Effect of Allo- and Xenotransplantation of Embryonic Nervous Tissue and Umbilical Cord Blood-Derived Stem Cells on Structural and Functional State of Cerebral Cortex of Albino Rats in Posttraumatic Period

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Comparative study of the structural and functional state of cerebral cortex of adult albino rats after intracerebral allo- and xenotransplantation of embryonic nervous tissue and intravenous injection of umbilical cord blood-derived stem cells at different terms after diffuse-focal cerebral trauma revealed the best cerebroprotective effect on day 7 of posttraumatic period in animals receiving embryonic nervous tissue.

Key Words: *brain injury; cerebral cortex; cell technologies; embryonic nervous tissue; stem cells*

Antenatal brain injury, birth trauma, gunshot and surgical brain injuries (BI), and brain ischemia and hypoxia lead to brain atrophy, enhancement of paroxysmal activity associated with activation of immune reactions against brain antigens, LPO stimulation, and impairment of associative and integrative function of the brain, which aggravate the course of posttraumatic period [10].

Intracerebral allo- and xenotransplantation of fetal nervous tissue after focal microinjuries to the brain in experimental animals reduces reactive gliosis, preserves the numerical density of neurons, increases the number of functioning capillaries, activates neosynaptogenesis, prevents synaptic hypertrophy and hyperactivity [5] and enhancement of convulsive activity [4,7], inhibits LPO [2], and normalizes cognitive function of the brain [3].

Here we compared structural and functional state of the brain in adult albino rats after intracerebral allotransplantation of embryonic nervous tissue and intravenous injection of stem cells from human umbilical cord blood at different terms after diffuse-focal brain injury.

MATERIALS AND METHODS

Experiments were carried out on 130 mature Wistar rats weighing 180-200 g. All surgical manipulation were performed under chloral hydrate narcosis (325 mg/kg intraperitoneally, 3% solution). The animals were sacrificed under ether rauch-narcosis. The animals were divided into 10 groups: group 1 consisted of intact rats, group 2 comprised rats with BI without transplantation; groups 3 and 4 were sham-operated rats on days 2 and 7, groups 5 and 6 comprised rats with bilateral intrahippocampal

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allotransplantation of neocortex tissue on days 2 and 7 of the posttraumatic period, group 7 and 8 included rats with bilateral intrahippocampal xenotransplantation of the anterior cerebral vesicle wall on days 2 and 7; groups 9 and 10 comprised rats with single intravenous injection of umbilical blood stem cells on days 2 and 7 of the posttraumatic period.

Dosed severe BI was modeled under ether narcosis using a special device [11]; a load fell along the parietal midline with individual (calculated for each animal) force impulse. Animal mortality, clinical course of the posttraumatic period, the state of higher nervous activity, and morphological picture of the cortex served as criteria of BI severity.

For bilateral stereotactic heterotopic intrahippocampal allo- and xenotransoplantation on days 2 and 7 after severe closed diffuse-focal BI we used fragment of the sensorimotor cortex from 15-17-day-old Wistar rat embryos and fragments of the anterior cerebral vesicle wall from 7-12-week-old human embryos. Transplantation was carried under sterile conditions using stereotactic atlas of rat brain [13] into CA1 and CA3 hippocampal fields. The time from embryo isolation to transplantation was 15-20 min, the time before xenotransplantation was 1 h. The accuracy of stereotactic manipulations and transplant take were verified morphologically. The increase in the volume of transplanted tissue, vascularization of the transplant, and the presence of neurons without morphological abnormalities on paraffin and celloidine 6-7-μ sections stained with thionine after Nissl and hematoxylin and eosin served as criteria of graft take.

Mononuclear fraction containing stem cells was isolated by centrifugation of umbilical blood in a one-step density gradient [12]. Cell suspension (1 ml) containing 10^6 cells was injected into the caudal vein on day 2 and 7 days after trauma.

The cognitive function of the brain (learning capacity, long-term memory) and behavioral reaction were studied using conditioned passive avoidant (PA) paradigm according to modified method proposed by J. Bures *et al.* [3]. The animals were trained PA reaction 6 days after trauma and transplantation. The initial time of stay in illuminated compartment was measured over 3 min for 4 days before PA conditioning. PA was successfully conditioned if the animal not less than 80% time (>144 of 180 sec testing time) spent in the safe compartment. If memory traces were absent within the first 3 days after PA conditioning, training session was repeated. The number of repeated sessions was determined. AP performance as well as the start and dynamics of its quenching were assessed daily until day 15, every 5th day until day 30, and every 15th day until day 60. AP quenching was complete, if the animal spent no more than 20% time (36 sec) in the safe compartment. Horizontal (number of visits to the dark compartment, burrow) exploratory and vertical (rearings) orientation activities, number and duration of grooming acts, freezing for the entire testing time

