Effect of Transplantation of Bone Marrow Cells on Morphology of Rat Myocardium after Cryodestruction

S. A. Afanasyev, E. V. Rogovskaya, I. N. Sviridov, L. P. Falaleeva, V. P. Shakhov, S. V. Popov, and R. S. Karpov

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We studied the effects of bone marrow cell transplantation on myocardium of the prenecrotic zone in Wistar rats. Intramyocardial cell transplantation reduced the severity of hypertrophy of myocardium and the degree of its cicatricial degeneration on day 40 after cryodestruction. The morphology of the myocardium in the prenecrotic zone depended on the type of transplanted cells. The course of inflammation was swifter; vascularization of the myocardium was more intensive. The best effect, evaluated by the number of new vessels, was observed after MSC transplantation. Hence, the positive effect of bone marrow cell transplantation is realized at the expense of more rapid structural organization of the damaged site and stimulation of myocardial vascularization.

Key Words: *cryodestruction; cell transplantation; mesenchymal stem cells; angiogenesis*

Along with drug therapy and surgery [8], the development of alternative therapies for heart failure is now receiving more and more attention. One of prospective trends is transplantation of cell material. Bone marrow (BM) cells attract special interest in cell therapy. Their important advantage is the possibility of autotransplantation. Bone marrow mononuclear fraction can be rapidly obtained and these cells produce a great spectrum of bioactive substances [2]. However, the content of stem cells in this fraction is low. Their count can be increased and their directed differentiation can be realized by preculturing. However, the efficiency of this therapy is ambiguous judging form published results of clinical use of BM cell transplantation to cardiological patients [12].

Institute of Cardiology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences. ***Address for correspondence:*** lpf@cardio.tsu.ru. S. A. Afanasyev

We compared the morphology of rat myocardium after destruction and subsequent intramyocardial transplantation of various types of BM cells.

MATERIALS AND METHODS

Experiments were carried out on 50 male Wistar rats (230-250 g) handled in accordance with the European Convention for Protection of Vertebrates Used for Experimental and Other Scientific Purposes.

Myocardial injuries were induced by cryodestruction. This method allows creation of lesions of virtually the same volume and location [4]. The thorax of narcotized animals was opened and the left ventricle (apical area) was subjected to cryodestruction with a metal rod cooled in liquid nitrogen. A flat end of the rod (6 mm in diameter) served as the working surface. It could be easily applied to the heart surface. The duration of rod

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contact with the heart surface was 10 sec. The wound was then sutured layer-by-layer.

After 9 days, the thorax was reopened and 100 μl culture medium was injected intramyocardially into 5-6 points along the perimeter of the damaged area. The animals were divided into 4 groups: 1) culture medium without cell material; 2) culture medium with BM mononuclear fraction (3×10^6 cell/ml); 3) MSC (2×10^5 cell/ml); and 4) medium with predifferentiated MSC (2×10^5 cell/ml). The concentrations of transplanted cells were selected on the basis of our findings and published data [5].

Cell material was obtained from 8 animals. The mononuclear fraction (MNF) was derived from femoral BM [5].

Mesenchymal stem cells were isolated from pre-isolated MNF by the standard method [5]. Directed differentiation of MSC was induced by adding 5-azacitidine (3 μmol/liter) [6].

All experimental and 10 intact animals were sacrificed on day 30 after transplantation. Body and heart weights were measured. The left ventricle was separated and its weight was measured after Avtandilov [1]. The weight of damaged myocardial area was measured in 5 animals of each experimental group. The damaged myocardial area was visualized by nitroyellow tetrazolium staining [13].

Histological studies were carried out on hearts without staining. Left ventricular fragments from the focus of destruction and fragments of intact myocardium were collected. The specimens were fixed in 10% neutral formalin. Serial paraffin sections were stained with hematoxylin and eosin [4]. Vessels in the zone of myocardial injury were counted in 10 visual fields at ×400 and their mean number per visual field was evaluated [4]. The data were processed using nonparametric Mann-Whitney *U* test.

