Evaluation of Psychoemotional Status of Rats after Brain Injury and Systemic Transplantation of Mesenchymal Stem Cells

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We studied the effects of systemic transplantation of mesenchymal stem cells obtained after culturing of autologous bone marrow on psychophysiological status of Wistar rats after diffuse brain trauma. Two months after systemic injection of mesenchymal stem cells we observed a decrease in manifestations of emotional behavioral reactions (anxiety and excitability) and shortening of the time of realization of drinking behavior in a T-maze. A significant positive effect of systemic transfusion of mesenchymal stem cells on avoidance conditioning in a shuttle box was observed 3 months after brain injury.

Key Words: *mesenchymal stem cells; brain injury; psychophysiological status of rats*

Mesenchymal stem cells (MSC) are characterized by considerable plasticity and can differentiate into mesenchymal and non-mesenchymal cells, including neurons and astrocytes [6,11,14]. Twelve neural genes and 8 genes related to the dopaminergic system are expressed in MSC [5]. Moreover, MSC constituently express native immature neuronal proteins such as nestin and Tuj-1 and after five passages *in vitro* MSC can already express more mature neuronal and glial proteins, such as TH, MAP-2, and GFAP. Expression of these proteins considerably increases in a special medium promoting differentiation of MSC into neurons and astrocytes [13]. Published data suggest that bone marrow stromal cells significantly alleviate motor and neurological deficit in animals with traumatic brain injury [7]. Our previous studies showed that systemic administration of syngeneic MSC produces proliferotropic, angiogenic, and probably neurotrophic effects [4].

Here we studied the effects of systemic transplantation of MSC during the acute period after brain injury (BI) on the recovery of brain functions in rats.

MATERIALS AND METHODS

Experiments were carried out on 3-month-old male Wistar rats weighing 160-180 g. The animals were divided into 3 groups: group 1 (control) comprised sham-traumatized animals (they were subjected to all manipulations, but the trauma was not inflicted); group 2 consisted of rats with BI, and group 3 included rats with BI and systemic transplantation of MSC. BI in the parietal area was modeled with a 50-g load falling through a hollow tube (diameter

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1.2 cm) from a height of 110 cm. The area of the impactor striking surface was 0.5 cm^2. MSC (2×10^6 cells in 0.5 ml physiological saline) were injected to group 3 animals on the next day after trauma. The primary culture of bone marrow MSC from adult rats was prepared as described previously [3]. Physical and neuropsychic status of experimental animals was evaluated 2 weeks and 2 and 3 months after BI. The following tests were used. In the "beam" test, animal motion on a beam (3-cm wide and 60-cm long) positioned at a height of 50 cm was evaluated using a 3-point scale; in the treadmill test their ability to keep on a rotating cylinder (6 cm diameter, 10 rpm) was assessed. In the inclined grid test, spatial orientation of the experimental rat on a grid fixed at an angle of 45° (negative geotaxis) at a height of 40 cm was evaluated (absence of the reaction – 1). Locomotor activity and mental state of rats were routinely evaluated in the open field test, extrapolation escape reaction was studied using diving test (1 min test duration, 2 presentations with 30-sec interval) [2]. Rat behavior under conditions of emotional discomfort was evaluated in the plus maze test. The maze consisted of 2 closed and 2 open illuminated arms of the same width and length and central open area and was positioned at a height of 50 cm above the floor. The rat was placed onto the central area with the head directed towards the open arm. The latency of entry into closed arm, the number of peepings out from these arms, the total duration of stay in closed areas, and the total number of runs in the maze (spontaneous motor activity under conditions complicated by height) were assessed over 3 min. Drinking behavior conditioning was studied in a T-maze. The maze consisted of 2 opaque perpendicular tubes with removable semitransparent covers with ventilation holes. The animal entered the maze through a tambour of the short tube separated by a movable wall; the drinking bottle was placed at one of the ends of the long tube. One day before the experiment, the animals were deprived of water but had free access to briquetted food. At the day of the experiment, the animals were adapted to the maze in the tambour for 10 sec and the latencies of entry into the maze and start of drinking were recorded. Each animal was given 3 presentations (3 min each) Searching reaction and learning capacity were analyzed.

