Transplantation of Cultured Human Neural Stem Cells in Rabbits with Experimental Laser-Induced Damage to the Retina

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Cultured neural stem/progenitor cells from human fetal brain were transplanted into the retrobulbar and suprachoroid space in rabbits with laser-induced damage to the retina. Transplanted cells survived, retained multipotent activity, migrated into the zone of injury, and stimulated reparation and regeneration in the traumatized retina.

Key Words: *human neural stem/progenitor cells; retina; laser damage; xenotransplantation; immunohistochemistry*

Recovery of the structural organization and functional rehabilitation of the retina during posttraumatic disturbances, retinal disorders in systemic diseases, and dystrophies of different etiology are actual problems of modern ophthalmology. Transplantation of neural stem cells (NSC) holds much promise for the therapy of these disorders. NSC are the potential source of various cells in the central nervous system, including neurons, astrocytes, and oligodendrocytes [4,10]. Populations of stem cells (SC) were revealed in the ciliary body of the eye in fishes, reptiles, birds, and mammals. They are capable of differentiating into various retinal structures in adult animals [5,13,14]. This prompted the search of these cells in humans. Nestin-positive SC were found in the *ora serrata* of the retina in adult humans [8]. These findings are contradictory to modern notions that human retinal tissue cannot regenerate and restore their structural organization in response to damage. In light of this much attention was given to stimulation of host SC capable of substituting lost retinal cells and to replacement therapy with exogenous SC. In ophthalmology the therapeutic potential of SC is studied on animals with hereditary retinal diseases similar to senile macular degeneration and pigment retinitis (RCS rats and *rd* mice) [11,15]. Experiments are performed with mechanical [9] and ischemic damage to the retina [6]. Most studies demonstrated that transplanted SC undergo differentiation into glial cells (astrocytes and Muller cells), neurons, and opsin-expressing photoreceptor-like cells [12]. The development of transplanted cells in the retina has a strong positive effect and prevents degeneration of retinal structures.

Here we studied the effect of NSC transplantation on reparation and regeneration of the retina under conditions of experimental laser-induced damage. We also examined the state of human neural stem/progenitor cells transplanted into the suprachoroid space of rabbit eye.

MATERIALS AND METHODS

Experiments were performed on 30 Chinchilla rabbits weighing 2.5-3.0 kg. Retinal damage was modeled using an argon laser. Coagulates (20 per eye) were

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applied to the lower half of the fundus through a pane of a 3-mirror lens (mean radiation power 400 mW). The duration of exposure was 0.1 sec. The diameter of the spot was 100 μ. According to the classification of L'Esperance, this treatment produced flash burn III. Thermal lesion of the retina produced by direct exposure to laser heat develops in the pigment epithelium and is accompanied by nuclear necrosis in the outer granular layer and necrosis of inner and outer segments in photoreceptors [2].

We performed 2 series of experiments. In series I the suspension of 6 million cultured NSC in 1 ml physiological saline was injected into the retrobulbar space of the right eye in rabbits with laser trauma of the retina (n=20, experiment). Physiological saline (1 ml) was administered into the left eye (control). In series II, the suspension of NSC was administered into the suprachoroid space (SCS) of both eyes in rabbits with laser-induced damage to the retina (n=10). The animals were intramuscularly narcotized with Relanium-Ketonal mixture before transplantation. Incision of the conjunctiva was made in the upper outer quadrant. The sclera was cut (2 mm) at a distance of 6 mm from the limbus. NSC suspension (1 million cells in 0.05 ml physiological saline) was administered toward the posterior pole of SCS using a Hamilton microsyringe. Immunosuppression was not performed.

NSC were isolated from the brain of human fetuses after medical abortion (9-12 weeks' gestation), dissociated, and inoculated into NPBM growth medium (2×10^6 cells/ml, Neural Progenitor Basal Medium, Clonetics). The medium contained standard growth factors, including human fibroblast growth factor, human nerve cell survival factor, epidermal growth factor,

