FEATURES OF CYTOKINE ADJUSTING MALFUNCTION FOR PATIENTS WITH GOUT DEPENDENT UPON THE STAGE, LEVEL OF COMORBIDITY AND TREATMENT

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Abstract. It has been established that in patients with gout, dependent on the stage of its development and intensity of polymorbidity and comorbidity adjustings of chronic inflammatory process progress and cytokine chain gets disturbed. Attainment of clinical remission of articular syndrome in patients with gout and significant expression of imbalance of pro- and anti-inflammatory cytokines is indicative of pathogenic "contribution" of comorbidity pathological conditions, especially metabolic syndrome, to support the chronic inflammatory process.

Key words: gout, comorbidity, cytokine, treatment.

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CORD BLOOD DERIVED NUCLEAR CELLS USAGE IN ANIMAL MODEL OF NEOVASCULAR RETINOPATHY

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Abstract. The effect of cord blood derived nuclear cells on neovascular retinopathy in experimental rats was studied.

Regression of neovascular tissue, retinal structure normalization was found after 45 day post single intravitreal injection of cord blood derived nuclear cells.

Key words: cord blood, neovascularization, retinopathy, stem cells.

Introduction. Over ten years ago quiescent neural stem cells in mammalian retinas were found [16]. Besides promising aspects of the biology of stem cells in experiments, the use of these cells has been suggested as a treatment to the retinal diseases [15].

The field of stem-cell research holds great potential for the treatment of retinal disease. However, stem-cell therapy application in practice is always balancing between a successful transplantation of cells in experiments and controversial results of the first clinical trials [5].

Most blinding retinal pathologies in its pathogenesis pass through a neovascularization phase. These diseases include diabetic retinopathy, wet age-related macular degeneration, retinopathy of prematurity, and neovascularization due to central retinal vessels occlusion.

Despite significant advances in traditional treatment, prevention of complications and progression of the retinal pathology and optic nerve [11-14], we could not restore lost visual functions to date. In addition, the question about the multiplicity of injections of anti-VEGF drugs and the amount of repeat procedures remains uncertain.

Successful results and broad application prospects of cryopreservated stem cells in different areas of medicine sparks interest in their use in ophthalmology.

Stem cells (SC) have a high proliferative capacity and the ability to reproduce themselves to maintain homeostasis tissue they belong to. The resulting current data indicate a positive impact of the stem cells use for damaged tissue regeneration. There are several sources of retinal neurons, including neuronal SC, ciliary margin cells, embryonic SC, bone marrow and cord blood [5].

15 years ago Japanese scientists showed successful transplantation of neuronal SC in the damaged retina in experiment [10]. However, these stem cells derived from hippocampus and subventricular zone, therefore their getting is a complex, time-consuming and expensive process.

In earlier experiments, the ciliary epithelium cells were also considered to be possible sources of stem cells in the retina [16]. However, according to Kharuta marginal cells lose their ability to regenerate shortly after transplantation [8].

Embryonic stem cells (ESCs) have high differentiation ability. Human embryos are most fre-

quently destroyed as the stem cells are harvested. A recent study created new stem lines by deriving human ES cells from single blastomeres, which allowed obtaining the material without destroying embryos [9]. Unfortunately, this source is not available for Ukrainian researchers.

Auto bone marrow cells (BMCs) do not suffer in transplantation from the problem of the human leukocyte antigen (HLA) barrier, but, since these BMCs would also carry the same abnormal gene as the retinal neural cells (RNCs) in hereditary diseases, the disease(s) could redevelop.

Cord blood stem cells (CBSCs) express the same markers as ESC, and can differentiate into the three embryonic layers: mesoderm (endothelium-like cells), ectoderm (neuron-like cells) and endoderm (insulin producing cells). CBSCs are less demanding in terms of division compared to embryonic cells. However, it should be noted that the potential for long-term proliferation of CBSCs is significantly reduced during prolonged cultivation [17].

It can be assumed that further optimization of cryopreservation conditions, low temperature storage and cultivation will improve the proliferative potential of CBSCs.

Moreover, from a technical standpoint, getting CBSCs is much simpler than ESCs. Thus, cord blood cells are the most suitable source of stem cells. Development of slow-release formulations of the drug substance and methods of gene therapy to stimulate retinal cells released neurotrophic factors are solutions to the problem of therapy duration. The use of stem cells as long-acting agents may be the most affordable way [7].

The aim. To study retinal morphological changes after intravitreal injection of cryopreserved cord blood stem cells to experimental animals with neovascular retinopathy.

