MORPHOLOGY AND PATHOMORPHOLOGY

Xenotransplantation of Stem/Progenitor Cells from Human Fetal Brain to Adult Rats with Spinal Trauma

G. A. Stepanov, D. O. Karpenko, M. A. Aleksandrova*, O. V. Podgornyi*, R. A. Poltavtseva*, A. V. Pevishchin, M. V. Marey***, and G. T. Sukhikh*****

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In vitro grown neural stem cells from human fetal brain were transplanted to adult rats with spinal trauma. The spinal cord was examined morphologically using histological and immunohistochemical methods on days 5, 15, 30, and 110. Human neural stem/progenitor cells were viable, migrated, and differentiated into neurons and glia in the traumatized spinal cord in adult rats.

Key Words: *human neural stem/progenitor cells; xenotransplantation; spinal cord; immunohistochemistry*

The inability of injured nerve fibers to regenerate is the cause for serious complications of spinal cord trauma. The therapy of patients with severe disorders of the spinal cord accompanied by damage to conducting axons, degeneration of nerve cells, and formation of glial scars and cysts is often unsuccessful or leads to the development of paraplegia and tetraplegia. Surgical, neurophysiological, pharmaceutical, biotechnological, and other methods are used to treat these disturbances. Transplantation of native or modified cells (genetic engineering) is used in biotechnology to construct a cellular bridge in damaged regions or for neurotrophic stimulation [4]. Treatment with neural stem cells holds much promise in this respect [5,7-13]. Embryonic and fetal neural stem cells were grown, cultured *in vitro*, and used for neurotransplantation to recover functions of the central nervous system [2,6].

Here we studied the state of cultured stem/progenitor cells from human fetal brain after transplantation into the traumatized spinal cord of adult rats.

MATERIALS AND METHODS

Experiments were performed on 30 female Wistar rats weighing 250-300 g. Trauma of the spinal cord was followed (n=15) or not followed (n=15, control) by transplantation of stem/progenitor cells from human fetal brain.

Laminectomy (projection Th8-9) was performed in animals intraperitoneally narcotized with 2 ml 2% calupsol. Spinal cord tissue was contused with a weight (10 mg) falling from a height of 6.5 or 12 cm. Stem/progenitor cells from human fetal brain were transplanted into the spinal cord immediately after trauma.

The cells were transplanted using a stereotactic device equipped with a Hamilton microsyringe. The

Central Institute of Traumatology and Orthopedics, Moscow; *N. K. Kol'tsov Institute of Developmental Biology, Russian Academy of Sciences, Moscow; **Institute of Ecological and Evolutional Problems, Russian Academy of Sciences, Moscow; ***Center for Obstetrics, Gynecology, and Perinatology, Russian Academy of Medical Sciences, Moscow

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cells were administered 5 mm proximally and distally from the epicenter of injury (6×10^5, 3 μl, depth 1.5-2.0 mm). Immunosuppression was not performed.

Cultured stem cells from human brain were isolated and grown as described elsewhere [1,10]. Brain cells of human fetuses (9-12 weeks gestation) obtained after medical abortions were dissociated and placed in NPBM growth medium (Neural Progenitor Basal Medium, Clonetics; 2×10^6 cells/ml). Fibroblast growth factor (hFGF), nerve cell survival factor (NSF-1), epidermal growth factor (hEGF), and gentamicin/amphoterin (NPMM, Clonetics) were added to the medium. The cell suspension was cultured in flasks for 7-10 days. True neurospheres were pipetted, transferred to fresh medium (1×10^5 cells/ml), and cultured for 3-4 weeks. Then neurospheres became heterogeneous by cell composition, because some stem cells spontaneously differentiated into neurons and glia [1,10].

Before transplantation the cells were stained with luminescent dye bisbenzimide in a concentration of 20 μg/ml (Hoechst 33342, Sigma). The suspension of single cells and small neurospheres (2×10^5 cells/ml) was used for transplantation.

