Mesenchymal Stem Cells Restore Orientation and Exploratory Behavior of Rats after Brain Injury

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Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 1, pp. 26-28, January, 2011
Original article submitted March 4, 2010

We studied the effects of intravenous and intracerebral transplantation of MSC on restoration of orientation and exploratory behavior of Wistar–Kyoto rats after removal of the left motor cortex. Removal of the motor cortex led to a significant reduction of the number of behavioral acts in the open field test. Two weeks after removal of the motor cortex and intravenous transplantation, the animals were as inhibited as the controls, but during the next 10 weeks, the behavioral status of these rats remained unchanged, while controls exhibited further behavioral degradation. After injection of MSC into the brain, the behavior of rats with trauma did not change in comparison with intact rats over 10 weeks.

Key Words: *mesenchymal stem cells; orientation and exploratory behavior; motor cortex*

Experiments demonstrated that transplantation of MSC after ischemic stroke or brain injury leads to restoration of the cognitive functions, orientation and exploratory behavior, and neurological status of animals [3,4]. Positive effect of cell therapy is mainly assumed to be due to the paracrine function of MSC, which is realized when the cells are located in the focus of tissue injury. MSC release trophic and growth factors (BDNF, vascular endothelial growth factor (VEGF), NGF, hepatocyte growth factor) stimulating the inflammatory reaction in the focus and angiogenesis, support neuronal viability, inhibit apoptosis in the borderline zone, promote more rapid formation of the glial cicatrix occupying a lesser area [5]. As a result of these processes, secondary degeneration of the brain tissue involves lesser volume in comparison with the control animals.

We studied the efficiency of intravenous and intracerebral transplantation of autologous MSC for restoration of orientation and exploratory behavior of animals after complete removal of the motor cortex (RMC) in the left hemisphere.

MATERIALS AND METHODS

Experiments were carried out on adult male Wistar–Kyoto rats (n=190; 200-250 g). The animals were kept under standard vivarium conditions at natural light with free access to water and food. The studies were carried out in accordance with the regulations of the European Convention for Protection of Vertebrates Used for Experimental Purposes (Strasbourg, 1986).

The left motor cortex was removed as follows. The rats were narcotized with Zoletil 100 (30 μl intraperitoneally). A hole (2×3 mm) was drilled in the parietal bone of the left side of the skull and the dura mater and the entire motor cortex were removed (AP= -1-4 mm from the bregma, SD=1.0 mm laterally from the sagittal suture). The depth of the injury was no more than 2 mm. The skin wound on the head was sutured.

MSC were isolated from the rat BM by the standard method [2].

Phenotyping of rat MSC was carried out by flow cytofluorometry on an Epics XL flow cytofluorometer (Beckman Coulter). MSC culture consisted of

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0007-4888/11/1511-0130

3% $CD45^+$ cells (hemopoietic) and 97% $CD90^+$ cells (MSC proper), of these, 15% were $CD106^+$ cells.

The animals were divided into 5 groups. Group 1 were intact rats (n=30). Group 2 (control I; n=40) animals received an injection of 100 µl culture medium into the caudal vein immediately after RMC. Group 3 (cell therapy I; n=40) animals received an injection of 5×10^6 MSC in 100 µl culture medium into the caudal vein immediately after RMC. Group 4 (control II; n=40) rats were intracerebrally injected with 20 µl culture medium immediately after RMC. Group 5 (cell therapy II; n=40) animals were intracerebrally injected with 200,000 MSC in 20 µl culture medium immediately after RMC.

Intracerebral transplantation consisted of two injections, 10 µl each, of culture medium or cell suspension into the brain area adjacent to the focus of injury frontally and dorsally to the depth of no more than 2 mm (to the neocortex only).

Open field behavior was evaluated several days before RMC and 2, 4, 6, and 10 weeks after the intervention.

The open field was a round area 80 cm in diameter with nontransparent walls 30 cm high with 16 holes, 3 cm in diameter, at regular distances from each other. An animal was placed into the center of the field and the number and duration of behavioral acts (locomotion, sniffing, movements on the spot, grooming, rearing with and without support, hole exploration, sitting) were recorded.

