Reaction of Oligoglia to Spinal Cord Injury in Rats and Transplantation of Human Olfactory Ensheathing Cells

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Translated from *Kletochnye Tehnologii v Biologii i Medicine*, No. 1, pp. 27-31, January, 2010
Original article submitted April 22, 2009

In experiments on rats with lateral T_{VIII} hemisection of the spinal cord and transplantation of ensheating olfactory cells, we studied structural changes at the lesion site and adjacent rostral and dorsal regions of the spinal cord. The state of oligodendrocytes and myelin fibers and motor function in experimental animal were analyzed. Open field testing (BBB test) showed that motor functions steadily increased (by 13% on average) within the interval from day 21 to day 53 after transplantation. Histological examination showed that groups of transplanted cells carrying human nuclear marker (HNu^+ cells) were still present at the lesion site 30 days after surgery. Some of these cells migrated in the rostral and caudal directions from the injection site to a distance up to 6 mm. At the initial period after hemisection, the number of oligodendrocytes ($O4^+$-cells) in the immediate vicinity to the lesion site decreased 2-fold, but no significant changes in the number of neurons were found in the rostral and dorsal fragments of the spinal cord compared to the corresponding parameter in controls. Sixty days after transplantation, the cross-section area in the rostral part of the spinal cord at a distance of 3 mm from damage site increased by 15.3% compared to the control. The number of $O4^+$-cells at the lesion site and in adjacent rostral and caudal parts of the spinal cord by 22.8% surpassed that in the control group. The number of remyelinated axons also increased. These findings suggest the absence of pronounced structural changes in the rostral and caudal parts of the spinal cord compared to lesion site at early stages after damage and cell transplantation. At the same time, pronounced activation of oligodendrocytes in this region suggests their involvement together with Schwann cells into remyelination of regenerating axons, which can serve as a factor of partial restoration of motor functions after spinal cord injury.

Key Words*: spinal cord; lesion; cell transplantation; myelination; oligodendrocytes*

Traumatic injuries of the spinal cord are associated with local loss of neurons and demyelination of axons of ascending and descending spinal tracts. Numerous experimental and clinical studies showed that regeneration of the spinal cord (spontaneous and inducted by pharmacological agents and cell therapy) is associated with regeneration of damaged neurons and new formation of myelin sheath around the preserved and regenerated fibers of the spinal tracts, which results in partial compensation of impaired motor and sensory functions [7-9]. In these studies, the most contradictory issue is conception concerning the recovery of myelin sheaths observed mainly after transplantation of glial cells from the olfactory system (olfactory ensheating cells). Previously obtained data on the role of these cells in remyelination after spinal lesion [11] were not confirmed in later studies, where the main

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role was assigned to Schwann cells [10,13]. At the same time, the data about the state of oligodendroglia and its involvement into remyelination of spinal cord tract fibers outside the damaged area during cell therapy are extremely limited.

Here we studied the state of oligodendrocytes in damaged spinal cord and their role in regeneration of myelin sheaths after transplantation of olfactory epithelium cells.

MATERIALS AND METHODS

Experiments were carried out on 14 mongrel rats weighted 200-250 g. The animals were kept under standard conditions with free access to food and water. After laminectomy under urethane anesthesia (600 mg/kg intraperitoneally), local hemisection of the spinal cord at the right side at T_{VIII} level was performed. This method of hemisection allowed using the undamaged side of the spinal cord for evaluation of the character and extent of posttraumatic processes at the damaged area. Olfactory epithelium (OE) cells were used for transplantation; the cells were obtained from Department of Applied and Fundamental Neurology (V. P. Serbskii Research Center of Social and Forensic Psychiatry) and cultured as described previously [1]. After hemisection, 200,000 cells in 5 µl Ringer solution were injected to experimental group animals (n=8) in two points 1 mm rostrally and caudally from the hemisection plane and 0.5 mm laterally from the midline towards the damaged side. Control animals (n=6) received 5 µl Ringer solution at the same points. Locomotor function in control and experimental animals was regularly evaluated starting from day 2 to day 53 in the open field test (BBB test) [2].

The data were processed statistically using ANOVA and Mann–Whitney test.