The rats were narcotized with 500 mg/kg chloral hydrate and transcardially perfused with 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4) on days 5, 15, 30, and 110 days after trauma and transplantation. The brain was removed, placed in a fixative for 24 h, and maintained in 30% sucrose at 4°C. Slices (20-40 μ) were prepared using a cryostat and mounted on slices. The slices with transplanted cells were selected by luminescence of cells stained with bisbenzimide. The slices were stained with hematoxylin and eosin, Sudan III, and cresyl violet. Immunohistochemical staining was performed with primary antibodies anti Human Nestin (human cell nestin; Chemicon, 1:20), anti Neurofilament-70 (neurofilaments 70; Serotec, 1:500), anti Human Nuclei (human cell nuclei; Chemicon, 1:30), anti GFAP (glial fibrillary acidic protein, GFAP; DAKO, 1:250), and anti β-Tubulin III (β-tubulin III; Abcam, 1:200). The cells were treated with biotinylated secondary antibodies (1:200, Vector Laboratories) for 1 h and stained with streptavidin labeled with fluorescence dye Texas red (Jackson) or avidin-biotin-peroxidase complex (Vector Laboratories). Peroxidase was developed with diaminobenzidine. Double immunocytochemical staining was performed using secondary antibodies labeled with fluorescence dyes Texas red and Cy-2. The slices were washed 3 times, cleared with 50% glycerol in phosphate buffer, and examined in luminescent or combined light.

RESULTS

Microscopic examination showed that the brain of experimental animals contained transplants of cultured human stem/progenitor cells.

The study of spinal cord preparations 5 days after surgery revealed regions of severe tissue injury and hemorrhages extending for 4-12 mm in each direction. Hemorrhagic focuses appeared between completely broken fibers. Newly formed vessels were found at the boundary of tissue injury. It was related to the treatment with fetal cells stimulating revascularization.

Transplanted cells stained with bisbenzimide were detected in punctured structures. The density of transplanted cells decreased with increasing the distance

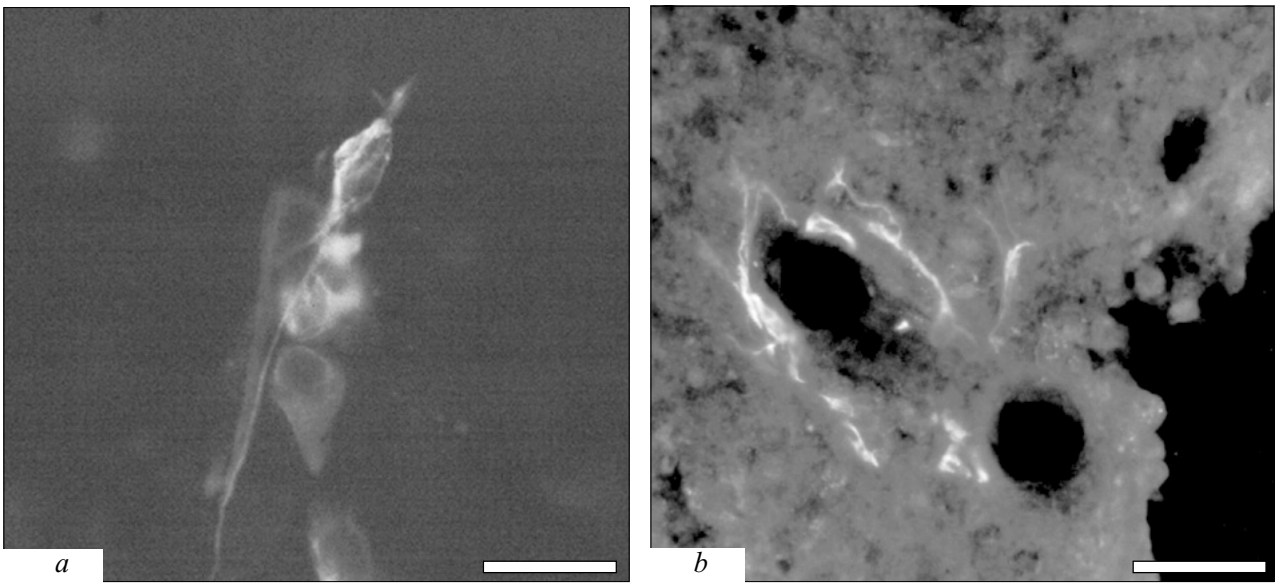

Fig. 1. Nestin-positive stem cells from human brain 15 days after transplantation into the traumatized spinal cord of adult rats: migration of single cells (*a*) and localization around vessels (*b*). Staining with antibodies against human nestin. Scale: 20 (*a*) and 80 μ (*b*).

