Effect of Transplantation of Cultured Human Neural Stem and Progenitor Cells on Regeneration of the Cornea after Chemical Burn

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We studied the effect of cultured human fetal (8-12 weeks gestation) neural stem and progenitor cells on regeneration of rabbit cornea after alkaline burn. A single subconjunctival injection of cell culture suspension after burn injury significantly accelerated regeneration of the anterior and posterior epithelium in comparison with the control group.

Key Words: *cell therapy; corneal regeneration; chemical burn*

Eye burns belong to the most severe injuries to the organ of vision with a very high percentage of unfavorable outcomes. Many therapeutic and surgical methods for the treatment of corneal burns are now proposed. Traditional therapy includes etiopathogenetic drugs: protease inhibitors, antibiotics, regeneration stimulators [1,5]. Despite numerous methods for the treatment of corneal burn, regeneration of the corneal tissues and recovery of their optical characteristics remains a pressing problem. Cell therapy is used in many fields of medicine for stimulation of damaged tissues with more complete and/or rapid regeneration of the damaged organ. The transplanted material in our experimental study was selected with consideration for the common origination of the anterior epithelium and neural stem cells, which suggests the use of unique properties of these cultured cells in regeneration of the cornea.

We studied changes in the status of corneal tissues after alkaline burn during treatment by means of transplantation of human fetal neural stem and progenitor cell culture. The state of the anterior epithelium was examined, specifically, the number of layers and degree of epithelium differentiation, thickness of the cornea, architectonics of fibrous components and cell composition of the stroma, and degree of posterior epitheliocyte loosening.

MATERIALS AND METHODS

Human fetal (8-12 weeks gestation) brain tissue served as the source of donor material. The cells were cultured in DMEM/F-12 medium (Invitrogen, GiBCO) with N-2 Supplement (1:100; Invitrogen, GiBCO), β-FGF (20 ng/ml; Calbiochem, Merk), EGF (20 ng/ml; Invitrogen, GiBCO), heparin (8 μg/ml; Sigma), penicillin (100 U/ml), streptomycin (100 μg/ml) (Invitrogen, GiBCO).

The cell suspension (2×10^6/ml) was cultured in 25 cm^2 culture flasks (Cornig, INC) at 37°C and 5% CO_2. Culture medium was replaced by 50-75% every 4-7 days. Formation of neurospheres was noted on days 7-9; they were isolated from the medium on days 14-21, treated with trypsin, and centrifuged. Cell suspension (1.5×10^6/ml) in normal saline with cell viability of 97-98% (according to Trypan blue staining) was used for transplantation.

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The study was carried out on 75 rabbits (2-2.5 kg) with modeled severe alkaline burns of the cornea. Disks (7 mm in diameter) of sterile chemical filter paper were impregnated in 10% NaOH for 5 min, transferred to cuvette walls for removal of excess alkali, and placed onto the corneal surface (strictly central area) for 40 sec. After removal of the disks the eyes were washed with saline (20 ml) for 30 sec. All manipulations were carried out under total anesthesia with 5% ketamine (50 mg/kg), 0.5% relanium solution (2 mg/kg), and 1% diphenhydramine solution (1 mg/kg), injected subcutaneously, and local analgesia of the cornea (1% dicaine).

Immediately after inflicting the burn, experimental animals received a single subconjunctival injection of suspension of human fetal neural stem and progenitor cells (3×10^5 cells in 0.2 ml saline). Controls were injected with 0.2 ml saline. Local treatment with levomycetin (0.25% solution 3 times daily) was carried out for preventing secondary infection in the eyes. The eyes were enucleated after sacrifice on days 1, 3, 7, 14, 22, 30, and 60 of the experiment. Histological studies were carried out on paraffin sections (5-7 μ) stained with hematoxylin and eosin.

RESULTS

No anterior epithelium was seen in the histological preparations on day 3 after burn in experimental and control animals. The growth of flattened epitheliocytes (one cell layer) from the paracentral to the central optical zone was observed later, and after fussion of the leading edges the number of layers increased, but the anterior epithelium had a trend to desquamation (Fig. 1, *a*, *b*). By day 30 the anterior epithelium of the central optical area consisted of 2 layers of epitheliocytes in controls and 4 layers in experimental animals. However, by this period no pronounced differentiation of the epithelium into basal, pricky, and squamous cell layers was observed in any of the groups. By day 60 central optical area of the cornea in control animals had up to 3 layers of undifferentiated epitheliocytes,

