УДК 616-005.8 616-008.1

CELL THERAPY FOR ISCHEMIC AND NON-ISCHEMIC DISEASES IN ANIMAL MODELS

© Victor N. Alexandrov[1,2], Vasily S. Sidorin[1], Alexander V. Kriventsov[1], Albina A. Kondratenko[1], Ekaterina V. Mikhailova[2], Lidia I. Kalyuzhnaya[1], Natalia V. Pak[1], Arina A. Kokorina[1]

[1] Military Medical Academy named after S.M. Kirov. 194044, Saint-Petersburg, Academician Lebedev str., 6
[2] Saint-Petersburg State Pediatric Medical University. 194100, Saint-Petersburg, Litovskaya str., 2

Contact information: Albina A. Kondratenko — junior researcher. E-mail: kondraa24@gmail.com

Abstract: To date, classical approaches to the treatment of many diseases of ischemic and non-ischemic genesis are successfully complemented by stem cell transplantation, in particular mesenchymal stem cells. The use of cell products based on cultured cells carries a potential risk of microbiological and virological contamination, as well as genetic instability and mutations. A cell product based on a mononuclear fraction of the bone marrow, as a product subjected to minimal manipulations, has no less potential for clinical use with an optimal protocol for its use. The aim of experimental study was to highlight some top issues of cell therapy for ischemic and non-ischemic diseases. It has been established that transplantation of the mononuclear fraction of allogeneic and autogenous bone marrow has a therapeutic effect in experimental models of critical lower limb ischemia, brain ischemia, and dilated cardiomyopathy. The administration of bone marrow mononuclear fractions to laboratory animals led to the following results: an increase in the area of the microvasculature in the ischemic extremities of rats; recovery of behavioral reactions in rats undergoing cerebral ischemia; restoration of myocardial morphology, systolic function of the left ventricle, ejection fraction, elimination of signs of blood stasis in the systemic and pulmonary circulation of rats and rabbits with dilated cardiomyopathy. It should be noted that the degree of effectiveness of cell therapy depended on the state of target tissues, methods for delivering cells to diseased organ, and also the number of cells in a cell product. Thus, a minimally manipulated cell product, which is the mononuclear fraction of the bone marrow, can be effective in treating the diseases presented in our study by experimental models.

Keywords: cell therapy; bone marrow mononuclear fraction; ischemia; heart failure; experimental model; cell therapy protocol; paracrine effect; angiogenesis.

КЛЕТОЧНАЯ ТЕРАПИЯ ИШЕМИЧЕСКИХ И НЕИШЕМИЧЕСКИХ ЗАБОЛЕВАНИЙ НА ЖИВОТНЫХ МОДЕЛЯХ

© Виктор Николаевич Александров[1,2], Василий Сергеевич Сидорин[1], Александр Викторович Кривенцов[1], Альбина Александровна Кондратенко[1], Екатерина Вячеславовна Михайлова[2], Лидия Ивановна Калюжная[1], Наталья Викторовна Пак[1], Арина Александровна Кокорина[1]

[1] Военно-медицинская академия им. С.М. Кирова. 194044, Санкт-Петербург, ул. Академика Лебедева, д. 6
[2] Санкт-Петербургский государственный педиатрический медицинский университет. 194100, Санкт-Петербург, Литовская ул., 2

Контактная информация: Альбина Александровна Кондратенко — младший научный сотрудник. E-mail: kondraa24@gmail.com

Резюме: На сегодняшний день классические подходы к терапии многих заболеваний ишемического и неишемического генеза с успехом дополняются трансплантацией стволовых клеток, в частности мезенхимальных стволовых клеток. Применение клеточных продуктов на основе культивированных клеток несет потенциальный риск микробиологической и вирусологической контаминации, а также генетической нестабильности и мутаций. Клеточный продукт на основе мононуклеарной фракции костного мозга как продукта, подвергнутого минимальным манипуляциям, имеет не меньший потенциал клинического применения при оптимальном протоколе его использования. Целью экспериментального исследования было осветить некоторые актуальные вопросы клеточной терапии ишемических и неишемических заболеваний. Установлено, что трансплантация

мононуклеарной фракции аллогенного и аутогенного костного мозга оказывает терапевтический эффект на экспериментальных моделях критической ишемии нижних конечностей, ишемии головного мозга и дилатационной кардиомиопатии. Введение мононуклеарной фракции костного мозга лабораторным животным привело к следующим результатам: увеличение площади микрососудов в ишемизированных конечностях крыс; восстановление поведенческих реакций у крыс, перенесших церебральную ишемию; отсутствие морфологических признаков повреждения миокарда, улучшение систолической функции левого желудочка, фракции выброса, устранение признаков застоя крови в системном и малом круге кровообращения крыс и кроликов с дилатационной кардиомиопатией. Следует отметить, что степень эффективности клеточной терапии зависела от состояния тканей-мишеней, способов доставки клеток к больному органу, а также от количества клеток в клеточном продукте. Таким образом, минимально манипулированный клеточный продукт — мононуклеарная фракция костного мозга — может быть эффективным при лечении заболеваний, представленных в нашем исследовании на экспериментальных моделях.

