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D.V. Dombrovsky, V.V. Savin, V.V. Maksymyuk*
Regional Clinical Hospital, the Department of Vascular Surgery, Chernivtsi;
**HSEE of Ukraine "Bukovinian State Medical University", Chernivtsi*

TRANSPLANTATIONS OF THE CORD BLOOD STEM CELLS UNDER CONDITIONS OF EXPERIMENTAL ISCHEMIA. MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISTICS

ТРАНСПЛАНТАЦІЯ СТОВБУРОВИХ КЛІТИН КОРДОВОЇ КРОВІ В УМОВАХ ЕКСПЕРИМЕНТАЛЬНОЇ ІШЕМІЇ. МОРФОЛОГІЧНА І ІМУНОГІСТОХІМІЧНА ХАРАКТЕРИСТИКА

Резюме. У статті наведено результати порівняння морфологічних і імуногістохімічних досліджень процесів, у результаті моделювання ішемії нижніх кінцівок і трансплантації стовбурових клітин кордової крові лабораторних щурів. У результаті експерименту виявлено, що введення кордової крові на тлі ішемії кінцівок у дослідних тварин відмічено постійну структурну стимуляцію регенераторних процесів ангіогенезу. Також відмічається зниження та відсутність фіброзування. Наведені результати обґрунтовують перспективність проведення подальших досліджень у даному напрямку.

Ключові слова: хронічна ішемія нижніх кінцівок, кордова кров, трансплантація.

About 171.000 patients with chronic ischemia of the lower extremities are operated on every year in Europe, herein, the number of amputations constitutes 150-280 by1 mln of the population. 160.000 reconstructions are performed in the USA, till 200 amputations by 1 mln /year. In Ukraine chronic ischemia of the lower limbs constitutes 20-40% of all chronic obliterating diseases of the lower extremities arteries. The extremity is successfully preserved in 55% of patients, initial amputation of the limb is performed in 25% and 20% pass away [1, 2]. Individual methods of the lower limbs revascularization such as transversal sympathectomy, fasciotomy, osteotrepanation, transplantation of the skin patches on pedicle, autotransplantation of the vascular pedicle cap, autotransplantation of the bone marrow aspirate, transplantation of multipotent cells of adipose tissue [3] are more often put into practice for the treatment of the patients, where it is impossible to perform reconstructive operations.

Bone marrow, peripheral blood, umbilical blood, adipose tissue, tissue of the umbilical cord, amniotic fluid, skin, tooth apex, nasopharynx mucous – are the most studied sources of the stem cells of the adult organism. Lately the presence of the stem cells has been revealed in the breast milk, hyppocamp, subventricular zones and in the areas of the olfactory bulbs [4].

Cord blood in its content contains a great number of biologically active substances such as: specific placental proteins, hormones, cytokines, hemopoietic factors, interleukins, opioid peptides, enzymes and proenzymes, vitamins and microelements, reproductive immunomodulators, growth factors. These substances are in the balanced concentrations and form biologically active complex, necessary for the organism, which develops, and normalizes the metabolism when introduced into the adult organism. The influence of a strong enzymatic system of the placental origin, determining the normal course of pregnancy and labor, is added to the effect of the cord blood biological activity. The cells of the cord blood produce a great number of cytokines, which, as a rule, have pleiotropic action [5].

Cord blood is used in the treatment of the diseases of the blood, nervous system, immunodeficiency, for the renewal of the inhibited blood formation [6], therefore banks of umbilical blood exist almost in every developed country, as well as in the majority of the developing countries.

Aim of the research: the aim of our research is studying the influence of the stem cells of the cord blood on angiogenesis processes under conditions of experimental ischemia.