Materials and methods. In our study we used newborn Wistar rats (n=30). We used brinzolamide-induced retinopathy as a model of neovascular retinal pathology. Brinzolamide was injected intraperitonealy to 20 rats in dose 200 mg/kg, twice daily from days 2 to 7 [3-4]. After 5 days of recovery the rats were randomized to either:

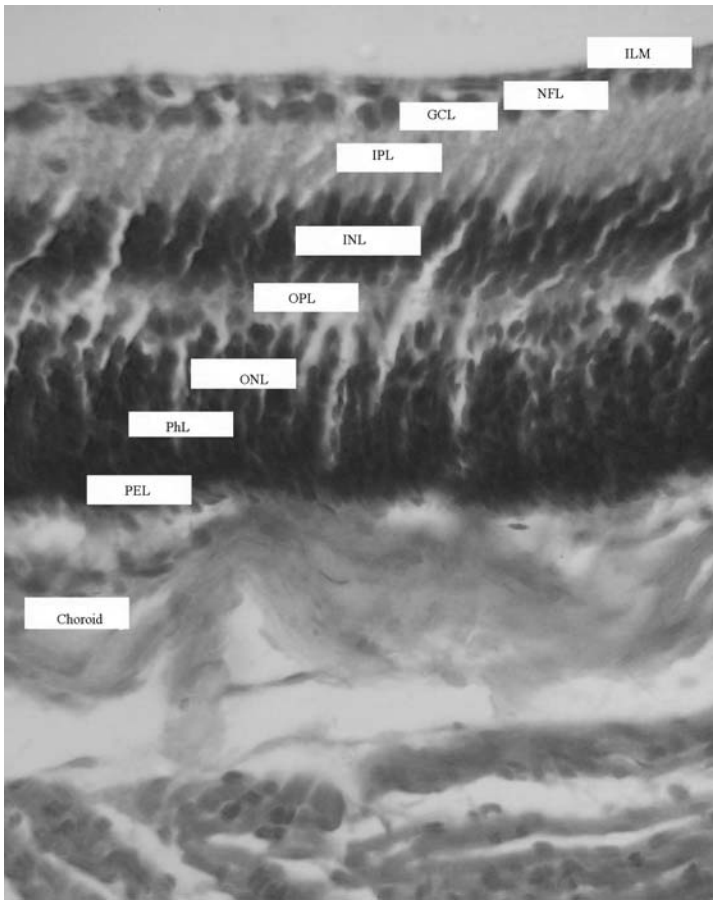
First group – experimental. Rats were given intravitreal nuclear cells injection n=10 (20 eyes);

Second group – control. Animals with no treatment, n=10 (20 eyes);

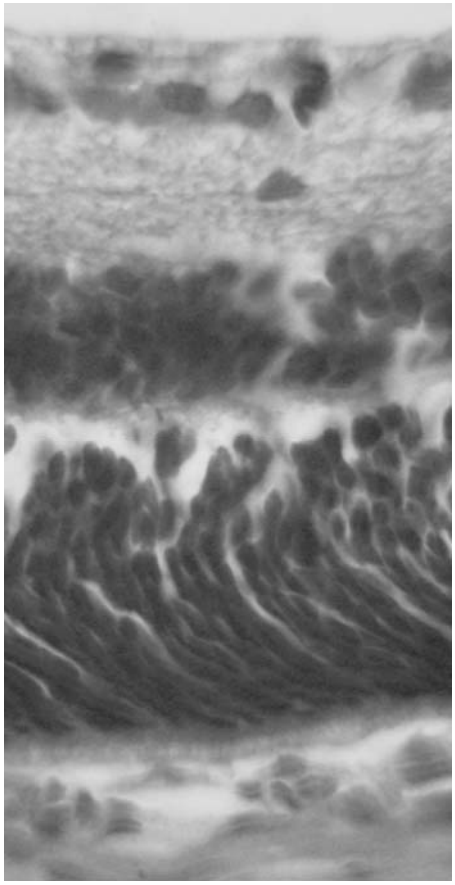
Third group – intact. Normal rats, n= 10 (20 eyes).

For experimental treatment we used cryopreserved cord blood derived nuclear cells (CB NC). We performed single intravitreal injection of CB NC in dose 0, 0125 ml (100 000 cells) to animals from experimental group.