Ключевые слова: клеточная терапия; мононуклеарная фракция костного мозга; ишемия; сердечная недостаточность; экспериментальная модель; протокол клеточной терапии; паракринный эффект; ангиогенез.

INTRODUCTION

Stem cell transplantation and mesenchymal stem cells (MSCs) in particular, is firmly part of the arsenal of modern approaches to the treatment of a number of diseases, despite a clearly insufficient understanding of their nature and regulation [1, 3]. The clinical use of MSCs requires ex vivo cultivation. Their properties will depend on the source and cultivation conditions. An assessment of the absence of microflora contamination and the virological safety of MSCs are required. During cultivation, genetic instability and MSC mutations may occur. At the same time, mononuclear fraction of the bone marrow (MFBM) — a minimally manipulated cell product having just as much therapeutic potential and posing a minimal danger when used properly, is in the background. In this context, we attempted to demonstrate the optimal efficiency and safety of cell therapy options for various socially significant human diseases. The advantages and therapeutic mechanisms of action of various types of cells for cell therapy of pathological conditions of various origins require further study. The optimal protocol, as shown by our studies, consisted of the optimal number of cells in the cell product, the optimal route for their delivery, the optimal state of the target tissue/organ, and the time of the actual procedure.

The aim of this study was to describe the protocol of the optimal variant and the result of cell therapy for ischemic and non-ischemic diseases in animal models as well as to get experimental models of critical limb ischemia, brain ischemia, dilated cardiomyopathy. We also carried out cell therapy for critical limb ischemia, brain ischemia, dilated cardiomyopathy in accordance with the developed optimal protocol for cell therapy.

MATERIALS AND METHODS

120 female and 174 male Wistar rats weighing 250–300 g and 40 female Chinchilla rabbits weighing 1.2–1.5 kg were used as experimental animals. The animals were randomly divided into groups in accordance with the models used in the work (Table 1).

The animals were kept in standard conditions. While working with them, they were guided by the requirements of the Directive of the European Community. A model of critical limb ischemia was modeled by femoral artery ligation at the point where it exits from under the Poupart's ligament. Brain ischemia was initiated by coagulation of the middle cerebral artery. Dilated cardiomyopathy was reproduced with rubomycin (daunomycin), a drug from the group of anthracyclines used in hemoblastosis, one of the side effects of which is the damage to the cardiovascular system with the development of heart failure. A mononuclear fraction of allogeneic bone marrow from both femur and tibia of rats or autogenous bone marrow from ilium of the rabbit pelvic bones obtained by Ficoll density gradient centrifugation at a density of 1.077 g/ml was used as a cell product. The isolated suspension of mononuclear cells was washed twice with an isotonic sodium chloride solution. In the trypan blue test, cell viability, which is usually not less than 95%, was evaluated, the number of cells was calculated in a Goryaev's chamber and diluted with 0.9% sodium chloride solution to a predetermined concentration. In preliminary studies, an optimal variant of cell therapy for each animal model was searched for. It was based on the criterion of survival and effectiveness of therapy depending on the number of transplanted cells, their delivery to the target organ/tissue, and time of transplantation. All manipulations, both in terms of reproducing experimental models and in the process of implementing the stages of preparation and execution of cell therapy, were performed under aseptic conditions.

Table 1

Distribution of animals by group

Experimental models	Number of animals in a group	
	rats	rabbits
Critical limb ischemia	54	–
Brain ischemia	120	–
Dilation cardiomyopathy	120	40

Angiography was performed to visualize the vessels of the damaged limb. An X-ray contrast drug (Omnipack 240) was administered in a volume of 1.5–2.0 ml after laparotomy, puncture and catheterization of the rat abdominal aorta. Radiographs were obtained on an Arman apparatus according to a standard technique. Functional changes in the activity of the central nervous system were judged by behavioral reactions in the Open Field technique. Systolic function of the left ventricle was examined echocardiographically. Myocardial perfusion was assessed by the accumulation of a radiopharmaceutical by perfusion scintigraphy. For histological examination, internal organs were fixed in 10% neutral formalin and histological sections were prepared by staining with hemotoxylin and eosin.