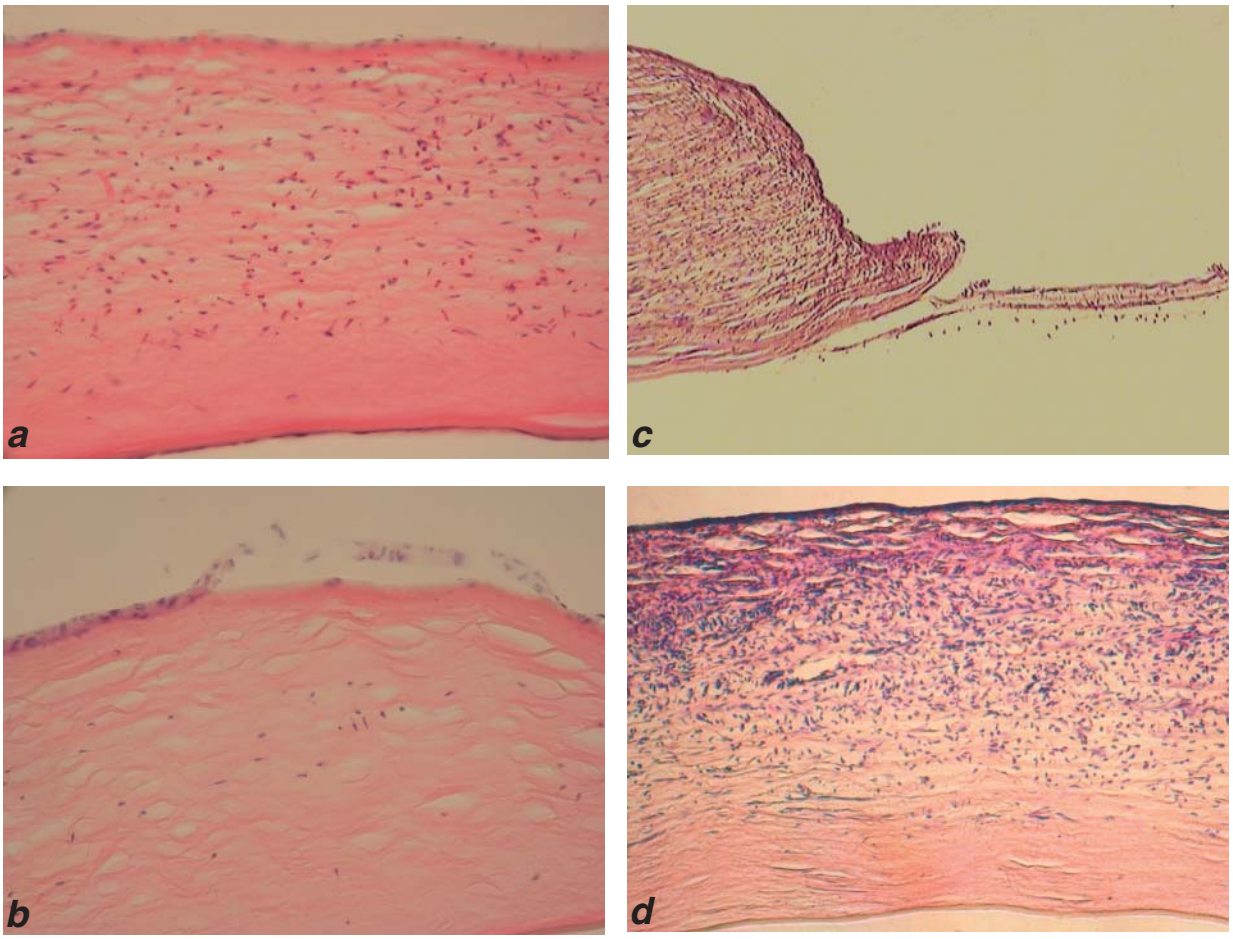

Fig. 1. Paracentral area of the cornea. *a*) 3 days after burn, control; *b*) 3 days after transplantation, experiment; *c*) 21 days after burn, control; *d*) 21 days after transplantation, experiment.