Histological and immunocytochemical examination of the spinal cords from the experimental and control animals were carried 30 and 60 days after the intervention. Anaesthetized animals were transcardially perfused with phosphate buffer (pH 7.4) and then with 4% paraformaldehyde on phosphate buffer. After laminectomy, a 20-mm fragment of the spinal cord

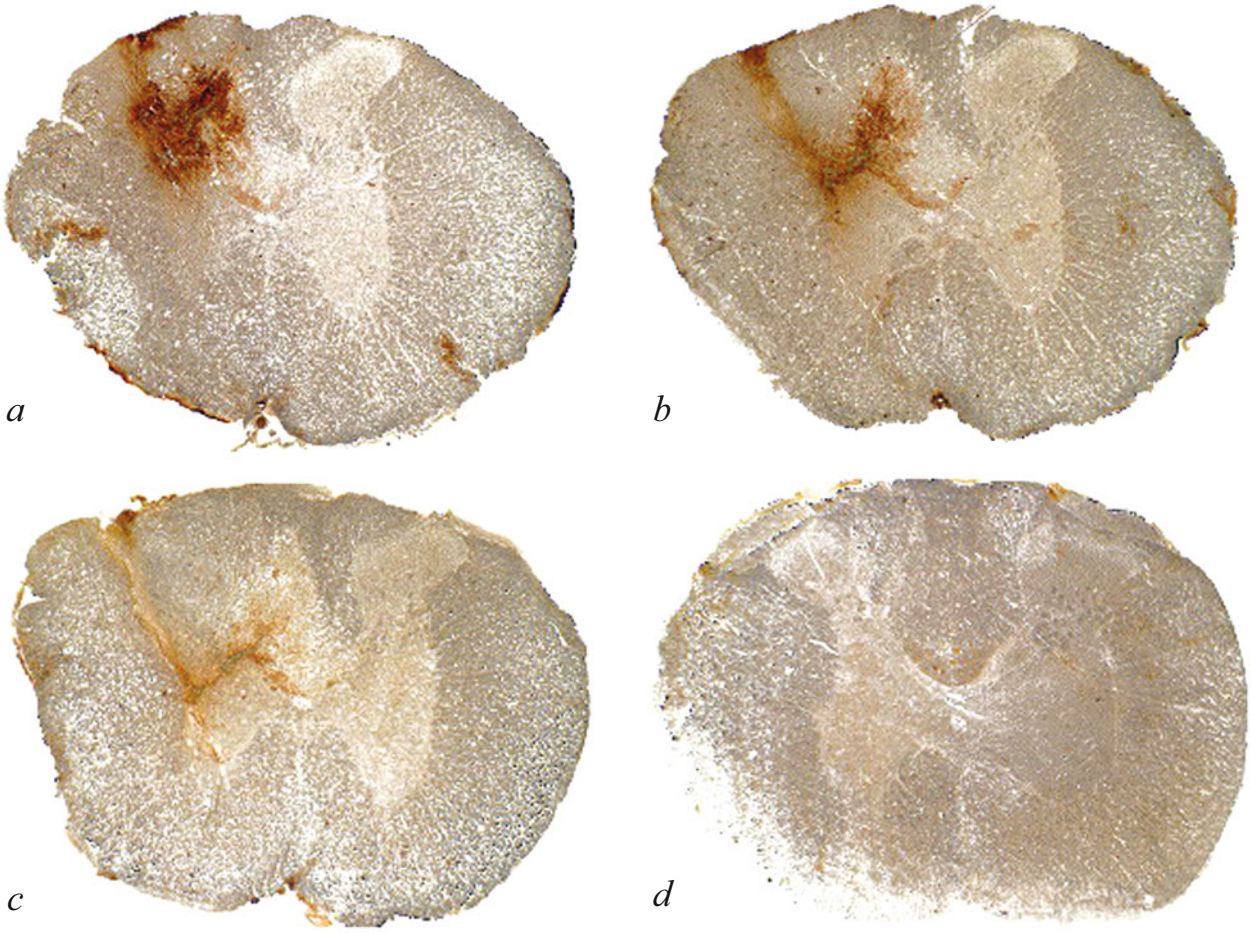

Fig. 1. Transplanted human OE cells in rat spinal cord: immunohistochemical reaction with antibodies to human cell nuclei (HNu) 30 days after intervention. Distance from hemisection plane toward the rostral direction: *a*) 1 mm, *b*) 3 mm, *c*) 5 mm, *d*) 7 mm.

with hemisection plane in the center was isolated. The material was additionally fixed in neutral formalin or glutaraldehyde and embedded in paraffin and a mixture of epon and araldite resin according to standard techniques. On cross-sections stained with methylene blue, the total area of section, area of the damaged site, and area of preserved grey matter were measured at the distance from 1 mm to 10 mm caudally and rostrally from the hemisection plane [12]. On day 60 after the intervention, the number of neurons was calculated on sections stained with methylene blue. The number of oligodendrocytes was determined after immunohistochemical reaction with O4 antibodies (1:100, R&D Systems, Inc.) with red fluorescent label Cy3 (1:300, Dianova). For nucleus identification, the sections were incubated in a medium with fluorescent dye Dapi (1:1000; Dianova). The number of myelin fibers was determined at a distance of 5 mm rostrally and caudally from the hemisection plane after staining of semithin sections of the spinal cord with toluidine blue. Migration of cells stained with antibodies to human cell nuclei (HNu, 1:100, Chemicon) was evaluated 30 days after their transplantation into the site of damage. Immunocytochemical examination was performed using an Axioscope 2 fluorescent microscope and Image J software. Statistical significance of obtained data was assessed using ANOVA, Mann–Whitney, and Student tests.

