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Regenerative effects of mouse aortic endothelial cells in a murine model of critical limb ischemia

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ABSTRACT

Critical limb ischemia is a serious disease that threatens a significant decrease in working ability and disability of patients. Cell therapy may be useful in correcting the endothelial dysfunction that accompanies this disorder.

THE AIM *of the study was to evaluate the effectiveness of local transplantation of mouse aortic endothelial cells (MAECs) in a model of critical limb ischemia in mice.*

MATERIALS AND METHODS. *Critical limb ischemia in FVB mice was modeled by femoral artery ligation. The primary culture of endothelial cells was obtained from the murine aortic intima. The endothelial phenotype of cells for the expression of CD31, CD38 and CD309 markers was confirmed by flow cytometry and $1 \cdot 10^6$ MAECs were transplanted intramuscularly into ischemic limb. Tissue perfusion was assessed by laser Doppler flowmetry as well as descriptive histology was used to analyze changes in ischemic muscle after cell transplantation compared to the control group.*

RESULTS. *After MAECs transplantation in animals with modeled critical limb ischemia, the skin of the foot kept pink color and the corresponding temperature of the healthy limb without signs of necrosis of the distal phalanges in contrast to animals of the control group. According to laser Doppler flowmetry data, a significant difference ($p \leq 0.05$) in perfusion of ischemic and sham-operated limbs in animals of the control group remained at the level of $\Delta = 45.7 \pm 13.1$ %. In animals after MAECs transplantation, the difference of these indicators between limbs was only $\Delta = 14.0 \pm 8.23$ % and was not statistically significant. A histological examination of muscle tissue after MAECs transplantation demonstrated the signs of compensatory processes characterized by hyperplasia and hypertrophy of myocyte's nuclei and lightening of the nucleoplasm with well-defined nucleoli in some myofibrils. In the cytoplasm of myocytes, intermediate Z-discs were clearly visualized, and the number of myofibrils in muscle fibers increased.*

CONCLUSION. *In animals with the model of critical limb ischemia, the transplantation of aorta-derived endothelial cells recovers the perfusion of ischemic limbs and improves the histological indicators of muscle tissue.*

KEY WORDS: *critical limb ischemia; mouse aortic endothelial cells; cell transplantation; tissue perfusion; laser Doppler flowmetry*

Critical limb ischemia (CLI) refers to a condition caused by chronic ischemic injury of legs that is characterized by at-rest pain, ulcers, or gangrene related to peripheral artery disease (PAD). The incidence of CLI is approximately 500 to 1000 per million people, with the highest rates among older subjects, smokers and diabetics [1]. It is estimated that > 200 million people have PAD worldwide, with a spectrum of symptoms from none to severe [2]. The risk of major amputation and cardiovascular events is at least 30–50 % in the first year in patients with CLI who do not have conventional revascularization [3]. As a result, a multidiscipline approach involving specialists in endovascular revascularization, open surgical revascularization, podiatry, wound care, and other specialties is often required to maximize patient outcomes [4].

In the pathogenesis of this disorder, the dysfunction of the endothelium plays a key role, as the result of endogenous and exogenous pathological impacts. Endothelial dysfunction accompanying ischemic tissue injury has been associated with impaired regeneration dependent on circulating and tissue-specific stem or progenitor cells [5].

Considering that, in case of CLI where the key is a violation of tissue

trophism at the level of vessels, cell therapy can be useful promoting proangiogenic effects. Peripheral blood-derived or bone marrow-derived mononuclear cells, mesenchymal stem cells, or marker-specific subsets of cells with angiogenic properties may hold promise for no-option PAD patients [6, 7]. Injected cells may exert beneficial actions by enhancing local angiogenesis (either through maturation of endothelial progenitors, or through the secretion of angiogenic mediators), or by transducing cytoprotective signals that preserve tissue structure [8].