The significance of differences between the two samples was evaluated using nonparametric Mann–Whitney U test (Statistica 6.0 software; $p\leq0.05$).

RESULTS

All animals exhibited marked motor and exploratory activity during the initial open field test. Removal of the left motor cortex, a severe brain injury, led to significant changes in individual animal behavior. Two weeks after RMC (Fig. 1, *a*) group 2 animals were inhibited: the number of locomotor acts decreased by 2.5 times. Exploratory motivation of animals manifested in hole exploration, sniffing, and rearing with support was reduced more than 3-4 fold in comparison with intact animals. The orientation and exploratory behavior of group 4 animals was less changed: the number of locomotion acts virtually did not differ from that in intact animals, while the exploratory behavior was disturbed only as regarded the rearing with support (Fig. 1, *b*). The animals of groups 2 and 4 spent more time in two positions: motionless (sitting) or moving the fore paws (movement on the spot). This behavior indicated that the animals were emotionally unstable, anxious, fearful, and frustrated (unable to make decisions). Animal behavior in these groups during 10 weeks after RMC was characterized by significant reduction of the motor and orientation and exploratory activity and a longer time spent motionless and frustrated (Fig. 2, *a*, *b*).

The orientation and exploratory activity of animals with injury intravenously injected with MSC sharply decreased similarly as in controls. After 2 weeks, the number of the main acts decreased 2-4-fold (Fig. 1, *a*). The time spent without motions significantly increased. However, while in the controls (groups 2 and 4) the degradation progressed during 10 weeks, the behavior of group 3 animals did not change and after 10 weeks they were 1.5-2 times more active, though did not reach the level of intact animals (Fig. 2, *a*). This was an expected result: cell therapy could not restore lost fragment of the brain tissue. It could hardly be expected that structures of the intact hemisphere would completely compensate for the lost functions after complete removal of the contralateral motor cortex.

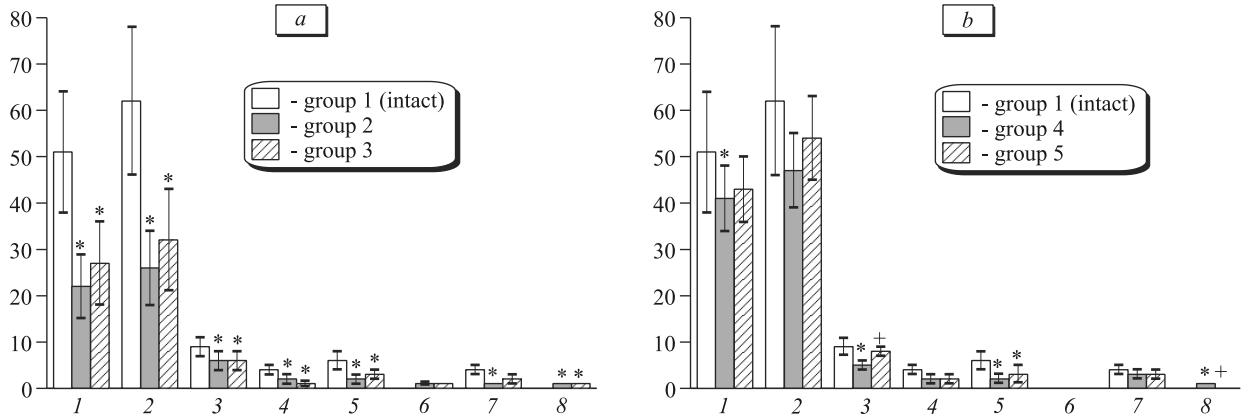

Fig. 1. Mean number of behavioral acts in open field test 2 weeks after RMC of the brain. Here and in Fig. 2: *a*) injection of MSC into the caudal vein; *b*) injection of MSC into the brain. *1*) locomotion; *2*) sniffing; *3*) movements on the spot; *4*) grooming; *5*) rearing with support; *6*) rearing; *7*) hole exploration; *8*) sitting. $^*p\leq0.001$ compared to intact animals (group 1); $^+p\leq0.004$ compared to group 2.