For statistical processing of the obtained results, Student's criterion was used. The differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

Cell therapy for critical limb ischemia in animal models was carried out 30 days after its initiation, i.e., in the absence of local and systemic manifestations of inflammation., as a response to tissue necrosis of the ischemic limb. Isolated cells 2×10^7/1.0 ml were transplanted into an invalid limb and according to the previously developed protocol, part of the cells (1×10^7/0.5 ml) was transplanted through the femoral artery, and the other equal part (1×10^7/0.5 ml) was subjected to five injections into the lower third of the thigh muscles. In the next 30 days of the posttransplant period [6, 8], reparative regeneration at the level of the invalid limb was visualized as the development of collateral arterioles, branching of capillaries, and formation of de novo microvessels (Fig. 1), which, when compared with clinical practice, determines the chance of limb preservation [10, 11].

Angiogenic, anti-inflammatory, neuroprotective, anti-apoptotic, paracrine effects of the cells that make up MFBM turned out to be sufficient to ensure a positive result of cell therapy [2, 7, 12]. The zone of ischemic brain necrosis in rats, into which 2×10^6 MFBM cells in the volume of 50 μl were transplanted through the common carotid artery on the 14th day after the stroke [4, 5], favorably differed from that in the animals of the control group, who received placebo (sodium chloride 0.9% solution), all other things

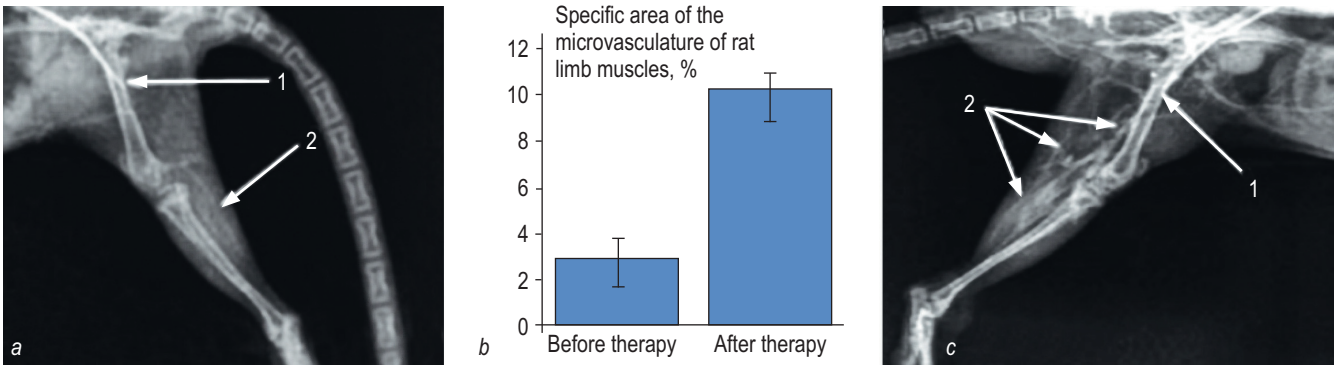
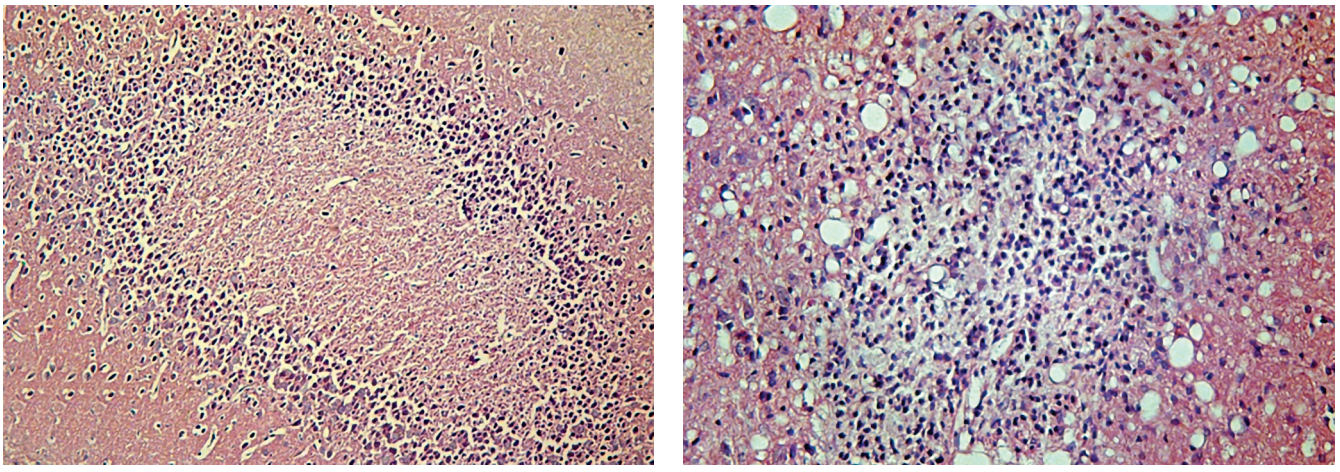
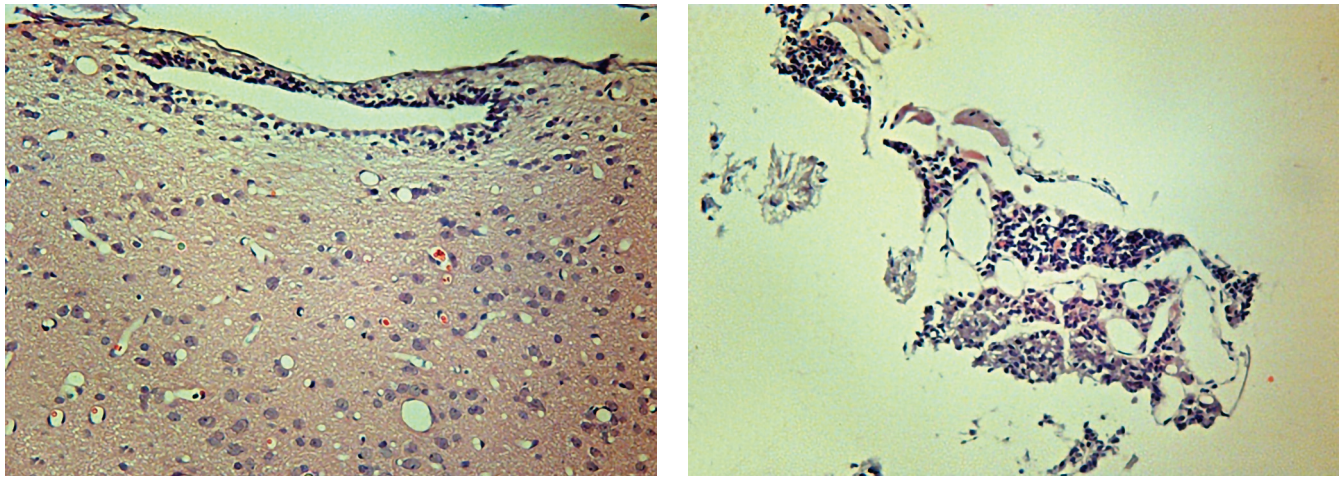