Material and methods. *Experimental investigations on 30 rats, the average weight of which was 240 g, aged 6±1.2 months, being under the room temperature and usual laboratory diet intake, have been carried out. The animals were divided into 2 groups: group I – the animals, which underwent ischemia*

modelling, group II – animals to which cord blood cells were introduced against a background of simulated limb ischemia. All conditions of aseptics and antiseptics were kept while carrying out investigations. Modelling of the limb tissue ischemia in rats was attained by means of putting two kapron ligatures round the vascular pedicle of the femur, thus, artery, vein and nerve are ligated, strengthening ischemic phenomena in the extremity (T.A. Kniazeva method) [7]. Cryoconservative cell suspension was obtained from the umbilical blood bank of LLC "Institute of cell therapy". Frozen samples were preserved in liquid nitrogen at temperature 196°C in cryodepot "38K w/Kryos Controler".

Cellular suspension was used in such parameters: content of nuclear cells – from 0.11×10 9 till 3.7×10 9, the number of mononuclears – 15-60%, CFU of hematopoietic cells – (50±10) × 10.3/ml, content of hematopoietic cells, carrying CD34+ CD45+ and CD117+ CD45+[8] markers on their surface, was equal (0.85±0.20) and (1.52±0.39)% correspondingly. Cell viability – (80±10)%. Stem cells of the cord blood were introduced into ischemic limbs on the 3rd day following ischemic modelling subfascially with thin strip on a medial surface of the femur.

The general quantity of the introduced cord blood constituted (50±5) ml. Statistic processing of the data obtained was carried out according to Стьюдент with determination of t-criterion by "BioStat" program.

Muscular tissue of the medial and lateral surfaces of the femur on the side of experimental conduction was taken on the limb on the 3-5, 10-14, and 21-25 days after ischemia modelling. Histological investigation was carried out by N.Z. Slinchenko method, immunohistochemical investigation of the obtained muscular tissue by means of determination of vimentin expression and Villebrand factor.

Electronic microscope Delto Optical Evolution 100 (Poland) with the object-glass 10^x (planokhromt) and ocular 10^x on the basis of Olympus SP-550 UZ camera was used to conduct electronic-microscopic research of the muscular tissue. Thickness of sections is 5 mcm. Immunohistochemical research was carried out by means of Reagents sets on the basis of polymeric system of detection with peroxidase mark and visualization by means of diaminobenzodine stain (Dako).

Results and discussion. In both groups oedema of the muscle interstitium and myositis necrosis are observed in both groups of the muscular system. Separate interstitium cells have vimentin-positive staining that indicates their mesenchymal origin. Location and shape of these cells correspond to fibroblasts and fibrocytes.

In the first group of the animals under study progressive desquamation and necrosis of endotheliocytes with obliteration of the vascular lumen are observed. Separation of fibers and vascular wall oedema occur there as well. Haemorrhages foci and separate multipotent cells against a background of oedema of intermuscular areas are observed. Oedema increase of the muscle interstitium lasts till 10-14 days with its further decrease to 21-25 days (fig. 1). Separate endothelial cells were marked too.

A part of the muscular fibers remains in the state of necrosis or is replaced by the connective tissue. It proves to be true in case of arrangement of histochemical technique on vimentin. Separate cells have vimentin-positive staining on the 3-5 days of the experiment indicating their mesenchymal origin; their shape and size correspond to fibroblasts and fibrocytes.

Vimentin expression was the biggest till 14 day from the moment of ischemia simulation, in intermuscular fibers, surrounding vascular fascicle, as well as in the membranes of the vascular walls of the venous and arterial types, that is the evidence of intermediate mesenchymal filaments.

Vimentin expression in perimysium round the vessels. Polymeric method of determining vimentin expression (monoclonal antibody against vimentin, V9) with peroxidase mark and diaminobenzodine counterstaining. Oc.10, Vol.10.

During the whole term of the experiment positive staining on Villebrand factor was not revealed in the animals of group I.

So, the evident changes, characterized by circulation disorder in the vessels, oedema of interstitium of the muscular tissue, decreasing only on the 25thday, were observed in the animals of group I till 14 day owing to ischemia simulation, however, pronounced sclerosis of the muscular tissue appears there.

