Radiofrequency Ablation as a Possible Method for Preparing Pathologically Altered Myocardium for Intramyocardial Cell Transplantation

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We studied the effects of radiofrequency ablation on the results of intramyocardial transplantation of bone marrow NSC into the myocardium of rats with postinfarction cardiosclerosis. It was shown that exposure of the pathologically changed myocardium to radiofrequency radiation led to destruction of formed connective tissue. Transplantation of MSC into sites exposed to radiofrequency radiation promoted the development of regenerative processes (abundant infiltration with mononuclear cells, presence of granulation tissue, and numerous newly formed blood vessels). We concluded that preliminary radiofrequency irradiation of the myocardial areas promotes realization of the regenerative potential of cell transplantation.

Key Words: *myocardium; postinfarction remodeling; cell transplantation; mesenchymal stem cells; radiofrequency ablation*

Most studies of the efficiency of cell therapy recognize the necessity of targeted application of the cell material. For instance, intramyocardial cell transplantation is a promising approach to correction of postinfarction remodeling and treatment of heart failure [6]. However, in case of successful targeted delivery of the cell material, a new problem arises: cell transfer from optimal conditions of cell culture into pathologically changed myocardium negatively affects their survival after transplantation.

Postinfarction remodeling manifests in not only changes in heart geometry, but also structural alteration of the myocardium [7]. Connective tissue growth in the zone of necrosis and adjacent zones of the myocardium increases rigidity of extracellular matrix. These conditions not only promote ischemic death of transplanted cells, but also induce their differentiation into wrong direction. It is however known that inflammatory reactions are accompanied by destruction of extracellular matrix and release of endogenous bioactive substances promoting regeneration [11]. This is usually taken into account when determining the terms of cell transplantation in myocardial infarction. However, activity of endogenous reparative processes decreases at late stages of postinfarction remodeling, while the growth of extracellular matrix is going on.

Local irreversible destruction of myocardial structures can be induced by exposure of the myocardium to radiofrequency radiation [12] causing practically momentary warming of irradiated myocardium [1] followed by the development of aseptic inflammation and the formation of connective tissue cicatrix. This mechanism was used for creation of special devices, radiofrequency destructors. Modern radiofrequency destructors enable strictly dosed exposure, which ensures disintegration of the extracellular matrix, cell membranes, and cytoskeleton [10]. Activation of regenerative processes after radiofrequency irradiation creates favorable conditions for realization of the effects of transplanted cell material.

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Here we studied the possibility of using radiofrequency ablation for preparation of pathologically altered myocardium for intramyocardial transplantation of cell material.

MATERIALS AND METHODS

Experiments were carried out on 30 male Wistar rats weighing 230-250 g. The animals were kept in accordance with European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. For initiation of postinfarction remodeling of the heart, the upper third of the descending coronary artery was ligated. On day 45 after coronary occlusion, postinfarction cardiosclerosis (PICS) developed. Similar coronary occlusion procedure was described elsewhere [3,8].

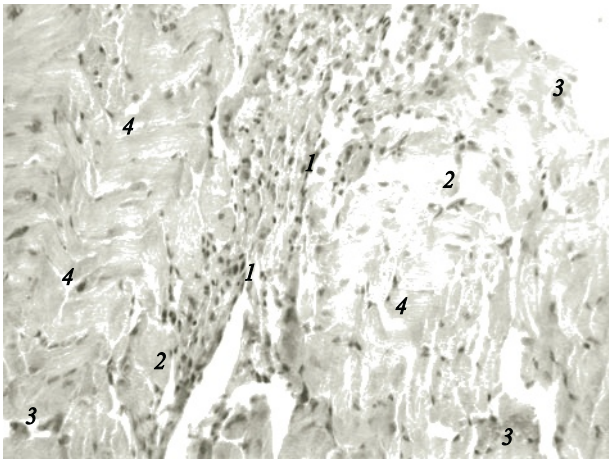

Fig. 1. Morphological picture of rat myocardium with PICS and intramyocardial transplantation of MSC. Hematoxylin and eosin staining, ×200. *1*) postinfarction cicatrix; *2*) hypertrophied muscle fibers; *3*) plethoric vessels; *4*) twisted muscle fibers.