Fig. 1. Angiogram of the state of the microcirculatory bed in the rat's invalid limb before (a) and after (c) cell therapy. The arrows indicate: 1 — the site of femoral artery ligation; 2 — collaterals. Specific area (%) of the microvasculature of rat limb muscles (b)



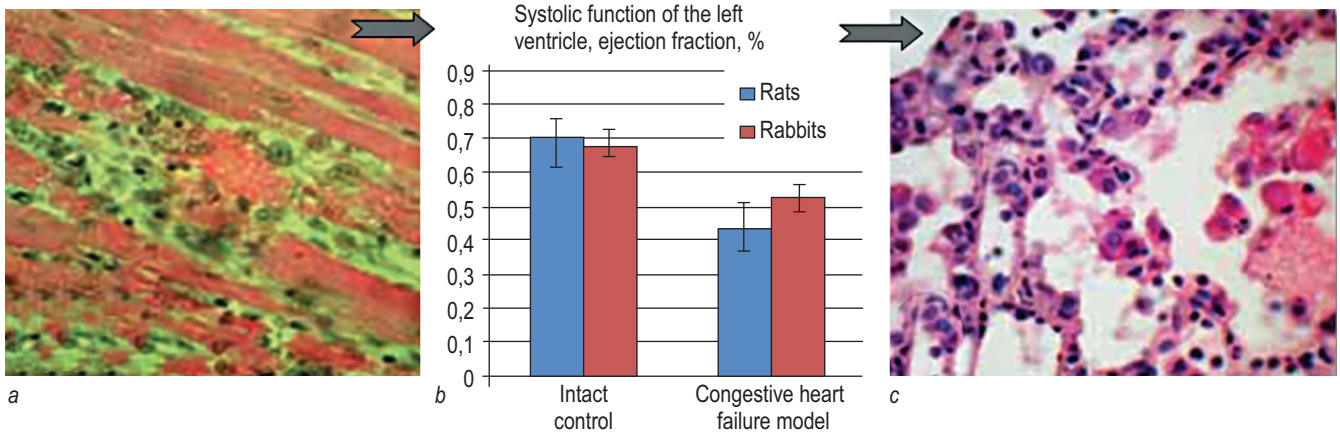
a *b*

Fig. 2. Infarct-like focus of ischemic necrosis, surrounded by a zone of reactive microglial and astrocytic proliferation with hemistocytic transformation (a); reactive gliosis with the formation of a glial micro-node in the perifocal zone of infarction (b). Hematoxylin and eosin staining, ×200