For the first time, endothelial progenitor cells (EPCs) were isolated from circulating blood by T. Asahara in 1997 [9]. EPCs can be isolated from the bone marrow and CD133/VEGFR2 cells represent a population with endothelial progenitor capacity. However, increasing evidence suggests that there are additional bone marrow-derived cell populations (e.g., myeloid cells, "side population" cells, and mesenchymal cells) and non-bone marrow-derived cells, which also can give rise to endothelial cells [10, 11]. Given that EPCs percentage is only 1 % of all circulating mononuclear cells and their endothelial differentiation potential has only been confirmed *in vitro*, the search for other sources of resident EPCs is actual [12, 13]. Li et al. demonstrated that the structural integrity of adult cardiac endothelium following myocardial infarction was maintained through clonal proliferation by resident EPCs in the infarct border region, without significant contributions from bone marrow cells or endothelial-to-mesenchymal transition. [14]. Microvascular-derived EPCs can be isolated also from the lung, brain, liver, adipose tissue, and placenta [15-19]. The most accessible and widespread method is the isolation of endothelial cells from large vessels (aorta, umbilical vein) which allows to obtain a pure population of EPCs devoid of other types of cells [20].

The **AIM** of our study was to evaluate the effectiveness of local transplantation of mouse aortic endothelial cells (MAECs) in a model of critical limb ischemia in mice.

MATERIALS AND METHODS

The study was carried out at the Department of Cell and Tissue Technologies of the Institute of Genetic and Regenerative Medicine (IGRM) on mature (4-5 months-old) males of FVB mice. The animals were kept under standard conditions at the experimental clinic of IGRM with free access to water and food. All experiments with experimental animals were carried out in compliance with the Law of Ukraine "On the Protection of Animals from Cruelty", "European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes", as well as the principles of bioethics and biological safety norms [21].

Obtaining primary cultures of endothelial cells from murine aorta.

Animals were euthanized by cervical dislocation after intraperitoneal injection of 2.5 % avertin solution (*Sigma*, USA) at a dose of 400 mg/kg. Under sterile conditions, aortas were prepared and placed to Petri dishes with RPMI-1640 medium (*Sigma*, USA), supplemented with 1 % antibiotic mixture PenStrep (*Sigma*, USA). Fragments of para-aortic adipose tissue were removed under a stereomicroscope and the inner lining of the aorta (tunica intima) was dissected. Using microscissors, the prepared tissue was minced into fragments about 1 mm^2 and incubated in a 0.1 % solution of collagenase type I (*Sigma*, USA) for 30-40 minutes at a temperature of +37 °C. Fermented suspension was washed with 10 mL RPMI-1640 medium (*Sigma*, USA) with 1 % PenStrep mixture, pellet were resuspended with complete nutrient medium and seeded into 25 cm^2 fibronectin-coated culture flasks (*Greiner bio-one*, Austria). The complete culture medium consisted of DMEM/F-12 medium (*HyClone*, USA) supplemented with 10 % fetal bovine serum (FBS), 1 % PenStrep antibiotic mixture, 10 ng/mL EGF epidermal growth factor and 20 ng/mL basic fibroblast growth factor bFGF (all *Sigma*, USA). Cultivation of primary cells was carried out under standard conditions in an incubator in a humidified atmosphere containing 5 % CO_2 at 37 °C. The first replacement of the medium was done 7 days after the start of cultivation. Further replacement of the nutrient medium was carried out every three days. Subculturing was performed when reaching 80 % confluency of the monolayer using of 0.25 % trypsin-Versene mixture. Cells at 3rd passage were cryopreserved in liquid nitrogen using cryopreservation media containing of 90 % FBS and 10 % DMSO (*Sigma*, USA). The CoolCell® freezing container (*Corning*, USA) was used for cell freezing at the rate of -1°C/minute for -80 °C.

Immunophenotyping of cells by flow cytometry.