Psychophysiological status of experimental animals was evaluated 3 months after BI by the capacity of avoidance conditioning in a shuttle box. In our previous experiments we found that conditioning in the majority of adult rats was attained after 2-3 learning sessions consisting of 50-40 presentations of the stimulus combinations in an optimal sequence: light and sound for 4 sec followed by electrical painful stimulation on seconds 4-12 with 20-sec interval between the cycles. In this study, all rats were tested 3 times, each session consisted of 50 combinations according to the above scheme of stimulation. The first session was performed on days 82-83 after trauma and the second and third sessions were carried out on days 3 and 10 after the first one. Learning criteria for this test were described previously [1].

All quantitative parameters were compared using Student's t test; Fisher exact test, Wilcoxon, Mann—Whitney, and median χ^2 tests were also used. During avoidance conditioning, stabilization of the reaction latency was observed (in consecutive trials), the absolute value of avoidance and run latency tended to temporal limits of conditioned avoidance reaction. For quantitative characteristics of this component of the adaptive behavior, dispersion of actual latencies in the dynamics of learning was evaluated using two-way dispersion analysis [9].

RESULTS

After BI modeling we observed a clinical picture typical of this pathology characterized by clonic convulsions, lateral posture, and development of coma. About 50% animals died within 2-4 min due to respiration arrest with signs of cyanosis and convulsions. Survivors changed the lateral posture after few minutes, but usually were in a stupor. They demonstrated complete adynamia, the absence of reactions to tactile and painful stimuli, pareses of the limbs, some animals had tremor, sanious nasal discharge, sometimes liquorrhea. Survivors were divided into 2 experimental groups: one group comprised animals with BI only, while animals of another group received intravenous injection of MSC 1 day after BI. The groups were comparable by the severity of BI evaluated by the complex of symptoms and tests over the first 30 min after trauma.

Two weeks after BI, the parameters characterizing physical state and motor activity (beam, treadmill, and open field tests), extrapolation reaction, and learning capacity (diving test) did not differ in the two experimental groups We observed a trend to acceleration of learning in rats receiving MSC, which in preliminary tests more rapidly escaped the life-threatening situation in the diving test. Moreover, the rats receiving MSC demonstrated more pronounced weight gain (22.1±2.5%) compared to the control (9.3±0.9%) and experimental rats with BI without MSC injection (12.9±1.8%).

Two months after trauma, the animals receiving MSC and controls demonstrated the same duration

TABLE 1. Plus Maze Behavior of Wistar Rats 2 Months after BI

Group	Latency, sec	Time of stay in closed arms, sec	Number of peepings out	Number of transitions
Control (sham trauma; *n*=13)	8.6±4.1	129.5±15.9	3.3±0.5	4.8±1.2
BI (*n*=12)	4.8±1.0	167.8±5.0**	2.8±0.5	2.4±0.7**
MSC transplantation (*n*=7)	6.3±2.0	128.1±10.2+	6.4±0.8*+	8.3±1.4+

Note. *n*: number of rats; *p<0.01, **p<0.05 compared to the control, +p<0.01 compared to BI.

of stay in closed arms of the plus-maze (Table 1), while in animals with BI this time was longer by almost 25%. The mean number of peepings out and runs in rats receiving MSC surpassed the corresponding parameters in animals with BI by 2 and 3 times, respectively (∂<0.01). These findings suggest that the therapeutic administration of MSC to rats with BI produced an anxiolytic effect, *i.e*. reduced manifestations of emotional behavioral reactions (anxiety and excitability) probably due to more rapid recovery of the relevant brain structures (thalamus, hypothalamus, limbic system, *etc.*).

Considerable differences between the groups were found during T-maze conditioning (drinking behavior, Table 2). In healthy animals, the latency gradually decreased by the 3rd trail. After entering the maze, healthy animals exhibited exploratory activity and moved many times along the territory. After finding the drinking bottle, they did not start drinking immediately, but continued to explore the maze. This is well-known pattern of rat behavior under experimental conditions. The rats with BI spend more time for the realization of the drinking reflex due to inhibition and residual locomotor disturbances and sometimes due to long grooming reflecting psychic discomfort. The animals receiving MSC spent less time for the realization of the drinking reflex in the T-maze (p<0.05).