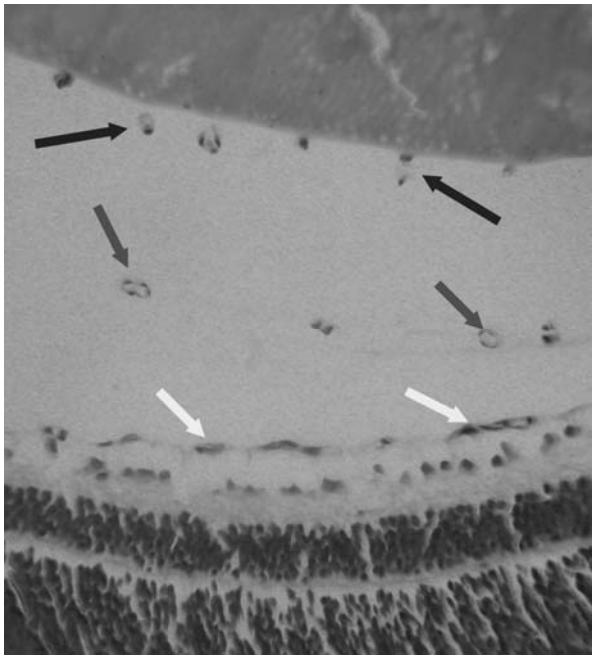
Animals were treated according to European Convention for the Protection of Vertebrate Animals



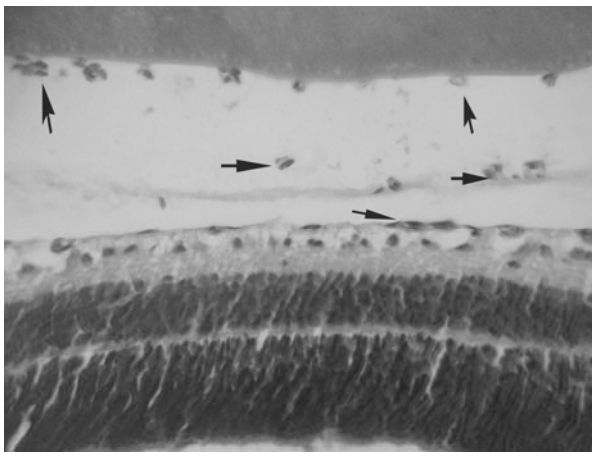
Pic. 1. Normal rat retina. Hematoxylin-eosin staining. ×400: internal limiting membrane (ILM), nerve fiber layer (NFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), photoreceptors layer (PhL), pigment epithelium layer (PEL)



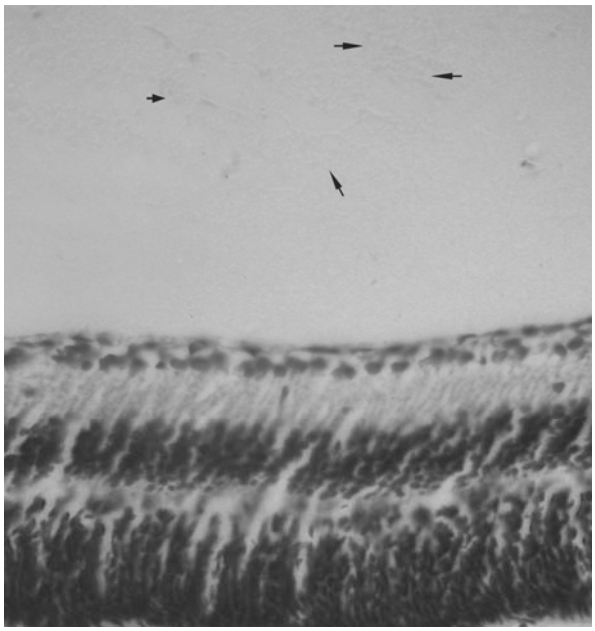
Pic. 2. Normal rat retina. Hematoxylin-eosin staining. ×1000, Immersion



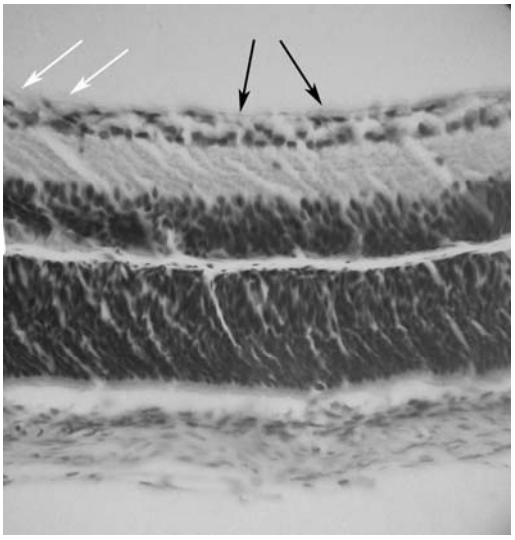
Pic. 4. Control rat retina. Hematoxylin-eosin staining. ×100. Arrows indicate neovascular tufts above ILM (white), in the vitreous cavity (grey), near by lens posterior capsule (black)