The obtained cell cultures from the aorta were phenotyped by flow cytometry using rat anti-mouse CD31 PE-Cy7 (cat. # 25-0311-82, *Invitrogen*, USA), CD38 APC (cat. # C2390-13D, US Biological, USA), and CD309 PE (cat. # 12-5821-82, *Invitrogen*, USA) monoclonal antibodies, according to the manufacturer's recommendations. In 5 mL polystyrene tubes, the suspension of 2•10^5 cells in 50 µL of DPBS (*HyClone*, USA) was incubated with antibodies at a 1:100 dilution for 20 minutes at +4 °C protected from light. Cells were washed with 1 mL of BD® CellWash buffer (*BD Biosciences*, USA), centrifuged at 200x g for 5 minutes at +4 °C and cell pellet was resuspended with 300 µL of DPBS supplemented with 1 % FBS. The control samples include unstained control and isotype control. The percentage of viable cells was determined by counting dead cells after the addition of 5 µL 7-AAD (*BD Biosciences*, USA). Measurement was performed on BD FACSAria cell-sorter (*Becton Dickinson*, USA) using BD FACS Diva 6.1.2 software.

Modeling of critical limb ischemia in mice.

Under general anesthesia (2.5 % avertin solution, 400 mg/kg) the hip was shaved and the skin was treated with a 70 % ethanol solution. A skin incision was made on the medial surface of the hip, the superficial fascia and inguinal adipose tissue were dissected using electrocoagulation to visualize femoral neurovascular bundle between the front and medial groups of muscles. Using microsurgical tweezers, the femoral artery was isolated from the vein and nerve from *a.epigastrica* branch to the *a.politea*. The fascia sheaths were dissected, double ligatures were applied in the proximal and distal points, the artery was transected, and the skin was sutured using synthetic suture.

Two weeks after the modeling, 1•10^6 thawed and washed mouse aortic endothelial cells were transplanted in 100 µL of 0.9 % saline by 3 injections into the leg muscles using 31G insulin syringe. Animals of the control group were injected with 100 µL of 0.9 % saline only.

Study of the limb perfusion

Under general anesthesia (2.5 % avertin solution, 400 mg/kg), the skin was treated with 70 % ethanol solution, and a 2 mm skin incision was made on the medial surface of the leg. The optical fiber of the laser Doppler flowmeter moorVMS-LDF-1 (*Moor Instruments*, Great Britain) was positioned perpendicular to the calf muscles and the perfusion level was recorded using the moorVMS, v.2.1 software. Data were recorded three times for 1 min for each limb. The wound was sutured with a synthetic suture.

Histological studies of the muscles

Animals were euthanized by cervical dislocation under general anesthesia (2.5 % avertin solution, 400 mg/kg). Using surgical instruments, the leg muscles were dissected and excised. Muscle tissue was fixed in 4 % formaldehyde solution on PBS (pH = 7.4) for 24 hours. After fixation, the samples were dehydrated according to the standard protocol in ethanol solutions of increasing concentration and benzene: 30 minutes in 70 % ethanol solution; 30 minutes – in 80 % ethanol solution; 30 minutes – in 90 % ethanol solution; 30 minutes – in 96 % ethanol solution; 12 hours – in 96 % ethanol solution; 30 minutes – in 100 % ethanol solution; 30 minutes – in the mixture of 100 % ethanol and benzene in a ratio of 1:1; twice for 30 minutes in benzene. The samples were kept in the mixture of benzene and paraffin in a ratio of 1:1 for 30 minutes at +37 °C, transferred three times to paraffin type 6 (*Richard-Allan Scientific*, USA) at 57 °C.

Using a rotary microtome HM 325 (*Microm*, Germany), 12-µm histological sections were prepared, transferred to glass slides and dried in a thermostat at +37 °C. Sections were deparaffinized twice for 5 minutes each in benzene and kept for 5 minutes in 96 % ethanol and 5 minutes in 70 % ethanol solution.

Sections were stained with Ehrlich's hematoxylin solution for 10 minutes, washed and stained with 0.1 % eosin solution for 2 minutes, as well as stained using Masson's trichrome technique. The samples were dehydrated in 96 % ethanol solution for 3 minutes, washed twice in benzene, embedded with Canadian balsam and covered with coverslips. The sections were examined using BX 51 microscope (*Olympus*, Japan).