TABLE 1. Long-Term Memory and Learning Capacity of Adult Wistar Rats in the Posttraumatic Period after Intracerebral Allo- and Xenotransplantation of Embryonic Nervous Tissue and Intravenous Injection of Human Umbilical Blood Cells

Group	Description	Number of animals	Number of animals with repeated PA conditioning	Number of animals with preserved PA reaction					
				15 days	20 days	25 days	30 days	45 days	60 days
1	Intact	10	0	6	6	6	4	7	6
2	BI	10	5	6	5	4	2	2	2
3	BI+sham-operation on day 2	10	6	5	6	5	3	2	2
4	BI+sham-operation on day 7	10	4	6	5	6	4	3	2
5	BI+AENT on day 2	10	2	5	4	4	3	3	3
6	BI+AENT on day 7	10	2	7	6	6	6	5	5
7	BI+XENT on day 2	10	3	5	4	4	3	3	2
8	BI+XENT on day 7	10	3	6	6	5	5	4	4
9	BI+XBSC on day 2	10	3	5	5	5	4	4	3
10	BI+XBSC on day 7	10	3	5	5	5	4	4	4

Note. Here and in Tables 2-4: AENT: allotransplantation of embryonic nervous tissue; XENT: xenotransplantation of embryonic nervous tissue; XBSC: xenotransplantation of blood stem cells.

(inhibition), and the number of urinations and boluses were evaluated.

For morphological examination, the brain was perfused through the ascending part of the aorta arch (15-20 min, 90 mm Hg perfusion pressure) with a solution containing 4 g paraformaldehyde, 1 g glutaraldehyde, and 5 g sucrose per 100 ml phosphate buffer (pH 7.4). After perfusion the brain was removed and processed for light microscopy [8,9].

The left hemisphere was embedded into paraffin. Frontal sections were stained with 0.1% toluidine blue after Nissl and with hematoxylin and eosin. The total numerical density of neurons and the content of neurons with reactive changes in layers III and V of the sensorimotor cortex were evaluated by light microscopy [8].

Intact and sham-operated animals served as the control.

The data were processed statistically using Statistica 5.0 and Excel software and presented as $M \pm m$. The critical level of significance was 5% [1,6].

RESULTS

Animal mortality within the first hour after BI and at later terms of the posttraumatic period (infectious and inflammatory complications) was 40 and 60%, respectively.

After BI (group 1) and sham-operation (without transplantation, groups 2 and 3) PA was preserved in 20% animals (Table 1). In 50% animals PA was conditioned after repeated training. Neurotransplantation increased the background time of stay in the illuminated compartment, "inhibition" was observed in 60% rats, in 80% animals PA was conditioned after the first training session. Orientation and exploratory activity and the level of anxiety decreased, while defensive-phobic behavior was activated.

Injection of umbilical blood containing stem cells less markedly affected cognitive function and behavioral reactions, than neurotransplantation. The normalizing effect of intracerebral allotransplantation of embryonic nervous tissue was more pronounced. Day 7 of the posttraumatic period is the optimal term for neurotransplantation and injection of umbilical blood stem cells. Intracerebral allo- and xenotransplantation of embryonic nervous tissue produces qualitatively similar effects; the differences were quantitative.

Diffuse-focal BI reduced the total numerical density of neurons in layers III and V of the sensorimotor cortex, increased the content of hyperchromic and pyknomorphic (shrunken hyperchromic) neurons, neurons with subtotal chromatolysis, and ghost cells (Tables 2-4). Cell transplantations partially protected neuronal populations, the most pronounced effect was observed after allotransplantation of embryonic nervous tissue, while intravenous injection of stem cells produced minimum effect. Transplantation on day 7 of the posttraumatic period was most effective (Table 2). Allotransplantation of the embryonic nervous tissue decreased the percentage of reversibly and irreversibly changed neurons. The neuroprotective effect of the transplant was higher after grafting on day 7 of the posttraumatic period (compared to transplantation on day 2, Tables 2 and 4).

The compensatory effect of neurotransplantation is determined by neurotrophic and neurotransmitter factors produced by the transplant [8,9]. Suspension of umbilical blood mononuclears produced a stimulatory effect on hemopoiesis and reduces the severity of polyorgan failure in the posttraumatic period.