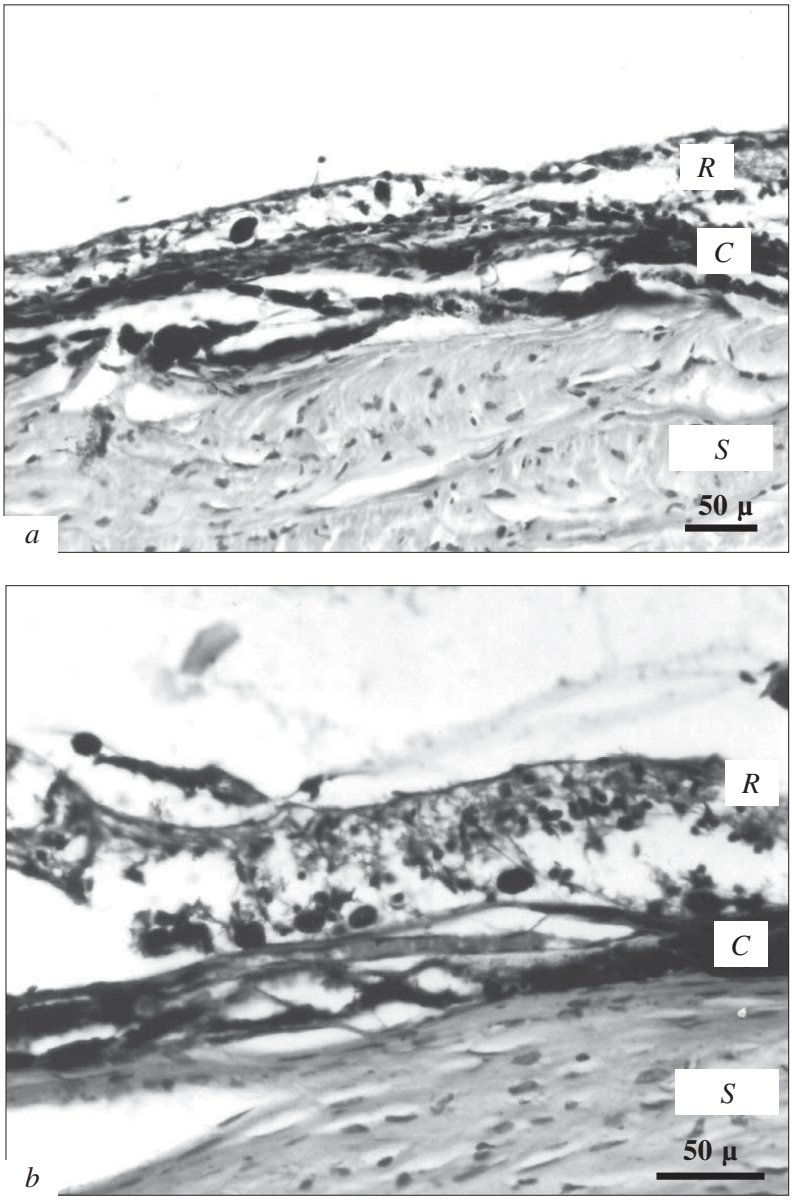

Fig. 1. Histological section of rabbit eye in the zone of laser-induced injury (after 3 weeks). Transplanted neural stem cells (NSC, *a*); control (*b*). *R*, retina; *C*, choroid; *S*, sclera. Hematoxylin-eosin staining, ×112.

and gentamicin/amphoterin (Clonetics). The cell suspension (2×10^5 cells/ml) was cultured in flasks for 7-14 days.

For histological visualization of human NSC they were stained with nuclear luminescent dye bisbenzimide in a concentration of 20 μg/ml (Hoechst 33342, Serva) before transplantation. Suspension of cells and small neurospheres was used for transplantation.

Morphological and immunohistochemical study of enucleated rabbit eyes was performed 1, 5, 10, 20, and 30 days after trauma and NSC implantation. The eyes were fixed with 10% formalin in phosphate buffer and processed using standard histological method. During an immunohistochemical study the sections with transplanted cells were selected by bisbenzimide luminescence, stained with primary antibodies against human cell nuclei (1:30, Chemicon) and human cell nestin (1:20, Chemicon), and examined in luminescent light under an Opton microscope.

RESULTS

In series I, histological examination of control eyes on day 10 after treatment showed complete alteration of the pigment epithelium and retinal layers (coagulation necrosis), thrombosis of choroid vessels, and hemorrhages. In experimental rabbits the pathological changes were less pronounced. We revealed only partial destruction of cells in the pigment epithelium. The inner layer of the retina was preserved in these animals. On day 21 after trauma and NSC transplantation the histological picture in control and experimental eyes differed significantly. In control animals pigment cells migrated into the inner layer of the retina and formed complexes, more coarse chorioretinal adhesions were seen. In experimental animals proliferation of pigment cells was observed only in the outer layer of the retina. Damaged regions were completely reconstructed (Fig 1). Our experiments showed that transplantation of NSC accelerates reparative regeneration of the retina. The stimulatory effect of NSC is related to secretion of various neurotrophic factors that activate compensatory processes in abnormal tissues [7].

In series II we studied the ability of NSC transplanted into SCS to survive, migrate, and integrate into the retina of recipients. The choroid is loosely connected to *lamina fusca sclerae* via long and short connective tissue plates. They are easily destroyed by mechanical factors. This anatomic feature allows using various biological materials for surgical treatment of senile macular degeneration. Most experiments are performed on subretinal or intravitreous transplants of SC. We believe that implantation of transplants into SCS is less traumatic and provides migration of cells in the zone of injury.