Statistical data processing

Statistical analysis was performed using the Student's t-test by STATISTICA v. 7.0 software (*StatSoft Inc.*, USA) [22]. The data are presented as the mean in each experimental group ± standard error of the mean (Mean ± SEM). Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Murine aortic intima explants were cultured in fibrin-coated flask for 10-14 days. Endothelial cells grew and migrated out of the explant pieces (**Fig. 1 A**). After the first passage the primary cell culture from aortic intima was represented by a homogeneous population of elongated cells, which were in tight contact with each other and formed typical "cobblestone-like" confluent monolayer (**Fig. 1 B**).

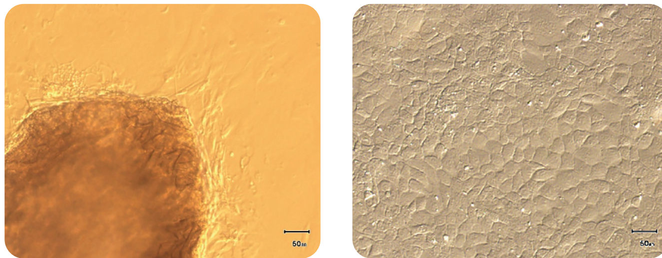

Fig. 1. Micrographs of primary murine aortic endothelial cell cultures in fibronectin-coated flask. A – migration of cells from the intima explant; B – cell culture at 1st passage. Light microscopy, phase contrast. Scale bar – 50 μm.

The immunophenotyping of MAECs cultures at the 3rd passage demonstrated the expression of endothelial cell adhesion molecule PECAM-1/CD31 (82.5 ± 3.1 %), the marker of activated endothelial cells CD38 (76.8 ± 56 %) and the receptor for vascular endothelial growth factor CD309 (84.8 ± 4.2 %), which is typical for endothelial cells (**Fig. 2**).

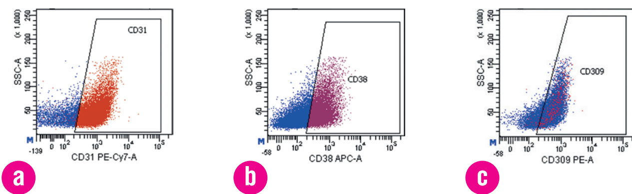

Fig. 2. Dot-plot histograms of the expression of CD31 (A), CD38 (B), and CD309 (C) markers in the culture of mouse aortic endothelial progenitor cells according to flow cytometry, passage 3.

Thus, according to the morphological characteristics and expression of specific surface markers, the obtained cell cultures were corresponded to the endothelial phenotype. Kobayashi et al. proposed the treatment of whole freshly excised aorta with collagenase followed by flushing the inside lumen to collect endothelial cells [23]. In the protocol of Wang et al., the freshly isolated aorta was cut into small segments and seeded onto a matrix with the endothelium facing down to allow for endothelial sprouting [24]. In our protocol, we additionally removed the outer layers of the aorta and prepared only the intima for further seeding to fibronectin-coated surface, which allows reducing the contamination of the culture by stromal elements.

In order to evaluate the regenerative effects of MAECs, ischemic injury of the limbs was modeled in mice by ligation of the femoral artery. Immediately after modeling, the effectiveness of occlusion was confirmed using laser Doppler flowmetry, which demonstrated a steady decrease in leg muscle perfusion by up to 60 % compared to the sham-operated limb (**Fig. 3**).

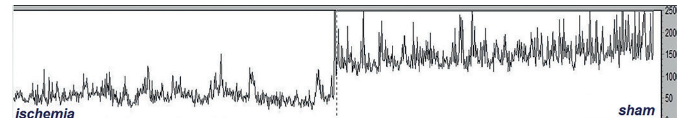

Fig. 3. Graph of murine limb perfusion according to laser Doppler flowmetry: left – ischemic limb, right – sham-operated limb (moorVMS v. 2.1 software).