Thus, cell transplantation produces a positive effect on structural and functional state of the sensorimotor cortex in the posttraumatic period. The effect of allo- and xenotransplantation of embryonic nervous tissue and xenotransplantation of umbilical blood stem cells produced qualitatively similar effects. The best preservation of cortical structure was noted after allotransplantation of embryonic nervous tissue on day 7 of the posttraumatic period.

TABLE 2. Total Numerical Density of Neurons (per 0.001 m^3) in Sensorimotor Cortex Layers III and V of Albino Rats after Allo- and Xenotransplantation of Embryonic Nervous Tissue and Xenotransplantation of Umbilical Blood Stem Cells on Days 2 and 7 of the Posttraumatic Period

Layer of sensorimotor cortex		Group				
		intact	BI	BI+AENT	BI+XENT	BI+XBSC
Treatment on day 2	layer III	68.0±4.3	40.1±4.2*	42.4±5.6*	39.6±4.7*	40.8±4.8*
	layer IV	41.9±4.3	27.2±2.6*	30.1±3.9*	29.6±3.2*	28.4±3.3*
Treatment on day 7	layer III	68.0±4.3	40.1±4.2*	59.1±5.6^{+o}	56.6±5.0^{+o}	52.9±4.4^{*o}
	layer IV	41.9±4.3	27.2±2.6*	36.1±3.3^{+}	35.7±3.1^{+}	34.2±2.8

Note. Here and in Tables 3 and 4: $p<0.05$ compared to: *intact rats; $^{+}$animals without transplantation; ^{o}transplantation on day 2.

TABLE 3. Content (%) of Reactively Changed Neurons in Sensorimotor Cortex Layers III and V in Albino Rats after Allo- and Xenotransplantation of Embryonic Nervous Tissue and Xenotransplantation of Umbilical Blood Stem Cells on Day 2 of the Posttraumatic Period

Parameter	Group				
	intact	BI	BI+AENT	BI+XENT	BI+XBSC
Layer III					
Normochromic	89.6±4.7	33.4±3.9*	37.2±64.6*	39.0±5.4*	37.1±4.9*
Hyperchromic	7.7±0.6	34.1±4.8*	35.1±3.9*	33.2±5.1*	33.0±4.7*
Pyknomorphic	0.6±0.1	15.2±2.0*	16.3±2.2*	14.6±2.3*	15.3±1.9*
Ghost cells	2.0±0.3	17.3±1.2*	11.4±1.7*+	13.2±1.5*+	14.6±1.9*
Layer V					
Normochromic	90.1±6.7	34.2±4.5*	33.5±3.6*	36.3±5.2*	34.8±3.7*
Hyperchromic	7.6±0.5	37.8±3.9*	37.3±4.1*	34.5±4.1*	38.0±3.9*
Pyknomorphic	0.4±0.1	18.3±2.6*	17.6±2.0*	19.1±1.8*	18.4±2.2*
Ghost cells	1.9±0.1	9.7±0.8*	11.6±1.7*	10.1±1.4*	8.8±1.3*

TABLE 4. Content (%) of Reactively Changed Neurons in Sensorimotor Cortex Layers III and V in Albino Rats after Allo- and Xenotransplantation of Embryonic Nervous Tissue and Xenotransplantation of Umbilical Blood Stem Cells on Day 7 of the Posttraumatic Period

Parameter	Group				
	intact	BI	BI+AENT	BI+XENT	BI+XBSC
Layer III					
Normochromic	89.6±4.7	33.4±3.9*	67.7±6.1*+o	58.3±6.0*+o	52.2±4.8*+o
Hyperchromic	7.7±0.6	34.1±4.8	15.9±1.7*+o	19.4±2.7*+o	25.8±3.1*
Pyknomorphic	0.6±0.1	15.2±2.0*	6.6±1.5*+o	8.8±1.3*+o	10.4±1.4*+
Ghost cells	2.0±0.3	17.3±1.2*	9.8±1.4*+	11.5±2.6*+	11.6±1.9*+
Layer V					
Normochromic	90.1±6.7	34.2±4.5*	69.2±6.7*+o	57.8±4.9*+o	52.0±5.6*+o
Hyperchromic	7.6±0.5	37.8±3.9*	19.1±1.8*+o	26.5±3.1*+o	30.0±3.8*
Pyknomorphic	0.4±0.1	18.3±2.6*	7.2±0.8*+o	9.4±1.2*+o	12.5±1.3*+o
Ghost cells	1.9±0.1	9.7±0.8*	4.5±0.4*+o	6.3±0.8*+o	6.5±0.5*+

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