Microscopy on days 1, 5, and 10 after treatment revealed transplants of cultured human NSC in animals of various experimental groups. Transplanted human cells were identified by positive staining of preparations and luminescence of cells stained with bisbenzimide and antibodies against human cell nuclei (anti Human Nuclei, Fig. 2, *a*). The density of transplanted cells decreased with increaseing the distance from the site of implantation. Cells migrated to the posterior pole of the eye and reached the zone of laser-induced injury on day 10 after transplantation. Donor cells immunopositive to human cell nuclei were localized individually or arranged in groups. Transplanted cells reached the zone of laser-induced injury and were found in the choroid and outer and inner layers of the retina (Fig. 2, *b*).

Double immunohistochemical staining with antibodies against human cell nuclei and nestin revealed

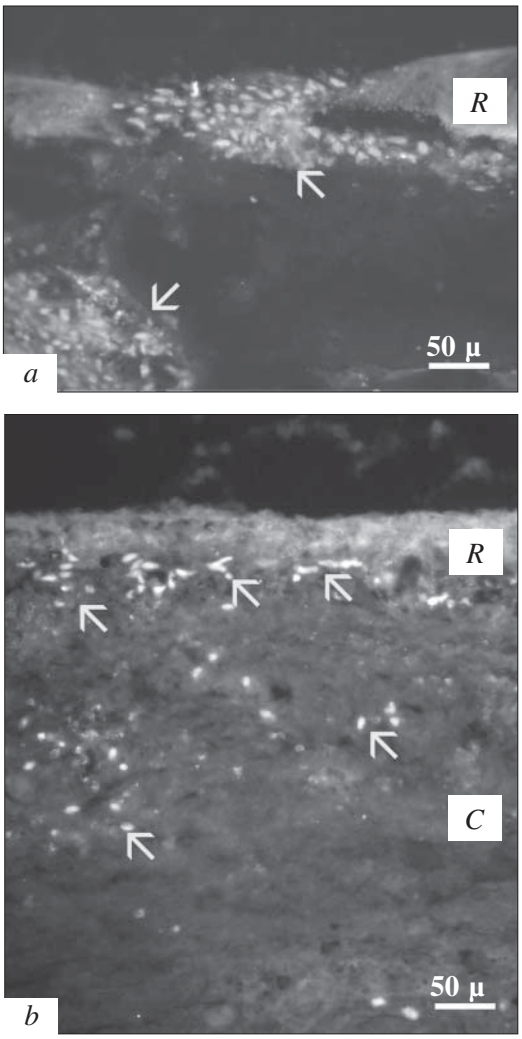

Fig. 2. Human NSC transplanted into subchoroid space in rabbits with laser-induced damage to the retina. Distribution of HSC (arrows) in the retina (*R*) and choroid (*C*) on days 5 (*a*) and 10 (*b*) after transplantation. Staining with antibodies to human nuclear proteins.

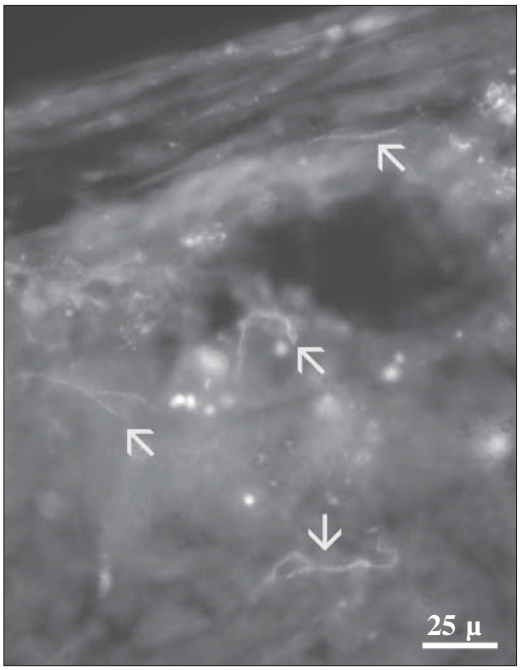

Fig. 3. Solitary nestin-positive stem cells (arrows) with long processes among transplanted NSC. Staining with antibodies to human nestin. Scale 25 μ.

the presence of true SC among transplanted cells. Several nestin-positive cells migrated from the site of implantation to the choroid and retina (Fig. 3).

Our results show the cells behave similarly after transplantation into the brain and spinal cord. *In vitro* cultured NSC retained viability for 30-110 days after xenotransplantation into the hypoxic brain and traumatized spinal cord of rats. They widely migrated, underwent partial differentiation into astrocytes and neurons, and produce a neuroprotective effect on degenerating neurons. It was manifested in normalization of learning and acquisition of a conditioned response in rats [1,3].

Our study illustrate that cultured human neural stem/progenitor cells injected into the retrobulbar space stimulate reparation and regeneration of the retina in rabbits with laser-induced injury, which contributes to the formation of a mild chorioretinal scar. Cells transplanted into SCS retained viability (30 days, no immunosuppression), widely migrated in the zone of laser-induced injury, and were localized in the outer and inner layer of the retina. These changes probably improve visual function.

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