from the site of injection. The cells migrated in distal and proximal directions between fibers and along vessels. Individual cells were positioned in cellular regions and zone of injury between blood cells. Immunohistochemical staining with antibodies against human nestin revealed the presence of not only transplanted cells, but also true stem cells. Single or clustered nestin-positive cells were localized at the boundary of injury.

Morphological signs of damage to the spinal cord tissue were similar in control animals. The average area of injury was 5-6 mm. Examination of preparations revealed severe tissue necrosis in the zone of mechanical injury and initial stage of cyst formation. Small cavities were easily detected; some of them merged into large cavities. In the peripheral zone of traumatized regions most motor neurons underwent various phases of degeneration. We revealed severe gliosis and initial stage of cicatrization.

Fifteen days after trauma and transplantation the zone of injury extended over the whole width of the spinal cord. Moreover, its zone spread in the distal and proximal directions for 6 mm or more from the epicenter of trauma. Fibers were characterized by serious damage. Large areas of necrosis and focal hemorrhages were formed in the zone of injury. Changes in the glia were insignificant. As differentiated from control animals, cysts were not formed in the spinal cord of rats receiving xenotransplants.

Transplanted cells stained with bisbenzimide were found in the site of injection and migrated to the zone of injury. Experiments with antibodies against human cell nuclei revealed single or clustered transplanted cells. Staining with antibodies against human nestin detected not only transplanted cells, but also true stem cells. Individual nestin-positive cells migrated from the site of injection in the brain tissue, while others were arranged in groups around vessels (Fig. 1).

The average area of damage to the spinal cord tissue in control rats was 4-5 mm. Pronounced lymphocytic reaction developed at the boundary of necrotic tissues. Glial scars and cyst were formed in this region (Fig. 2). In the zone of injury nerve cells underwent various pathological changes including chromatolysis and hyperchromia. Individual neurons retained normal structural characteristics.

Histological examination of preparations stained with cresyl violet showed that 30 days after trauma and transplantation of cultured stem cells the area of injury was 3-4 mm. Tissue edema and cyst formation were not characteristic of this period. Changes in the glia were pronounced in the zone of injury. However, focal glial scars were not found. A considerable number of small newly formed vessels were revealed in the zone of injury. In distal and proximal regions motor neurons and interneurons had normal morphological structure.

Examination of preparations from rat spinal cord revealed transplanted cultured cells stained with bisbenzimide. Immunohistochemical staining with antibodies against human cell nuclei showed that transplanted cells were localized around the zone of injury. Double staining for human cell nuclei and treatment with antibodies against GFAP or vimentin demonstrated that individual transplanted cells underwent differentiation into glial cells. Staining with antibodies against human cell nestin showed that many implanted cells remained undifferentiated. Nestin-positive cells were associated with vessels and practically did not migrate to the surrounding tissue.

On day 110 after transplantation the area of damage was 5 mm. Fibers localized near the surface layer were completely broken. In the zone of injury moderate changes in the glia were not accompanied by cicatrization. Nerve cells were morphologically unchanged. Cysts were not formed in the zone of injury.