Stability of pathological changes in critical limb ischemia model was tested in 2 weeks after the modeling using instrumental and histological methods. Macroscopic examination revealed paleness of the ischemic limb and a decrease in its temperature compared to the sham-operated limb. According to the laser Doppler flowmetry data, in all animals a stable decreased perfusion of the ischemic limbs in comparison with the sham-operated ones was showed (Δ = 50.6 ± 12.9 %, $p \le 0{,}001$) (**Fig. 4**).

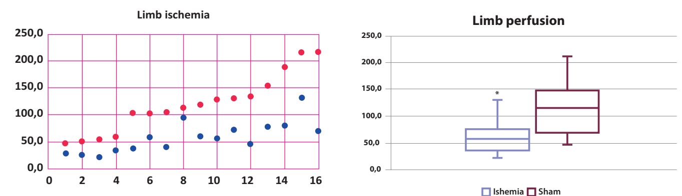

Fig. 4. Perfusion level in ischemic (blue) and sham-operated (red) limbs of mice for 2 weeks after the modeling.
A – perfusion in limbs of each experimental animal;
B – mean data (n = 16). Note: * – compared to sham-operated limb

Histological examination of muscle biopsies of ischemic limbs after 2 weeks detected destructive changes in muscle fibers in the form of homogenization of the cytoplasm of myocytes, the basophilic nuclei of which become thinner and heterochromic (**Fig. 5 A**). Myocytes showed a decrease in the number of muscle fibers. Areas of myofibril destruction with hypertrophy and hyperplasia of myocyte's nuclei, and moderate lymphocytic infiltration in the adjacent connective tissue were detected (**Fig. 5 B**). A significant decrease in the number of myofibrils in muscle fibers was revealed when the sections were stained using the Masson's trichrome technique (**Fig. 5 C**).

Fig. 5. Micrographs of histological sections of murine muscles 2 weeks after CLI modeling. Hematoxylin-eosin (A, B) and Masson's trichrome (B) staining, oc. x10, ob. x40. (description in the text)

Two weeks after the MAECs transplantation, a macroscopic, histological and second instrumental examination was performed. During the macroscopic examination, in the animals of the control group without cell transplantation, evident paleness of the ischemic limb with a cyanosis of foot, compared to the sham-operated one, as well as necrotic changes of the nail phalanges were revealed (**Fig. 6**). After MAECs transplantation in animals with modeled critical limb ischemia, the skin of the foot kept pink color and the corresponding temperature of the healthy limb without signs of necrosis of the distal phalanges.

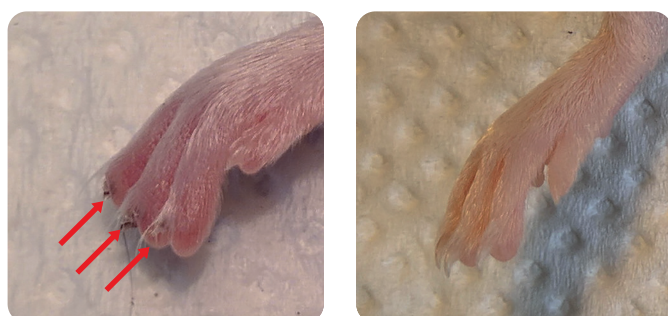

Fig. 6. Photographs of the feet of mice 4 weeks after modeling of critical limb ischemia A – arrows indicate necrosis of the nail phalanges of the fingers on the control limb with femoral artery ligation; B – foot with femoral artery ligation and transplantation of MAECs .

On the sections of animals' limbs in the area of MAECs transplantation, an increase in the vascular pattern due to the growth of arterioles, which formed anastomoses with each other, was detected. In animals of the control group with modeled critical ischemia, according to laser Doppler flowmetry data, a significant difference ($p \leq 0.05$) in perfusion of ischemic and sham-operated limbs remained at the level of $\Delta = 45.7 \pm 13.1$ % (**Fig. 7 A**) . At the same time, in animals after MAECs transplantation, the difference of these indicators between limbs was only $\Delta = 14.0 \pm 8.23$ % and was not statistically significant (**Fig. 8 B**).