RESULTS

Thirty days after spinal cord hemisection and transplantation of OE cells, groups of cells labeled with human nucleus marker (HNu^+-cells) were found in the zone of damage (Fig. 1). We showed that these cells migrated up to the distance of 6.20±1.23 mm in the rostral direction and up to 5.80±1.08 mm in the caudal direction through the spinal cord; however, according to previously obtained data, glial cells from OE transplanted to the spinal cord start to migrate 4 h after transplantation and by day 7 pass a distance equal to 1 mm in both rostral and caudal directions [4].

After injection of OE cells into the site of hemisection, the area of damage was smaller than in the control on days 30 and 60. Significant changes in the area of damage were observed 7 mm rostrally from the damage site 30 days after intervention (Fig. 2, *b*). Sixty days after transplantation, the cross-section area at a

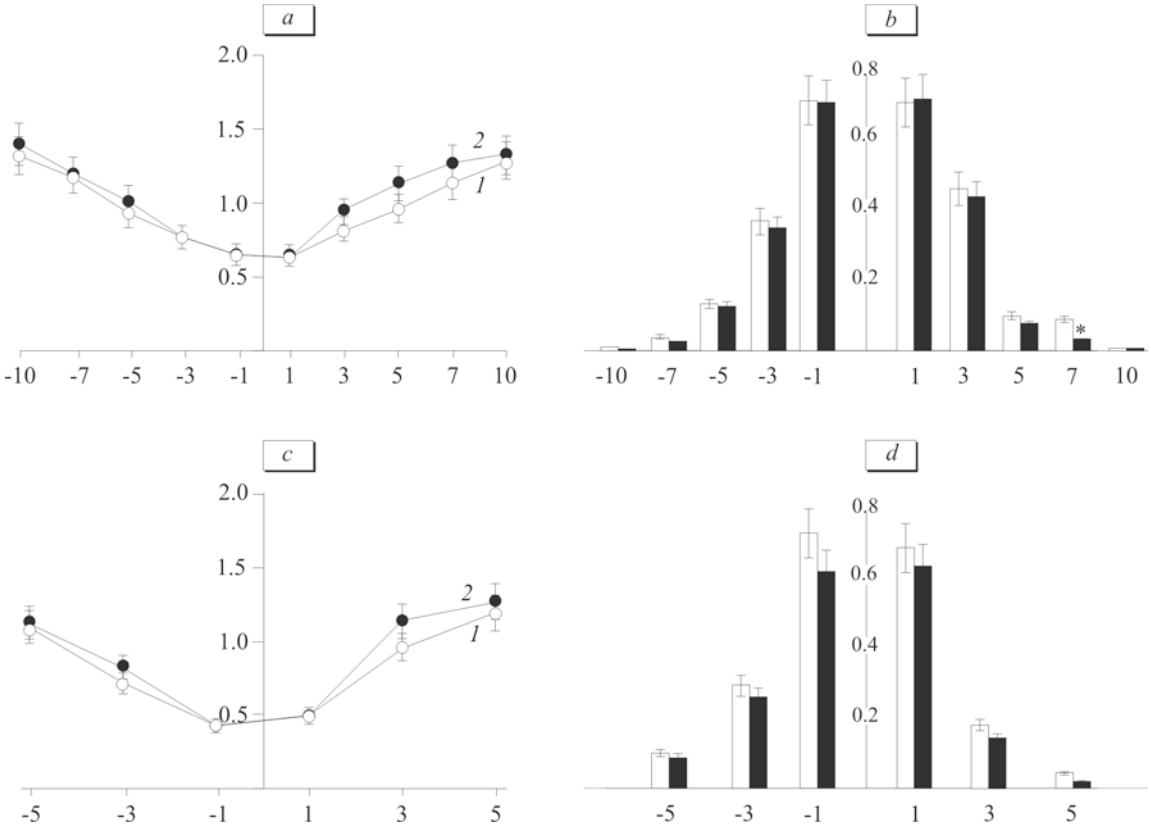

Fig. 2. Morphometric characteristics of rat spinal cord 30 (*a*, *b*) and 60 days (*c*, *d*) after transplantation of human OE cells in the site of hemisection. *a*, *c*) area of preserved grey matter; *b*, *d*) area of damaged site. Abscissa: distance from hemisection plane in rostral (+) and caudal (-) directions in mm. In *a* and *c*: *1*) control values (without cell injection), *2*) experimental values (cell injection). In *b* and *d*: light bars: control, dark bars: experimental group.