In animals of group II mosaic changes in myoplast – phenomena of dystrophy, myoplast destruction and interstitium oedema, took place on the 3rd day after transplantation. Endothelial cells densely colonize interstitium on the 10-14 days of the experiment, an increased number of fibroblasts is noted.

On the 10-25 days foci of angiogenesis and regeneration have been revealed, granulation tissue on the stages of maturing into scar with a significant quantity of collagenous fibers, a great quantity of cells (predominantly fibroblasts) and blood vessels, where circulation occurs, have been noted (fig. 2).

Mature vimentin- positive endotheliocytes of the blood vessels were revealed when applying immuno-

histochemical technique. Beginning from the 5th and, in particular, 10-14 days after the cord blood introduction, the signs typical for hypoxia, gradually disappeared. Interstitium is densely colonized with vimentin-positive multipotent cells. The presence of macrophages and formation of the initial vascular structures were observed.

On the 21th day angiogenesis and regeneration foci with multiple small vessels, located in the connective tissue and fibrous foci are evident. Mature vimentin-positive endotheliocytes of the blood vessels, as index of the stimulated angiogenesis have been revealed (fig. 3).

Small disseminated groups of cells with clearly positive staining on Villibrand factor, enabling to ascertain pronounced non angiogenesis in these rats just on the 10-14 day and subsequent term have been noted when studying the muscles of ischemic limb of group II animals.

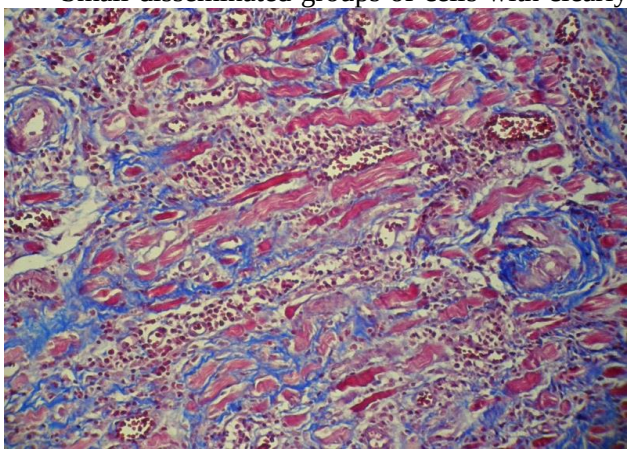

Fig. 1. Microphotography. The twenty fifth day of simulated ischemia. Sclerosis (fibrosis) of the muscular tissue. Staining according to N.Z. Slinchenko method. Vol.10; Oc.10

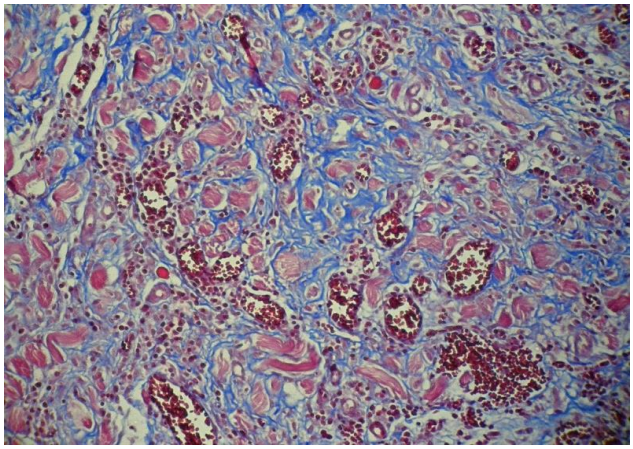

Fig. 2. Microphotography. Twenty fifth day following transplantation of the stem cells of the cord blood. Blood vessels where circulation occurs. Staining according to N.Z.Slinchenko method. Vol.10; oc.10