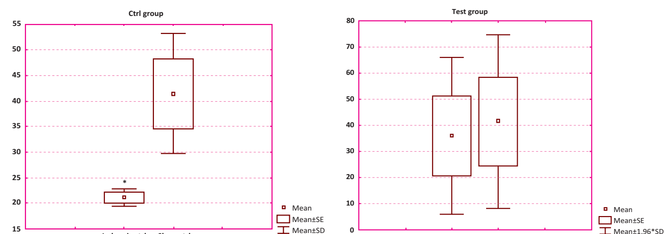

Fig. 7. Perfusion level of ischemic (left blocks) and sham-operated (right blocks) limbs in experimental mice according to laser Doppler flowmetry: A – control group without cell transplantation (n = 8), B – group with MAECs transplantation (n = 8).

Note: * – $p < 0.05$ compared to the sham-operated limb.

When comparing the relative difference in perfusion between ischemic and sham-operated limbs, a significant difference ($p < 0.01$) was established between the control group and the group of animals with MAECs transplantation (**Fig. 9**).

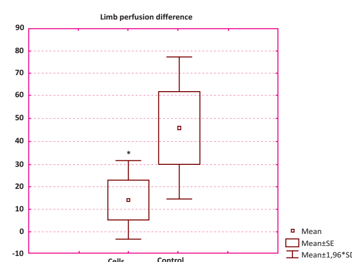

Fig. 8. Relative difference in lower limb muscle perfusion between the ischemic and sham-operated limb in animals of the control group (right block) and the group of animals with MAECs transplantation (left block) according to laser Doppler flowmetry (n = 8).

Note: * – $p < 0.01$ compared to control group.

During a histological examination in mice of control group without cell therapy, in the muscle fibers the homogenization and loss of eosinophilia of the cytoplasm of myocytes, as well as karyolysis as a signs of waxy degeneration of muscles are observed (**Fig. 10 A**). In the part of myocytes that has cytoplasm with the sites of edema and loss of eosinophilia and transverse striations, the karyoplasm becomes transparent in the nuclei, and the chromatin forms clumps. In addition, the infiltration of connective tissue and the formation of scars were found in the areas of myocyte necrosis (**Fig. 10 B**). In the lumen of arteries and veins in the connective tissue and in the stroma between muscle fibers, stasis, sludge, and adhesion of erythrocytes are observed.

In histological samples of animals after MAECs transplantation, signs of compensatory processes characterized by hyperplasia and hypertrophy of myocyte's nuclei and lightening of the nucleoplasm with well-defined nucleoli were observed in some myofibril fibers (**Fig. 10 C**). In the cytoplasm of myocytes, intermediate Z-discs were clearly visualized, and number of myofibrils in muscle fibers increased (**Fig. 10 D**). In addition, normal capillary blood filling was detected. In comparison with animals of the control group, the studied indicators according to descriptive histology were significantly better.