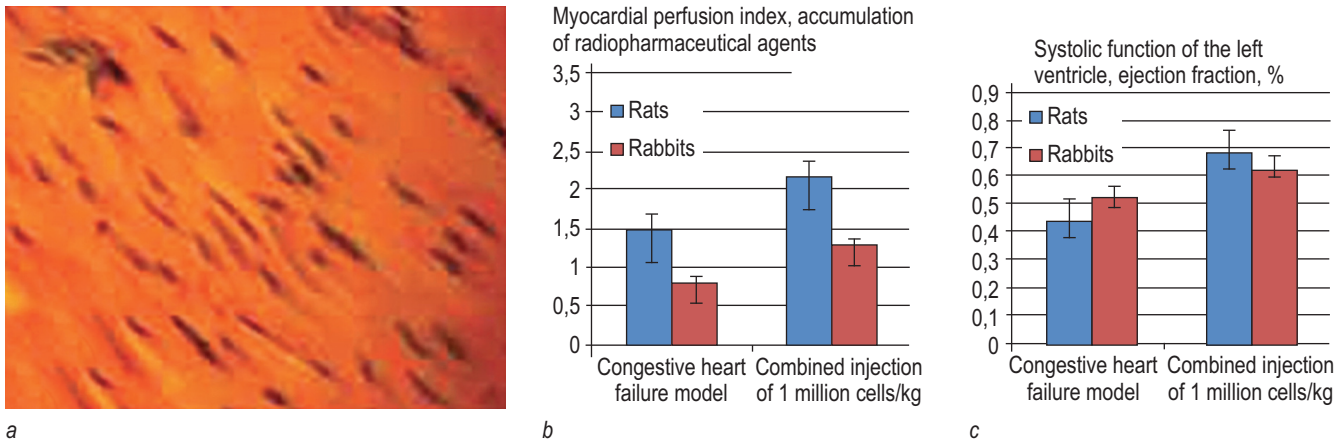
a b

Fig. 3. Ependymal proliferation in the wall of the III ventricle, ×200 (a); hemangio–meningotheliomatous proliferative micro-nodules in the soft medulla, ×100 (b). Hematoxylin and eosin staining, ×200



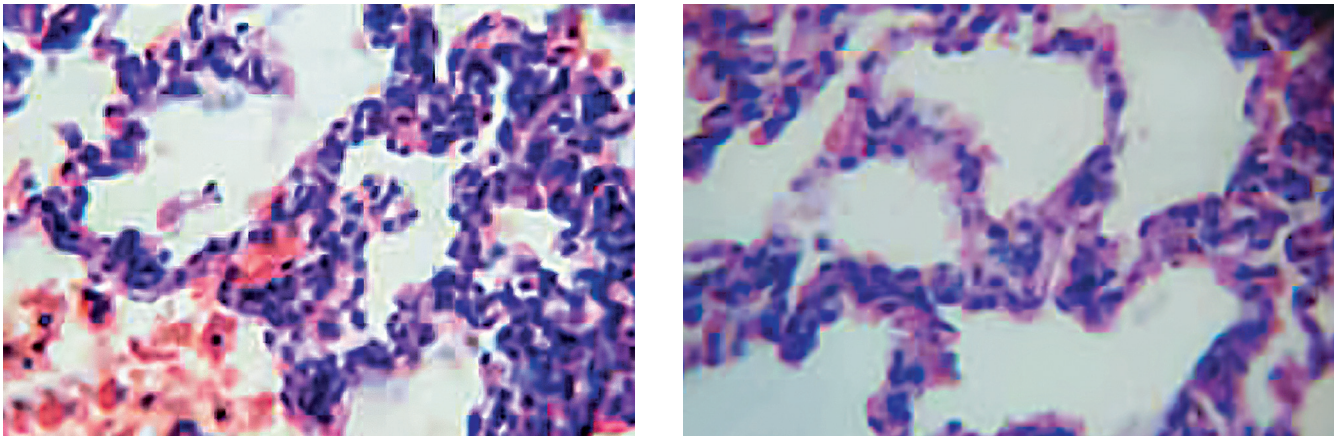
a b c

Fig. 4. Diffuse cardiomyocyte dystrophy with foci of necrosis (a); systolic function of the left ventricle, ejection fraction (b); lung congestion (c). Hematoxylin and eosin staining, ×200