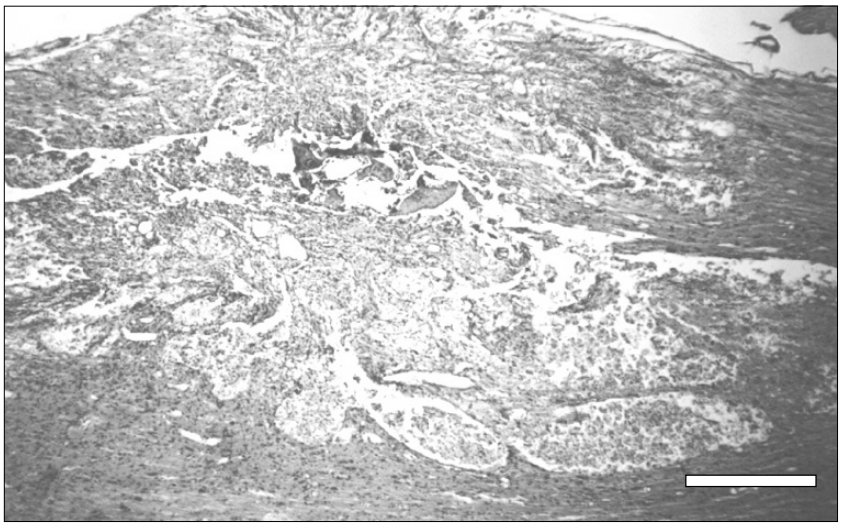

Fig. 2. Cyst formation in the traumatized spinal cord of control rats 15 days after injury. Staining with hematoxylin and eosin. Scale: 500 μ.

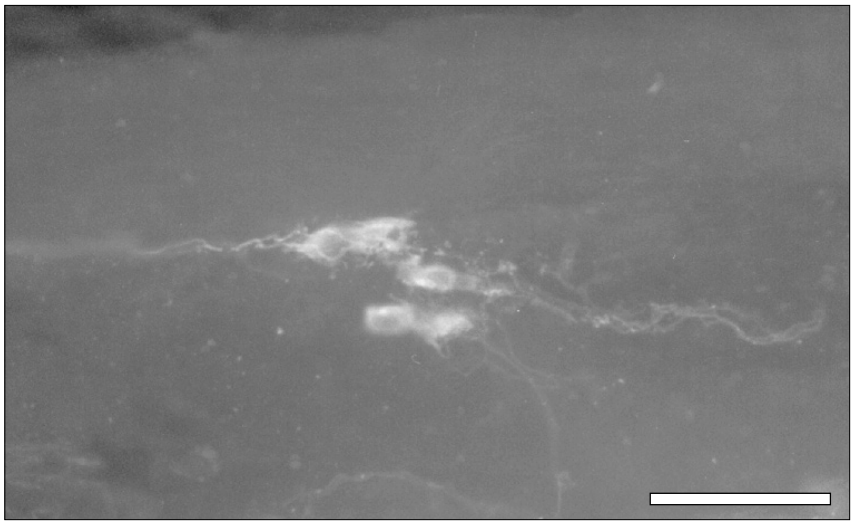

Fig. 3. Differentiation of transplanted stem/progenitor cell of human brain into neurons 110 days after transplantation into the traumatized spinal cord of adult rats. Staining with antibodies against β-tubulin III. Scale: 40 μ.

Transplanted cells were identified by bisbenzimide luminescence and staining with antibodies against human cell nuclei. Nestin-positive cells were absent. Staining with antibodies against β-tubulin III showed that individual implanted cells underwent differentiation into neurons, while other developed into astrocytes and expressed GFAP (Fig. 3).

Our results show that cultured stem/progenitor cells from human fetal brain transplanted into rat spinal cord after mechanical injury survived and retained the ability to migrate and differentiate for 110 days (without immunosuppression). The behavior of implanted cells was similar to that observed after their transplantation into the brain [3]. In the spinal cord transplanted cells migrated along fibers and vessels and were present in the zone of necrosis. Nestin-positive cells were detected among implanted cells up to the 30th day after transplantation. Individual cells were arranged in groups, while others migrated in the brain tissue of recipients. Clusters of stem cells were revealed during mild injury to the spinal cord. It was probably related to the influence of weak traumatic factors that stimulate migration and differentiation of cells [12].

Expression of vimentin, β-tubulin III, and GFAP showed that individual transplanted cells differentiate into neurons and glia.

Transplantation of cultured stem cells induced intensive revascularization, but not cyst formation in the zone of injury (as differentiated from control animals). Therefore, human stem/progenitor cells improve the course of posttraumatic processes in the spinal cord of adult rats.

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