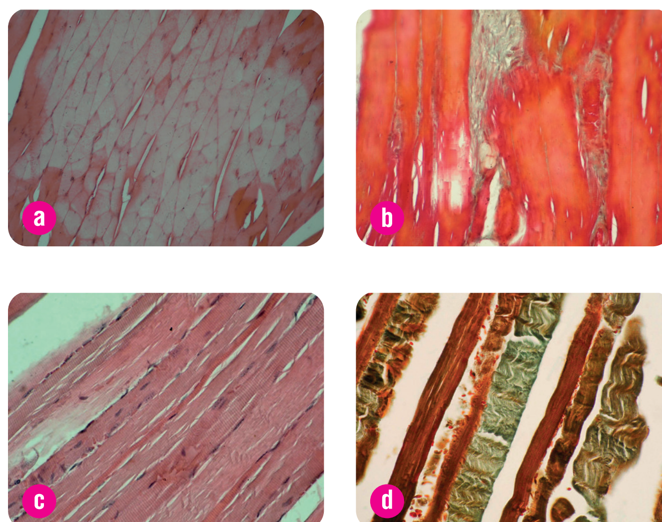

Fig. 9. Micrographs of histological sections of muscles of murine lower limbs 4 weeks after CLI modeling in control group (A, B) and after MAECs transplantation (C, D). Hematoxylin-eosin (A-C) and Masson's trichrome (D) staining, oc. x10, ob. x40 (description in the text).

Thus, the data obtained in our study demonstrate the ability of aorta-derived endothelial cells to improve regeneration in ischemic tissue in a murine model of critical limb ischemia, which confirms the results of other authors that transplanted progenitor cells of different origin.

Different types of cells are involved in the regeneration of ischemic damage through paracrine mechanisms by numerous cytokines, chemokines and growth factors. Intramuscular injection of conditioned medium derived from TNF-α-treated mesenchymal stem cells into the ischemic hindlimb resulted in attenuated severe limb loss and stimulated blood perfusion and angiogenesis in the ischemic limb. The injection of recombinant IL-6 and IL-8 proteins resulted in increased homing of intravenously transplanted EPCs into the ischemic limb and improved blood perfusion *in vivo* [25].

Direct intercellular interaction is also extremely important, which can be realized through the mutual complementation of the functions of different types of cells. Numerous preclinical studies suggest that optimized blood vessel formation may require paracrine and structural contributions from multiple progenitor cell lineages, pro-angiogenic-secretory myeloid cells derived from hematopoietic progenitor cells, tubule-forming endothelial cells generated by circulating or vessel-resident endothelial precursors, and vessel-stabilizing perivascular cells derived from mesenchymal stem cells [26].

In the experimental study of Park et al. in a murine hindlimb ischemia model, co-transplantation of endothelial cells with smooth muscle cells improved blood perfusion and increased the recovery rate in ischemic limbs compared to the transplantation of either endothelial or moth muscle cells alone. Moreover, such a co-transplantation stimulated angiogenesis and led to the formation of capillaries and arteries *in vivo* [27]. However, in other study of Amani et al., the combined therapy using mesenchymal stem cells and mast cells in rats with critical limb ischemia model resulted in a reduced, rather than enhanced, therapeutic effect. At the same time, the authors note that the combination of hydrogel and cell therapy generates a greater angiogenic potential at the ischemic site than cell therapy or hydrogels alone [28]. This is an additional confirmation that the spatial 3D organization of the graft and the direct contact interaction of the cells is quite important in realization its regenerative potential.

To date, numerous clinical trials of cell therapy in critical limb ischemia using various types of stem cells are known [29, 30, 31, 32]. In the randomized clinical trial SCELTA (NCT02454231) the safety and therapeutic effects of enriched circulating EPCs vs bone marrow mononuclear cells administration were compared. In EPCs-treated patients, there was a positive correlation between injected cell counts and the increase in muscle perfusion. The safety profile was comparable between the EPCs and BM-MNC treatment arms. In both groups, the number of deaths and major amputations was lower compared with eligible untreated patients and reference patients [33]. In prospective randomized single-blinded non-inferiority trial (NCT02089828) purified $CD34^+$ cells were not inferior to peripheral blood mononuclear cells at limb salvage in the treatment of angiitis-induced CLI and appeared to induce earlier ischemia relief [34].

At the same time, it should be noted that the regenerative potential of endothelial stem and progenitor cells can be significantly affected by limiting factors from both the donor and recipient side. In particular, Capla et al. showed, that proliferation, adhesion and migration of endothelial progenitor cells from diabetic patients in response to hypoxia was significantly reduced compared with controls. Also, cell mobilization from the bone marrow and recruitment into ischemic tissue were significantly reduced in diabetic mice. Normal cells injected systemically as replacement therapy in a diabetic mouse increased but did not normalize ischemic tissue survival [35]. Most clinical trials transferred autologous cells damaged by chronic disease that demonstrated poor survival in the ischemic environment and impaired function conferred by atherosclerotic or diabetic co-morbidities [26]. In addition, there are also a number of other limitations to the correct comparison of experimental and clinical studies, including standardization of disease severity, type, route, dose, and frequency of cell administration [36, 37].