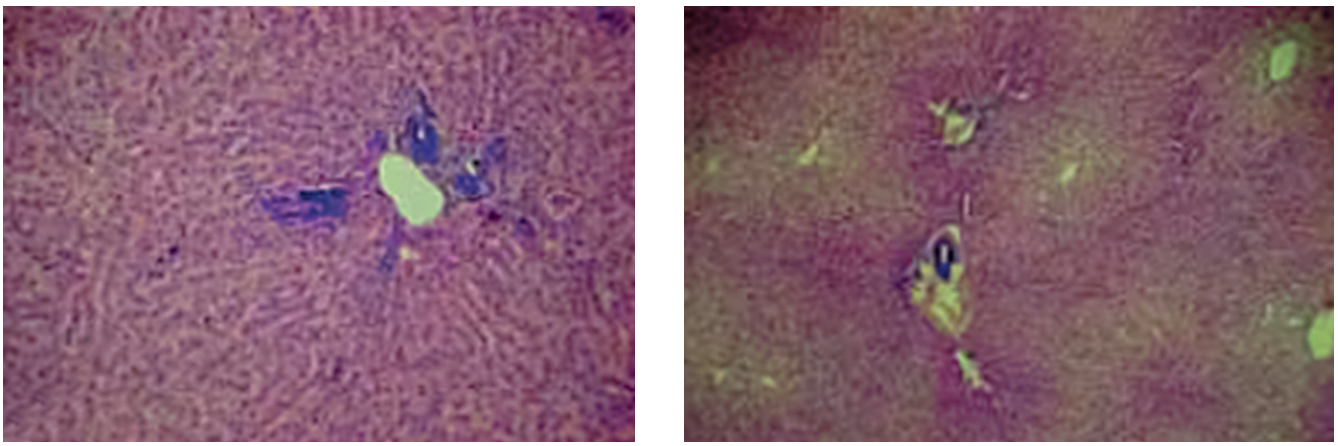
a b c

Fig. 5. Normal myocardial morphology. Longitudinally oriented myofibrils with transverse striation (a); hematoxylin and eosin staining, ×200, myocardial perfusion index (b); left ventricular systolic function, ejection fraction (c)



a *b*

Fig. 6. Morphology of rabbit lungs: vascular congestion and red blood cell diapedesis in dilated cardiomyopathy (a); normal histology of rabbit lungs after cell therapy (b). Hematoxylin and eosin staining, ×400



a *b*

Fig. 7. Vein overflow and inflammatory infiltrates in the liver in cases of dilated cardiomyopathy (a); normalization of liver perfusion after cell therapy (b). Hematoxylin and eosin staining, ×200 and ×50

being equal. Inflammation was initiated in the penumbra zone, accompanied by a cleansing of the infarction zone, the formation of a glial scar (Fig. 2a), and stabilization of brain tissue. In the control group, by the 30th day of the post-transplant period, the process of repair of ischemic injuries remained incomplete. The manifestations of gliosis and astrocytosis with the formation of glial micronodules in the perifocal surroundings of cerebral infarction were more common, compared with the experimental group (Fig. 2b).

At the same time, paracrine effects of transplanted cells caused the activation of angiogenesis, associated with the restoration of metabolism, the viability of neurons, and the stimulation of reparative regeneration. Moreover, the increase in proliferative activity is most pronounced on the part of the ependymal glia, vascular endothelium and meningothelium of the soft medulla (Fig. 3a, b).

The functional expression of the effectiveness of cell therapy for ischemic stroke, performed in accordance with the developed (optimal) Protocol, were signs of recovery of behavioral activity

Table 2

Behavioral activity of rats under the influence of cell therapy on the 14th day after coagulation of the middle cerebral artery

Type of behavioral activity	Number of animals	
	without cell therapy	with the use of cell therapy
Verticallocomotor activity	26,5±3,7	36,7±2,7*
Horizontal locomotor activity	4,3±0,6	5,6±1,0
Research activity by the number of visited burrows	1,7±0,3	3,6±0,3
Episodes of fur and face cleaning, (grooming)	2,7±0,1	3,6±0,1
Freezing	4,1±0,3*	1,4±0,1

*p <0,05.

of animals-recipients of the mononuclear fraction of the bone marrow (Table 2).

Diseases of non-ischemic origin are often the result of mutual influence of genetic and external factors, as is the case with dilated cardiomyopathy induced by rubomycin in the experiment (Fig. 4). However, the paracrine effects of MFBM cells extend to these forms of pathology [1, 9]. Transplantation of 2×10^6 allo- or autogenic MFBM cells in the volume of 100 μl by combined introduction of equal volumes of cell product intraaortically (during the systole with simultaneous compression of the aorta below the injection site) and intracardially (by four injections of cell suspension into both ventricles), contributed to the restoration of myocardial morphology, systolic function of the left ventricle, ejection fraction (Fig. 5), the disappearance of signs of blood stagnation in the small (Fig. 6) and large (Fig. 7) circulatory circles.

CONCLUSION

Bone marrow-derived mononuclear cells therapy for ischemic and non-ischemic diseases was performed according to the protocol taking into account the state of the target organ / tissue, optimal time of the procedure regarding the onset of pathology, optimal concentration of cells in the cell product and the optimal route for its delivery. Mononuclear therapy for ischemic and non-ischemic diseases has been demonstrated to be safe effective option or an independent procedure in the treatment of such diseases.