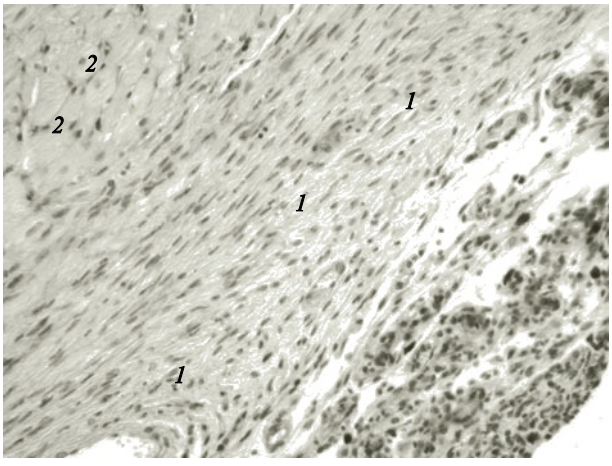

Fig. 2. Morphological picture of rat left ventricular myocardium with PICS and radiofrequency ablation. Hematoxylin and eosin staining, ×200. 1) young connective tissue with numerous cells; 2) hypertrophied cardiomyocytes with vacuolated cytoplasm.

MSC were obtained using BM mononuclear fraction from rat femoral bones; they were cultured for 14 days in a sterile CO_2-incubator at 37°C, 100% humidity, and 5% CO_2 [5,9].

The animals with developed experimental PICS were anesthetized, fixed on the operating table, and the thorax was opened. A myocardial area adjacent to the postinfarction cicatrix was chosen for subsequent MSC transplantation. This area was exposed to radiofrequency irradiation (5-6 pulses, 98 msec duration, 3 W power) so that the whole surface was covered). A Russian-made standard Elektropul's RF 100 TZ radiofrequency destructor was used. Immediately after irradiation, 100 μl culture medium containing $1\text{-}2\times10^5$ MSC was injected into the myocardium. The operation wound was sutured layer-by-layer. All manipulations were performed under sterile conditions and with sterile instruments.

The animals were divided into 3 groups. Experimental group 1 comprised animals receiving transplantation of MSC after radiofrequency destruction [4,5]. Animals of group 2 (injection of an equivalent volume of culture medium after radiofrequency ablation) and group 3 (MSC transplantation without radiofrequency ablation) served as the control. The animals were sacrificed in 15 days after treatment. The fragments of the left ventricle subjected to experimental procedures were isolated, fixed in 10% formalin, dehydrated in ascending alcohols, and embedded in paraffin. Serial paraffin sections were stained with hematoxylin and eosin [2,3].

RESULTS

Histological examination of myocardial sections revealed signs of PICS in animals of all groups. At the same time, morphological picture of the myocardium in each group had certain peculiarities. In group 3, minor cell infiltration presented by mononuclears and fibroblasts in equal amounts was observed in the studied myocardial samples on day 15 after intramyocardial transplantation of MSC against the background of PICS. Hypertrophy of muscle fibers, swelling of cardiomyocyte cytoplasm, and moderate capillary plethora were seen. Myocardial samples from this group were also characterized by twisted muscle fibers and the absence of cell infiltration in connective tissue layers. Figure 1 presents typical morphological picture of the myocardium in animals of this group.

In group 2 animals (radiofrequency ablation against the background of PICS), the myocardial zone subjected to destruction was characterized by the presence of young connective tissue. Fibroblasts were predominant cells in this zone, while mononuclears were rare.

Hypertrophy of muscle fibers and vacuolation of cardiomyocyte cytoplasm were seen along the periphery of postinfarction cicatrix. Arterial walls in the zone of destruction and in the adjacent myocardium were equally thickened due to sclerosis. Typical morphological picture observed in myocardial samples from this group is presented in Figure 2.