Evaluation of the psychophysiological status of rats 3 months after BI revealed a significant positive effect of systemic transfusion of MSC on avoidance conditioning in a shuttle box. This effect manifested in the rate of the increase in β-coefficient of regression of the ratio of the number of successful escapes from electrical stimulation to failed attempts and in the relative rate of learning after 3rd testing (days 92-93 after BI). The increase in the success/failure ratio in animals after transplantation of MSC almost 2-fold surpassed the corresponding parameter in the control group and in rats with BI (Table 3). The relative rate in rats receiving MSC was higher by 89% than in other 2 groups. In animals receiving MSC we observed a tendency towards improvement of the final efficiency of learning: the percent of rats demonstrating avoidance response increased, the number of refusals of transitions decreased, temporal parameters of avoidance response stabilized (dispersion of latencies decreased). At the same time, we observed some differences in avoidance conditioning between the experimental groups. The animals with BI were more emotional: they demonstrated increased anxiety, panic reactions, and chaotic movements in the chamber compartment, jumps, and frequent interstimulus transitions accompanied by vocalizations. In the groups with MSC transplantation and in controls these emotional reactions were observed only in few animals and disappeared within 3-5 min of testing. This can be explained by improvement of repair processes in brain structures re-

TABLE 2. Realization of Drinking Response in Wistar Rats 2 Months after BI

Parameter		Group		
		control (*n*=10)	BI (*n*=12)	MSC transplantation (*n*=7)
Latency, sec	1st trial	8.7±2.7	6.3±1.9	3.4±0.6
	2nd trial	6.8±2.1	4.4±1.5	9.9±4.4
	3rd trial	5.1±1.4	9.9±3.2	4.1±1.0
Start of drinking, sec	1st trial	71.1±14.2	116.6±18.6	44.9±15.8*
	2nd trial	73.6±18.8	84.8±22.0	50.4±12.6
	3rd trial	67.7±18.4	93.0±22.0	37.3±9.4*

Note. *p<0.05 compared to BI.

TABLE 3. Learning Capacity (Avoidance Conditioning) in Rats 3 Months after BI (Results of 3rd Trial)

Group	Regression of sign increase, b-coefficient		Relative rate of learning, %
	success/failure	percent of rats with avoidance response	
Control (*n*=13)	0.027±0.004	0.58±0.12	100
BI (*n*=12)	0.027±0.004	0.41±0.09	100
BI+MSC (*n*=7)	0.051±0.004*	0.81±0.13	189

Note. *$p<0.001$ compared to BI.

sponsible for emotional sphere under the effect of MSC, which reduced negative manifestations of stress (fear, anxiety, and increased excitability) persisting in delayed periods in rats receiving no MSC.

Thus, our findings suggest improvement of cognitive functions in rats receiving systemic transfusion of MSC at early terms after BI. At present, the mechanism of this effect can be only hypothesized. It is known that MSC injected intravenously to rats can enter the damaged brain and improve the neurological status [7]. Transdifferentiation of MSC and differentiation of neuronal precursors *in vivo* is regulated by microenvironment. The concentration of MSC at the site of BI probably promotes the recovery of the structure and function of the damaged nervous tissue, because these cells contain and release trophic factors, including IL-6, MCP, SCF, SDF-1, growth factors BDNF, VEGF [10], NGF, NT-3 [8], and brain natriuretic peptide [12]. This assumption is based on the results of our previous pathomorphological studies of structural changes in the damaged area of rat brain after diffuse trauma of the head and systemic transplantation of MSC. It was found that reactive, compensatory, and regeneration mechanisms with initiation of cell proliferation in zones of neurogenesis are activated at early terms after trauma. However, in untreated animals the phase of regeneration is characterized by prolonged course with slow repair processes in damaged tisse and cell structures of the brain. After injection of MSC, the therapeutic effect of stimulation of cell proliferation and angiogenesis was visually seen at the early terms of recovery of damaged tissue and cell structures [4]. This effect of systemic transplantation of MSC on the process of structural reparation in the damaged area of rat brain can, in turn, determine more efficient recovery of the psychophysiological status of experimental animals observed in our study.

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