ЛИТЕРАТУРА

1. Белевитин А.Б., Никитин А.Э., Цыган В.Н., Хубулава Г.Г., Кривенцов А.В., Александров В.Н., Сазонов А.Б., Свистов А.С., Румакин В.П., Калюжная Л.И., Сухов В.Ю., Талантов С.В., Полуботко Е.В., Бабашев Ф.А., Максимов А.Г., Перекрест С.В. Поиск оптимального варианта клеточной терапии застойной сердечной недостаточности неишемического генеза в эксперименте. Вестник Российской Военно-медицинской академии. 2010; 3 (31): 112–20.
2. Верлов Н.А., Трашков А.П., Пахомова М.А. и др. Биомоделирование ангиогенеза. Педиатр. СПб.: 2016; 7(2): 127–34. DOI: 10.17816/PED72127-134.
3. Калюжная Л.И., Александров В.Н., Кривенцов А.В., Мартынов Б.В., Михайлова Е.В. Эффективность клеточной терапии, проведенной в разные сроки у крыс с вызванной ишемией головного мезга. Вестник Российской Военно-медицинской академии. 2016; 4(56): 127−31.
4. Кораблев Р., Васильев А. Неоангиогенез и опухолевый рост. Russian Biomedical Research. 2017; 2(4): 3–10.
5. Маслянюк О.В., Александров В.Н., Чирский В.С., Кривенцов А.В., Максимов А.Г., Румакин В.П., Калюжная Л.И. Поиск оптимального варианта клеточной терапии критической ишемии конечностей в эксперименте. Клеточная трансплантология и тканевая инженерия. 2011; 3(4): 41–5.
6. Bai X., Xu J., Zhu T., He Y., Zhang H. The Development of Stem Cell-Based Treatment for Acute Ischemic Cerebral Injury. Current stem cell research & therapy. 2020; 15: 30.
7. Beltran-Camacho L., Jimenez-Palomares M., Rojas-Torres M., Sanchez-Gomar I., Rosal-Vela A., Eslava-Alcon S., Perez-Segura M.C., Serrano A., Antequera-Gonzales B., Alonso-Pinero J.A., Gonzalez-Rovira A., Extremera-Garcia M.J., Rodriguez-Pinero M., Moreno-Luna R., Larsen M.R., Duran-Ruiz M.C. Identification of the initial molecular changes in response to circulating angiogenic cells-mediated therapy in critical limb ischemia. Stem Cell Research & Therapy. 2020; 11: 106–26.
8. Carmona D., Cañadillas S., Romero M., Blanco A., Nogueras S., Herrera C. Intramyocardial bone marrow mononuclear cells versus b one marrow-derived and adipose mesenchymal cells in a rat model of dilated cardiomyopathy. Cytotherapy. 2017; 19(8): 947–61.
9. Farina M., Kajikawa M., Takaeko Y., Kishimoto S., Hashimoto H., Maruhashi T., Kihara Y., Nakashima A., Higashi Y. Long-Term Clinical Outcomes of Autologous Bone Marrow Mononuclear Cell Implantation in Patients with Severe Thromboangiitis Obliterans. Circulation Journal. 2020; 84(4): 650–55.
10. Fung E., Helisch A. Macrophages in collateral arteriogenesis. Front Physiol. 2012; 3: 11.
11. Hilal R., Poittevin M., Pasteur-Rousseau A., Cogo A., Mangin G., Chevauché M., Ziat Y., Vilar J., Launay J.-M., Gautier J.-F., Broqueres-You D., Levy B., Merkulova-Rainon T., Rubis N. Diabetic Ephrin-B2-Stimulated Peripheral Blood Mononuclear Cells Enhance Poststroke Recovery in Mice. Stem Cells Int. 2018; 17.
12. Kikuchi-Taura A., OkinakaY.,Takeuchi Y., Ogawa Y., Maeda M., Kataoka Y., Yasui T., Kimura T., Gul S., Claussen C., Boltze J., Taguchi A. Bone Marrow Mononuclear Cells Activate Angiogenesis via Gap Junction-Mediated Cell-Cell Interaction. Stroke. 2020; 51(4): 1279−89.
13. Qadura M., Terenzi D., Verma S., Al-Omran M., Hess D. Concise review: cell therapy for critical limb ischemia: an integrated review of preclinical and clinical studies. StemCells. 2018; 36: 161–71.

