their daily routine as they did previously. Three patients remained disabled (disability groups II-III). It seems that further transplantation treatment can be indicated for them within the framework of rehabilitation measures.

No serious complications of cell therapy were detected during the entire period of observation.

Five patients (females) with comatose state resultant from acute cerebral hypoxia also received cell transplantation. Intensive care measures led to recovery of spontaneous respiration and cardiovascular activity at the optimal level, but consciousness was not restored. Each of these patients received 3-5 transplantations. However, no expected awakening effect ensued, which we attributed to total hypoxic injury of the cerebral cortex (absence of target for transplanted cells effect).

On the whole, the results indicate that cell technologies can be effectively used for the treatment of patients with BI during the acute period of disease. However, it is still early to make conclusions about the efficiency of these technologies in combined rehabilitation of patients with BI; wide-scale studies according to a universal program should be carried out at several specialized centers.

The study was partially financed by the Social Foundation for Russian Medicine Support.

REFERENCES

1. I. V. Bratishchev, *Vestn. Intens. Ter.*, No. 4, 17-21 (2003).
2. A. S. Bryukhovetskii, *Nerve Cell Transplantation and Tissue Engineering of the Brain in Nervous Diseases* [in Russian], Moscow (2003).
3. G. G. Zhdanov and A. V. Kuligin, *Vestn. Intens. Ter.*, No. 3, 40-44 (2003).
4. V. V. Semchenko, S. I. Erineev, S. S. Stepanov, and G. G. Sergienko, *Transplantation of Immature Nervous Tissue in Experimental and Clinical Neurology* [in Russian], Omsk (2000).
5. G. T. Sukhikh, *Byull. Eksp. Biol. Med.*, **126**, Suppl. 1, 3-13 (1998).
6. J. W. Favcett, *Spinal Cord*, **36**, 811-817 (1998).
7. A. I. R. Maas, M. Dearden, and F. Servadei, *Curr. Opin. Crit. Care*, **6**, 281-292 (2000).
8. V. I. Seledtsov, I. V. Avdeev, A. V. Morenkov, *Immunobiology*, **192**, 205-217 (1995).