Pic. 3. Control rat retina. Hematoxylin-eosin staining. ×100. Arrows indicate neovascular tufts



Pic. 5. Experimental rat retina. Hematoxylin-eosin staining. ×400. Arrows indicate conglomerates of involutioned newly formed vessels



Pic. 6. Experimental rat retina. Hematoxylin-eosin staining. ×400. Arrows indicate ILM, that is clearly visualized, its surface is smooth (black arrows). At the extreme periphery of the retina there are newly formed vessels involution (white arrows)

Table 1

Dynamics of morphometric changes in the retina of animals in the experimental and control groups (в мкм)

	Experimental group		Control group	
	Mean	±SD	Mean	±SD
Central zone	180*	1,41	165	0,63
Periphery	139,8*	2,0	125	1,41
IPL	29,2*	0,75	23,2	0,98
OPL	7*	0,9	5	0,63
INL	24*	1,55	17,8	0,75
ONL	40,5*	1,38	31,8	1,17
GCL	19*	1,1	15	1,41

*- statistically significant ($p<0,05$) difference according to Student's criteria

Table 2

Dynamics of morphometric changes in the retina of animals in the intact and experimental groups (в мкм)

	Experimental group		Intact group	
	Mean	±SD	Mean	±SD
Central zone	180*	1,41	206	1,41
Periphery	139,8*	2,0	155	0,98
IPL	29,2*	0,75	38,2	0,75
OPL	7*	0,9	9,3	1,03
INL	24*	1,55	27,7	1,86
ONL	40,5*	1,38	43,7	1,03
GCL	19*	1,1	22	0,63

*- statistically significant ($p<0,05$) difference according to Mann-Whitney's criteria

used for Experimental and Other Scientific Purposes (Strasbourg, 18.III.1986). On the 45th day all the rats were euthanized. Each eye was enucleated and kept immersed for at least 21 days in a fixative solution containing 4 % paraformaldehyde [1]. Six paraffin-embedded sections (thickness, 5 μm) cut through the optic disc of each eye were prepared in a standard manner and stained with hematoxylin and eosin. Light microscope images were photographed using a digital camera.

To investigate the damage in each retinal layer after intraperitoneal brinzolamide or saline injections, we evaluated the number of cells in the ganglion cell layer (GCL) and the thicknesses of the inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), and outer nuclear layer (ONL). The cells in GCL and the thicknesses of the IPL, INL, and ONL at a distance between 375 and 625 μm from the optic disc were measured on the photographs in a masked fashion by a single observer.

Results & Discussion. Rat retinas from intact group animals is represented by ten distinct layers that had different thickness in the central and peripheral areas (204±2,16 μm and 154,06±1,61 μm, tabl.1). Photoreceptor outer segments were short, most were fragmentary. Pigment epithelial cells formed a brushy structure, contained dark areas. Thickness of IPL was 42±2,19 μm, OPL – 18±1,63 μm, INL – 40,94±1,77 μm, ONL – 55±1,89 μm, GCL – 23,06±1,76 μm (tabl. 1).

In the retinas of intact animals the internal limiting membrane (ILM) in the central and peripheral parts was thin, but well-defined (pic. 1).

This membrane was neuroglial and consisted mainly of astrocytes, there were no newly formed vessels in its structure (pic. 2).

Meanwhile, in the rat eyes from the control group were revealed morphological patterns of developed proliferative retinopathy, exciting medial and extreme peripheral areas of the retina (pic. 3).

Neovascular tufts located not only at ILM, but also on in the vitreous cavity, near by lens posterior capsule (pic. 4).

Internal limiting membrane had a vague outline and was poorly differentiated. On its surface newly formed vessels were defined. In addition, almost the entire surface of the ILM determined the formation of fibrous tissue (epiretinal membrane). Peripheral part of the retina was positive to traction deformation.

In the rat eyes from the experimental group at the mid-peripheral retina we observed newly formed vessels at different stages of involution. Latter formed conglomerates of membrane structures with the nuclei of endothelial cells (pic. 5).