Myocardial samples from group 3 animals (intramyocardial transplantation of MSC after radiofrequency ablation against the background of PICS) principally differed from the control samples (Fig. 3). They were characterized by abundant cell infiltration (with primarily mononuclear cells). The samples contained granulation tissue with preserved or degenerative cardiomyocytes and fields of young connective tissue. Numerous newly formed blood vessels were seen. Cardiomyocyte hypertrophy was minor; signs of inflammation were seen.

These findings attest to more favorable course of regenerative processes in animals of the experimental group. This suggests that radiofrequency ablation can be used for preparation of pathologically changed myocardium to intramyocardial cell transplantation. Such preconditioning creates conditions for better realization of regenerative potential of cell transplantation.

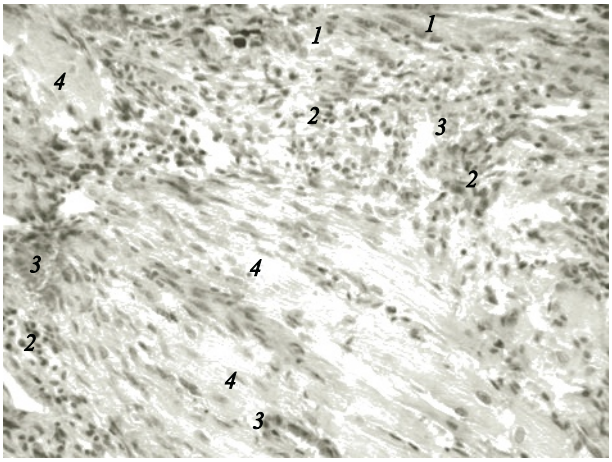

Fig. 3. Morphological picture of rat myocardium with PICS, radiofrequency ablation, and intramyocardial transplantation of MSC. Hematoxylin and eosin staining, ×200. *1*) granulation tissue; *2*) mononuclear infiltration; *3*) plethoric vessels; *4*) hypertrophied cardiomyocytes.

REFERENCES

1. V. N. Ardashev, A. V. Ardashev, and V. I. Steklov, *Therapy of Heart Rhythm Disturbances* [in Russian], Moscow (2005).
2. S. A. Afanas'ev, Yu. V. Rogovskaya, I. N. Sviridov, *et al., Klet. Tekhnol. Biol. Med.*, No. 2, 81-84 (2009).
3. S. A. Afanas'ev, Yu. V. Rogovskaya, L. P. Falaleeva, *et al., Byull. Eksp. Biol. Med.*, **148**, No. 6, 631-634 (2009).
4. S. A. Afanas'ev, I. N. Sviridov, V. P. Shakhov, *et al., Vestn. Aritmol.*, No. 47, 38-41 (2007).
5. S. A. Afanas'ev, I. N. Sviridov, V. P. Shakhov, *et al., Klet. Tekhnol. Biol. Med.*, No. 1, 29-33 (2008).
6. Yu. I. Buziashvili, S. T. Matskeplishvili, B. G. Alekyan, *et al., Vestn. Ross. Akad. Med. Nauk.*, No. 4, 65-69 (2005).
7. V. A. Kazakov, I. V. Sukhodolo, V. M. Shipulin, and A. A. Miller, *Sib. Med. Zh.*, **1**, No. 4, 6-11 (2009).
8. D. S. Kondrat'eva, S. A. Afanas'ev, L. P. Falaleeva, and V. Shakhov, *Byull. Eksp. Biol. Med.*, **130**, No. 6, 613-616 (2005).
9. N. A. Onishchenko, I. V. Potapov, L. V. Bashkina, *et al., Byull. Eksp. Biol. Med.*, **138**, No. 10, 403-407 (2004).
10. D. E. Haines, *Biophysycs and pathophysiology of lesion formation by transcatheter radiofrequency ablation. Catheter Ablation of Cardiac Arrhythmias. Basic Concepts and Clinical Applications,* Blackwell Futura, New York (2008), pp. 20-34.
11. I. Pountos and P. V. Giannoudis, *Injury*, **36**, Suppl. 3, 8-12 (2005).
12. J. G. Whayne, S. Nath, and D. E. Haines, *Circulation*, **89**, No. 5, 2390-2395 (1994).