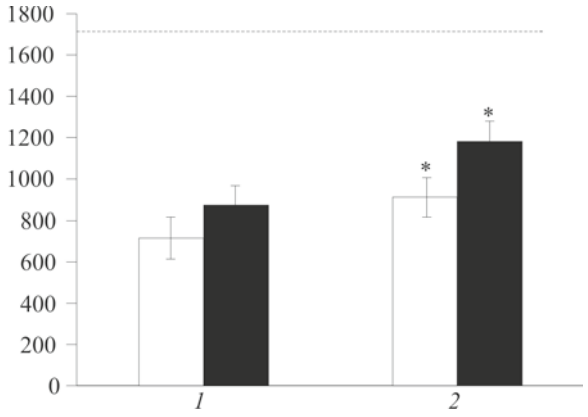

Fig. 3. The number of O4$^+$-cells in rat spinal cord at a distance of 3 mm in the caudal (*1*) and rostral (*2*) directions from the hemisection plane 60 days after intervention. Ordinate: total number of cells on the section; light bars: control, dark bars: experiment. Dotted line: number of O4$^+$-cells in the spinal cord of intact animals at T_{VIII} level. Here and on Fig. 4: *p<0.05 when control and experimental values are compared.

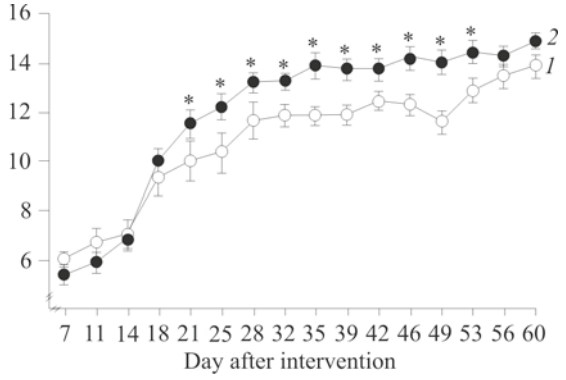

Fig. 4. Dynamics of recovery of motor function according to open field test (BBB test) after hemisection of rat spinal cord and injection of human OE cells into the site of damage. *1*) control; *2*) experimental group.

distance of 3 mm rostrally from the hemisection plane surpassed that in control animals by 15.3% (p<0.05). The area of preserved grey matter also tended to increase 30 and 60 days after transplantation of OE cells (Fig. 2, *a*, *c*).

The number of O4$^+$ cells in the immediate proximity to the site of damage decreased almost twofold on day 60 (Fig. 3), while the number of O4$^+$-cells at a distance of 3 mm rostrally from the hemisection plane at this term exceeded the corresponding parameter in control animals by 22.8% (p<0.05, Fig. 3). These data are important for understanding such substantial results of cell therapy of spinal cord injuries as maintenance of oligodendrocyte structure and function, inhibition of posttraumatic demyelination, and stimulation of remyelination of nerve fibers.

Motor function in the experimental animals was repeatedly evaluated from 21 day to 53 day after hemisection and transplantation of human OE cells. During this period, motor function in rats receiving transplantation of OE moderately improved according to BBB test by 12.8% on average, in comparison with control animals (Fig. 4). Our results agree with previous data obtained in similar studies [8].

It should to be emphasized, that the described improvement in motor functions directly correlated with an increase in oligodendrocyte number during spinal cord regeneration. These data can serve as the basis for understanding the role of oligodendroglia in one of major regeneration processes during cell therapy of spinal cord lesions, inhibition of demyelination of impaired nerve fibers and simulation of remyelination of regenerating axons in spinal tracts.

The increase in the number of oligodendrocytes after transplantation of olfactory epithelium cells into the damaged spinal cord can be explained by production of neurotrophic factors essential for oligodendrocyte survival by transplanted cells migrating to appreciable distances in the undamaged rostral and caudal parts of the spinal cord. The possibility of stimulation of differentiation and proliferation of oligodendrocyte progenitors by transplanted cells cannot also be excluded [6].

Remyelination of regenerated axons by Schwann cells after spinal cord damage is described in a number of reports. According to these studies, transported glial cells cannot form myelin sheaths, but stimulate migration of Schwann cells into the area of spinal cord damage and create microenvironment maintaining fiber myelination by Schwann cells [3,5,10,13].

Thus, according to our findings and previously obtained results, remyelination of ascending and descending spinal tracts and regenerating axons at the site of spinal cord damage during cell therapy is mediated by trophic interaction between transplanted glial OE cells, myelin-producing oligodendroglial cells, and Schwann cells.

The study was supported by Russian Foundation for Basic Research (grants No. 07-04-00746, No. 08-04-01167) and DZN 09-15.

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