TABLE 1. Thickness of the Cornea in the Central Optical Area (μ)

Group	Day						
	1	3	7	14	21	30	60
Control	311.8	266.6	133.3	250	8.3	275	500
Experiment	308.3	275	350	341.6	616.6	425	350

TABLE 2. Thickness of the Cornea in the Paracentral Area (μ)

Group	Day						
	1	3	7	14	21	30	60
Control	666.6	558.3	391.6	291.6	8.3	325	200
Experiment	412.5	625	600	375	300	225	233.3

while in experimental group the anterior epithelium formed up to 5 layers of epitheliocytes with signs of differentiation. Desquamation of the anterior epithelium of the central optical area of the cornea at early terms of the experiment resulted from continuing local inflammatory reaction, presenting on histological preparations as increased counts of lymphocytes, granulocytes, loss of stromal fibers, stromal edema and vascularization. Moreover, injury to the anterior corneal epithelium stimulates apoptotic death of underlying fibroblasts [10]. This is due to IL-1α and IL-β release from destroyed epithelium and subsequent activation of fibroblasts releasing growth factors. Later these factors activate proliferation of the anterior epithelium and, presumably, its hyperplasia, but inhibit differentiation. Up to day 3 of the experiment the stroma was presented by cell-free connective tissue. The severity of chemical burn of the cornea cannot be evaluated at once, because the effect of alkali with destruction of protein structures can develop for several days longer and lead to thinning and even perforation of the corneal membrane. Necrotically modified tissue is a chemical attractant for neutrophils, which migrate from dilated limbic blood vessels and induce migration of new neutrophils by production of special mediators. The phagocytic function of neutrophils is associated with the release of proteolytic enzymes, which also leads to ulceration of the cornea. In the control group we observed destructive changes in the cornea, manifesting by its thinning and in some cases complete perforation in the central optical area (Fig. 1, *c*, *d*). The thickness of the cornea is an important indicator of its optical characteristics; significant thinning or thickening of the cornea leads to loss of visual acuity. Normally, thickness of rabbit cornea in the central optical area is 300 μ. In the experimental group the thickness of this corneal area increased by this period, which seemed to be due to not only stromal edema, but also to increased count of fibroblasts and their synthetic activity. By day 30 and further on, the mean thickness of the cornea in experimental group was 425 μ. By day 60 of observation corneal edema was markedly reduced in experimental group (Tables 1, 2). This manifested by more compact packing of fibrous components of the stroma and less pronounced vascular infiltration. Corneal stroma at the early terms of the experiment was characterized by looseness of fibrous components in both groups, which promoted vascular growth from the corneal limbic area and intensive leukocyte and granulocyte (particularly, eosinophil) infiltration. Dilatation of blood vessels was observed in the limbic area 1 day after burn, increase of the diameters to 83.3 μ being observed in the control group, compared to maximum diameter of 41.6 μ in experimental group during the same period. By day 3 the counts of lymphocytes and polymorphonuclear cells migrating from the corneal limbic vessels increased appreciably in the paracentral area of corneal stroma.

The posterior epithelium (endothelium) was absent 1 day after the injury in the central part of the cornea as a result of its desquamation after burn. After 3 days sites, of endotheliocytes migrating from the adjacent areas were detected in the paracentral ocular area in both groups, with the mean distance between the cell nuclei 16.6 μ. On day 7 of the experiment the endothelium of the central optical area was restored in experimental group, with the mean distance between endotheliocytes 8.3 μ. In controls the endothelium of this area started to restore by day 30 of the experiment, with the mean distance between the cells 25 μ. Hence, the anterior corneal epithelium in rabbits regene-

rated by day 30 after burn in experimental group and by day 60 in controls. The posterior corneal epithelium started to restore by day 7 in experimental group and by day 30 in control group. The inflammatory reaction of corneal tissues (cell infiltration, vessels, loose fibrous components of the stroma) was less pronounced in experimental group compared to controls. The productive stage of inflammation (fibroblast proliferation and migration, restructuring of stromal intercellular substance, differentiation of the anterior epitheliocytes) was much more rapid in experimental group during the entire experiment in comparison with the control.

Positive facts of effective regeneration of the anterior epithelium, regeneration of the endothelial layer and corneal stroma in experimental group indicate the efficiency of transplantation of the culture of human fetal neural stem and progenitor cells for the treatment of corneal burns. Human stem and progenitor cells migrate after transplantation towards the focus of injury and are effective for neurotrophic stimulation [2-4,6,8,9], and hence, presumably these cells, injected subconjunctivally, stimulate reinnervation of the cornea, which is essential for recovery of its optical characteristics. The nervous system of the cornea regulates all its tissues, coordinating their activity and normal function under varying environmental conditions.

The results of histological studies indicate the efficiency of cell therapy after experimental alkaline burn.

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