Growths of fibrous tissue on the surface of the ILM were not determined. ILM was clearly visualized in all sections. At the retinal extreme periphery areas of desolated newly formed vessels as well as smoothing and flattening of the retinal layers were determined (pic. 6).

In addition, there was an increase in the retina thickness in experimental group treated with the CB NC, compared to those in control group (tabl. 1). The difference in the central and peripheral areas maintained. To note, the greatest changes were discovered in the inner nuclear layer. It is well known there are located horizontal, bipolar cells, as well as a large number of Muller cells. It is proved that the latter, having a high metabolic activity, deliver products of retinal neurons glycogen breakdown required for aerobic metabolism. Muller cells also carry out removal of metabolic products from neurons (carbon dioxide, ammonia, products of amino acid metabolism), and protect neurons from excessive release of neurotransmitters. It is important to indicate that the cells of Muller express retinoic acid that is important in the development of the retina, central nervous system, as well as the visual pigment metabolism [17].

Comparing the morphometry data from intact and experimental groups, we noticed that the retina of animals from the experimental group still remains more flattened, but there was a tendency of layers thickening, mainly the inner nuclear layer (tabl. 2).

Conclusions

1. Thus, the potential possibility for regression of newly formed blood vessels in the retina was demonstrated. It is promoted by administering the preparation of cryopreserved cord blood to the experimental animals. This fact was confirmed pathologically. After 45 days from the beginning of experiment, and 30 days after intravitreal injection it was morphologically identified as follows:

2. Newly formed vessels in the eyes of rats in the experimental group underwent involution and forms a conglomerate of membrane structures with few nuclei of endothelial cells;

Neovascularization was not accompanied by appearance of fibrous tissue and epiretinal membrane formation in the eyes of rats in the experimental group.

Neovascular retinopathy with active neovascular membrane and vitreo-retinal traction in the posterior segment of the eyeball was detected in the animals' eyes from the control group.

Thus, the newly formed vessels appearing after intraperitoneal administration of brinzolamide in a dose of 200 mg / kg and underwent involution gradually after single intravitreal injection of cryopreserved cord blood. Injection of cryopreserved cord blood hindered the development of an active proliferative retinopathy with vascular epiretinal membranes and high risk of retinal detachment. In addition, there was an increase in the thickness of the retinal layers (mainly inner nuclear layer) in the experimental group which suggests a high functional activity of these cell layers. These data suggest that the influence of cryopreserved cord blood cells may both reverse the development of existing new vessels and suppress neovascularization process in general.

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ПАТОМОРФОЛОГИЧЕСКИЕ АСПЕКТЫ ПРИМЕНЕНИЯ КРИОКОНСЕРВИРОВАННЫХ ЯДРОСОДЕРЖАЩИХ КЛЕТОК ПУПОВИННОЙ КРОВИ ПРИ НЕОВАСКУЛЯРНОЙ РЕТИНОПАТИИ У ЭКСПЕРИМЕНТАЛЬНЫХ КРЫС

Ю.А. Дёмин[1], П.В. Белецкая[2]

Резюме. В экспериментальной работе изучалось действие препарата криоконсервированной кордовой крови на сетчатую оболочку крыс с моделированной неоваскулярной ретинопатией.

Установлено, что на 45-ые сутки после однократного интравитреального введения ядросодержащих клеток пуповинной крови происходит обратное развитие новообразованных сосудов и нормализация архитектоники сетчатой оболочки.

Ключевые слова: криоконсервированная кордовая кровь, неоваскуляризация, ретинопатия, стволовые клетки.

ПАТОМОРФОЛОГІЧНІ АСПЕКТИ ЗАСТОСУВАННЯ ЯДРОВМІСНИХ КЛІТИН КОРДОВОЇ КРОВІ ПРИ НЕОВАСКУЛЯРНІЙ РЕТИНОПАТІЇ У ЕКСПЕРИМЕНТАЛЬНИХ ЩУРІВ

Ю.А. Дьомін[1], П.В. Білецька[2]

Резюме. В експериментальній роботі вивчалася дія препарату кріоконсервованої кордової крові на сітківку щурів із модельованою неоваскулярною ретинопатією.

Встановлено, що на 45-ту добу після одноразового інтравітреального уведення ядровмісних клітин кордової крові відбувається зворотний розвиток новоутворених судин і нормалізація архітектоніки сітківки.

Ключові слова: кріоконсервована кордова кров, неоваскуляризація, ретинопатія, стовбурові клітини.

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