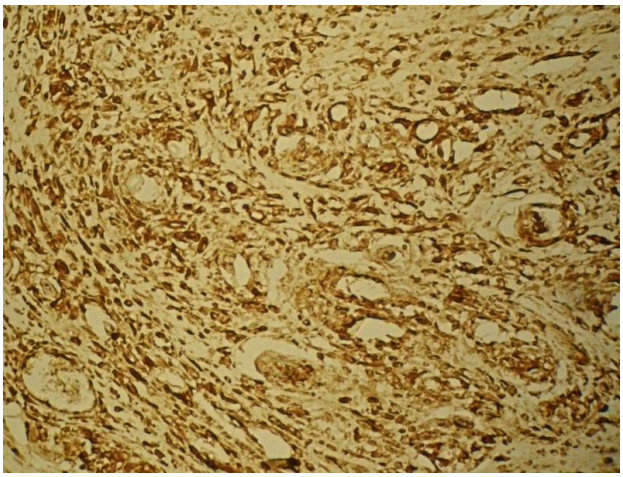

Fig. 3. Microphotography. The twenty first day following transplantation of the stem cells of the cord blood. Angiogenesis foci with numerous vessels. Polymeric method of vimentin expression determination (monoclonal antibody against vimentin V9) with peroxidase mark and counterstaining with diaminobenzodine. Oc.10, Vol.10

Thus, constant structural stimulation of the regenerative processes and angiogenesis was revealed in group II animals in case of introduction of the cord blood against a background of the limbs ischemia. The evident blood flow in the "young" vessels, confirmed by investigation of Villebrand factor expression, is observed on the 10-14 day of the experiment.

At the same time positive data concerning the decrease and absence of fibrous, typical for ischemia development, have been noted, that is confirmed by the research of mesenchymal factor of vimentin.

Conclusion. Thus, transplantation of the cord blood stem cells results in activation of compensatory-restoration reaction in ischemic muscular tissue and stimulation of angiogenesis process de novo, confirmed by means of immunohistochemical methods of investigation. The carried out experiment indicates substantial activation of the processes of the formation of new vessels against a background of ischemia, and it is advisable to consider it as preclinical stage of the given research with further studying of these processes in the clinical practice in patients with chronic ischemia of the lower extremities.

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ТРАНСПЛАНТАЦИЯ СТВОЛОВЫХ КЛЕТОК КОРДОВОЙ КРОВИ В УСЛОВИЯХ ЭКСПЕРИМЕНТАЛЬНОЙ ИШЕМИИ. МОРФОЛОГИЧЕСКАЯ И ИММУНОГИСТОХИМИЧЕСКИ ХАРАКТЕРИСТИКА

Резюме. В статье приведены результаты сравнения морфологических и иммуногистохимических исследований процессов, в результате моделирования ишемии конечностей и трансплантации стволовых клеток кордовой крови в лабораторных крыс. В ходе эксперимента выявлено, что при введении кордовой крови на фоне ишемии конечностей у подопытных животных отмечено постоянную структурную стимуляцию регенераторных процессов и ангиогенеза. Вместе с этим имеющиеся положительные данные об уменьшении и отсутствие фиброзирование. Вышеприведенные результаты позволяют определить перспективность дальнейшей разработки данного направления исследований.

Ключевые слова: хроническая ишемия нижних конечностей, кордовая кровь, трансплантация.

TRANSPLANTATIONS OF THE CORD BLOOD STEM CELLS UNDER CONDITIONS OF EXPERIMENTAL ISCHEMIA. MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL CHARACTERISTICS

Abstract. The results of comparison of the morphological and immunohistochemical investigations of the processes occuring after simulating ischemia of the limbs and transplantation of the cord blood stem cells in the laboratory rats are presented in the article. In the course of the experiment it has been revealed that in case of introduction of the cord blood against the ground of limb ischemia a constant structural stimulation of the regenerative processes and angiogenesis was noted in the animals under study. At the same time positive data concerning decrease and absence of fibrosis are evident. The afore-cited results enable to determine perspectives of further elaboration of the given direction of investigations.

Key words: chronic ischemia of the lower extremities, cord blood, transplantation.

Regional Clinical Hospital, the Department of Vascular Surgery (Chernivtsi);
*HSEE of Ukraine "Bukovinian State Medical University" (Chernivtsi)

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