REFERENCES

1. Belevitin A.B., Nikitin A.E., Cygan V.N., Hubulava G.G., Krivencov A.B., Aleksandrov V.N., Sazonov A.B., Svistov A.C., Rumakin V.P., Kalyuzhnaya L.I., Suhov V.Yu., Talantov C.B., Polubotko E.V., Babashev F.A., Maksimov A.G., Perekrest C.B. Poisk optimal'nogo varianta kletochnoj terapii zastojnoj serdechnoj nedostatochnosti neishemicheskogo geneza v eksperimente [The search of optimal variant for cell therapy of congestive heart failure of non-ischemic genesis in experiment]. Vestnik Rossijskoj Voenno-medicinskoj akademii. 2010; 3(31): 112–20. (in Russian).
2. Verlov N.A., Trashkov A.P., Pahomova M.A. i dr. Biomodelirovanie angiogeneza [Biomodeling of angiogenesis]. Pediatr. St. Petersburg: 2016; 7(2): 127–34. DOI: 10.17816/PED72127-134. (in Russian).
3. Kalyuzhnaya L.I., Aleksandrov V.N., Krivencov A.V., Martynov B.V., Mihajlova E.V. Effektivnost' kletochnoj terapii, provedennoj v raznye sroki u krys s vyzvannoj ishemiej golovnogo mozga [Efficiency of the cellular therapy conducted in different terms for rats' ischemic stroke model]. Vestnik Rossijskoj Voenno-Medicinskoj akademii. 2016; 4(56): 127−31. (in Russian).
4. Korablev R., Vasil'ev A. Neoangiogenez i opuholevyj rost [Neoangiogenesis and tumor growth]. Russian Biomedical Research. 2017; 2(4): 3–10. (in Russian).

5. Maslyanyuk O.V., Aleksandrov V.N., Chirskij V.S., Krivencov A.V., Maksimov A.G., Rumakin V.P., Kalyuzhnaya L.I. Poisk optimal'nogo varianta kletochnoj terapii kriticheskoj ishemii konechnostej v eksperimente [Search of an optimum variant of cell therapy of a critical limbs ischemia in experiment]. Kletochnaya transplantologiya i tkanevaya inzheneriya. 2011; 3(4): 41–5. (in Russian).
6. Bai X., Xu J., Zhu T., He Y., Zhang H. The Development of Stem Cell-Based Treatment for Acute Ischemic Cerebral Injury. Current stem cell research & therapy. 2020; 15: 30.
7. Beltran-Camacho L., Jimenez-Palomares M., Rojas-Torres M., Sanchez-Gomar I., Rosal-Vela A., Eslava-Alcon S., Perez-Segura M.C., Serrano A., Antequera-Gonzales B., Alonso-Pinero J.A., Gonzalez-Rovira A., Extremera-Garcia M.J., Rodriguez-Pinero M., Moreno-Luna R., Larsen M.R., Duran-Ruiz M.C. Identification of the initial molecular changes in response to circulating angiogenic cells-mediated therapy in critical limb ischemia. Stem Cell Research & Therapy. 2020; 11: 106–26.
8. Carmona D., Cañadillas S., Romero M., Blanco A., Nogueras S., Herrera C. Intramyocardial bone marrow mononuclear cells versus bone marrow-derived and adipose mesenchymal cells in a rat model of dilated cardiomyopathy. Cytotherapy. 2017; 19(8): 947–61.
9. Farina M., Kajikawa M., Takaeko Y., Kishimoto S., Hashimoto H., Maruhashi T., Kihara Y., Nakashima A., Higashi Y. Long-Term Clinical Outcomes of Autologous Bone Marrow Mononuclear Cell Implantation in Patients with Severe Thromboangiitis Obliterans. Circulation Journal. 2020; 84(4): 650–55.
10. Fung E., Helisch A. Macrophages in collateral arteriogenesis. Front Physiol. 2012; 3: 11.
11. Hilal R., Poittevin M., Pasteur-Rousseau A., Cogo A., Mangin G., Chevauché M., Ziat Y., Vilar J., Launay J.-M., Gautier J.-F., Broqueres-You D., Levy B., Merkulova-Rainon T., Rubis N. Diabetic Ephrin-B2-Stimulated Peripheral Blood Mononuclear Cells Enhance Poststroke Recovery in Mice. Stem Cells Int. 2018; 17.
12. Kikuchi-Taura A., OkinakaY.,Takeuchi Y., Ogawa Y., Maeda M., Kataoka Y., Yasui T., Kimura T., Gul S., Claussen C., Boltze J., Taguchi A. Bone Marrow Mononuclear Cells Activate Angiogenesis via Gap Junction-Mediated Cell-Cell Interaction. Stroke. 2020; 51(4): 1279−89.
13. Qadura M., Terenzi D., Verma S., Al-Omran M., Hess D. Concise review: cell therapy for critical limb ischemia: an integrated review of preclinical and clinical studies. StemCells. 2018; 36: 161–71.