However, there are certain known limitations of such therapy. There is a need for extended experimental *in vitro* and animal studies to identify the optimal cell types or their combinations capable to enhance regenerative potential at ischemic tissue injuries. The successful translation of the obtained fundamental results into further large, well-designed placebo-controlled clinical studies will increase the safety and effectiveness of cell therapy for peripheral artery disease.

CONCLUSION

On the model of critical limb ischemia in mice, using morphological and instrumental research methods, the regenerative potential of aorta-derived endothelial cells was established, which was manifested in the recovery of perfusion of ischemic limbs and improvement of histological indicators of muscle tissue.

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Регенеративні ефекти ендотеліальних клітин-попередників з аорти миші на моделі критичної ішемії кінцівок у мишей

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РЕЗЮМЕ

Критична ішемія кінцівок – важке захворювання, яке загрожує для пацієнтів значним зниженням працездатності та інвалідністю. Клітинна терапія може бути корисною для корекції ендотеліальної дисфункції, яка супроводжує цей патологічний стан.

МЕТА ДОСЛІДЖЕННЯ *— оцінити ефективність локальної трансплантації ендотеліальних клітин-попередників з аорти миші на моделі критичної ішемії кінцівок у мишей.*

МАТЕРІАЛИ І МЕТОДИ. *Критична ішемія кінцівок у мишей FVB була змодельована шляхом перев'язки стегнової артерії. Первинну культуру ендотеліальних клітин отримували з інтими аорти миші. Ендотеліальний фенотип клітин-попередників за експресією маркерів CD31, CD38 і CD309 був підтверджений проточною цитометрією та $1•10^6$ клітин трансплантовано внутрішньом'язово в ішемізовану кінцівку. Тканинну перфузію оцінювали за допомогою лазерної доплерівської флоуметрії, а також використовували описову гістологію для аналізу змін в ішемізованому м'язі після трансплантації клітин в порівнянні з контрольною групою.*

РЕЗУЛЬТАТИ. *Після трансплантації ендотеліальних клітин-попередників у тварин із змодельованою критичною ішемією кінцівки шкіра стопи зберігала рожевий колір і відповідну температуру здорової кінцівки без ознак некрозу дистальних фаланг на відміну від тварин контрольної групи. За даними лазерної допплерівської флоуметрії достовірна різниця ($p \leq 0,05$) перфузії ішемізованих та псевдооперованих кінцівок у тварин контрольної групи залишалася на рівні $\Delta = 45,7 \pm 13,1$ %. У тварин після трансплантації клітин різниця цих показників між кінцівками становила лише $\Delta = 14,0 \pm 8,23$ % і не була статистично значущою. При гістологічному дослідженні м'язової тканини після трансплантації ендотеліальних клітин-попередників виявлено ознаки компенсаторних процесів, що характеризуються гіперплазією, гіпертрофією та просвітленням ядер міоцитів з чітко вираженими ядерцями в деяких міофібрилах. У цитоплазмі міоцитів чітко візуалізувалися проміжні Z-диски, збільшувалася кількість міофібрил у м'язових волокнах.*

ВИСНОВОК. *У тварин з моделлю критичної ішемії кінцівок трансплантація ендотеліальних клітин-попередників з аорти миші відновлює перфузію ішемізованих кінцівок і покращує гістологічні показники м'язової тканини у них.*

КЛЮЧОВІ СЛОВА: *критична ішемія кінцівок; ендотеліальні клітини аорти миші; трансплантація клітин; перфузія тканин; лазерна доплерівська флоуметрія*