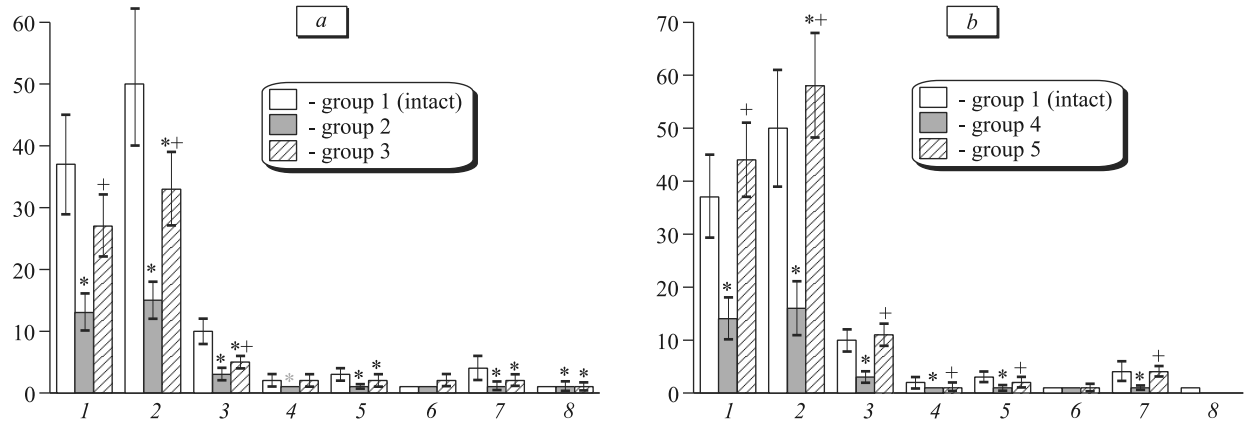

Fig. 2. Mean number of behavioral acts in the open field test 10 weeks after RMC.

A different behavioral algorithm was observed in group 5 animals injected with MSC directly into the brain. By all behavioral acts these animals virtually did not differ from intact rats over 10 weeks. Only the time spent in movements on the spot increased significantly, but after 10 weeks this parameter corresponded to the behavior of intact animals.

These data indicate that cell therapy with autologous MSC leads to positive shifts even after severe brain injury (unilateral RMC). Intravenous transplantation of MSC just stabilized animal status and prevented their further degradation, while intracerebral transplantation completely prevented the disorders in orientation and exploratory activity associated with RMC. Presumably, all aspects of therapeutic effect of MSC on damaged brain structures (stimulation of inflammatory reaction at the site of brain injury and formation of glial cicatrix, stimulation of angiogenesis, inhibition of apoptosis, neuroprotection in the zone adjacent to the focus, and hence, reduction of the volume of tissue subjected to secondary degeneration) can be realized after intravenous and intracerebral transplantation. It was shown that after intravenous injection of MSC after ischemic stroke the cells reached the brain only on day 3, even if the integrity of the blood-brain barrier was violated [1]. Hence, during the first days after RMC inflammation and secondary degradation of the nervous tissue developed without participation of exogenous MSC. After intracerebral injection two cell conglomerations formed from the very first minutes after injury in the zones bordering the focus (in sites of injections). It seems that MSC release growth and trophic factors directly modulating damaged but still viable neurons, preventing their death.

REFERENCES

1. N. N. Zinkova, E. G. Gilerovich, I. B. Sokolova, *et al.*, *Tsitologiya*, **49**, No. 11, 923-932 (2007).
2. I. Sokolova, N. Zinkova, A. Bilibina, *et al.*, *Klet. Transplantol. Tkan. Inzhener.*, **2**, No. 4, 54-62 (2007).
3. I. Sokolova, O. Fedotova, N. Zinkova, *et al.*, *Kletoch. Tekhnol. Biol. Med.*, No. 4, 202-205 (2006).
4. J. Chen, Y. Li, L. Wang, *et al.*, *Stroke*, **32**, No. 4, 1005-1011 (2001).
5. J. Chen, Y. Li, L. Wang, *et al.*, *Neuropathology*, **22**, No. 4, 275-279 (2002).