RESULTS

A characteristic macroscopic sign of cryodestruction is strong cardiac hypertrophy with a clearly seen connective tissue cicatrix; this picture was observed in control animals (Fig. 1). Cell transplantation significantly reduced the severity of these changes, which was seen from heart weights (Table 1). Heart weight and left-ventricular weight increased 1.6 times 40 days after cryodestruction. These parameters were different in animals treated by cell transplantation. Heart and left ventricular weights in these animals surpassed the normal by only 1.4 and 1.3 times. The weights of the cicatricial focus in groups 2, 3, and 4 were 1.29, 1.45, and 1.51 lower than in group 1, respectively.

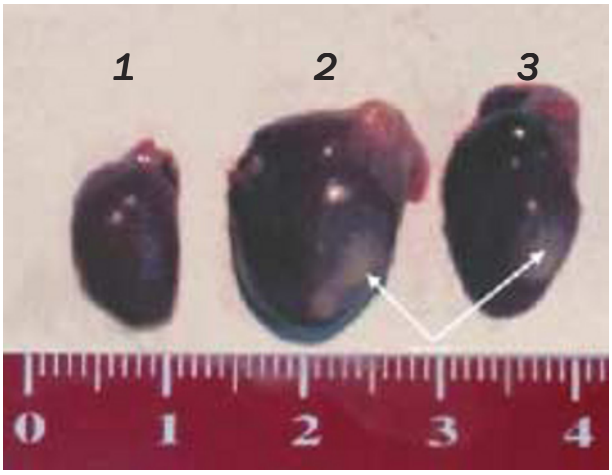

Fig. 1. Rat heart 40 days after cryodestruction and BM cell transplantation. *1*) intact; *2*) after cryodestruction; *3*) after cryodestruction and cell transplantation. Arrows show the sites of destruction.

Microscopy of the myocardium in group 1 showed pronounced cell infiltration, forming the demarcation zone, separating the focus from intact myocardium (Fig. 2, *a*). Greater magnification showed that the demarcation zone contained destroyed and intact cardiomyocytes forming separate islets (Fig. 2, *b*). Pronounced interstitial edema was seen in the adjacent intact myocardium; muscle fibers were unevenly hypertrophied.

The same changes in the myocardium were observed in other groups (Fig. 2, *c*, *d*). However, the severity of these changes was different, depending on the type of transplanted cells. The greatest cell infiltration in the demarcation zone was seen in group 2, with total cell count in a visual field 14±3. In groups 3 and 4, cell infiltration was minor: 7±2 cells per visual field.

These results do not contradict other published reports [9,11,14]. It was shown that tansplantation of mononuclear cells led to intensification of inflammatory processes due to more intense secretion of bioactive substances by these cells. Hence, slight cell infiltration after MSC transplantation can be indicative of their low proinflammatory effect.

The main difference between the groups was the intensity of angiogenesis processes. New vessels appeared only after cell transplantation. The least number of vessels was detected in group 2 animals (Fig. 3). The number of vessels after MSC transplantation (group 3) was almost 6-fold higher while after transplantation of predifferentiated MSC (group 4) the number of vessels increased only 4-fold. This result is in line with the report indicating that BM cell transplantation can initiate the formation of branched microcirculatory network [15]. The difference detected in our study can be

TABLE 1. Relationship between Intramyocardial Cell Transplantation and Weight Characteristics after Cryodestruction (*M*±*m*)

Parameter	Groups of animals				
	intact (*n*=5)	40 days after cryodestruction			
		1 (*n*=5)	2 (*n*=5)	3 (*n*=5)	4 (*n*=5)
Body weight, g	287±23	236±3	239±3	248±4	240±4
Heart weight, mg	955±44**	1501±20	131±17**	1224±16**	1283±6**
Left ventricular weight, mg	620±35**	1020±13	835±16**	791±15**	810±16**
Focus weight, mg	0	111±1	86±4*	76±3*	73±1*

Note. *p<0.01, **p<0.05 compared to group 1.

explained by different intensity of production of vascular growth stimulating factors by different transplanted cells [7]. Of these cells, MSC are the most committed and capable of transforming into other cell types, including endotheliocytes [2]. The fact that transplantation of predifferentiated cells less intensely activated neoangiogenesis indicates limited potential of these cells to develop into

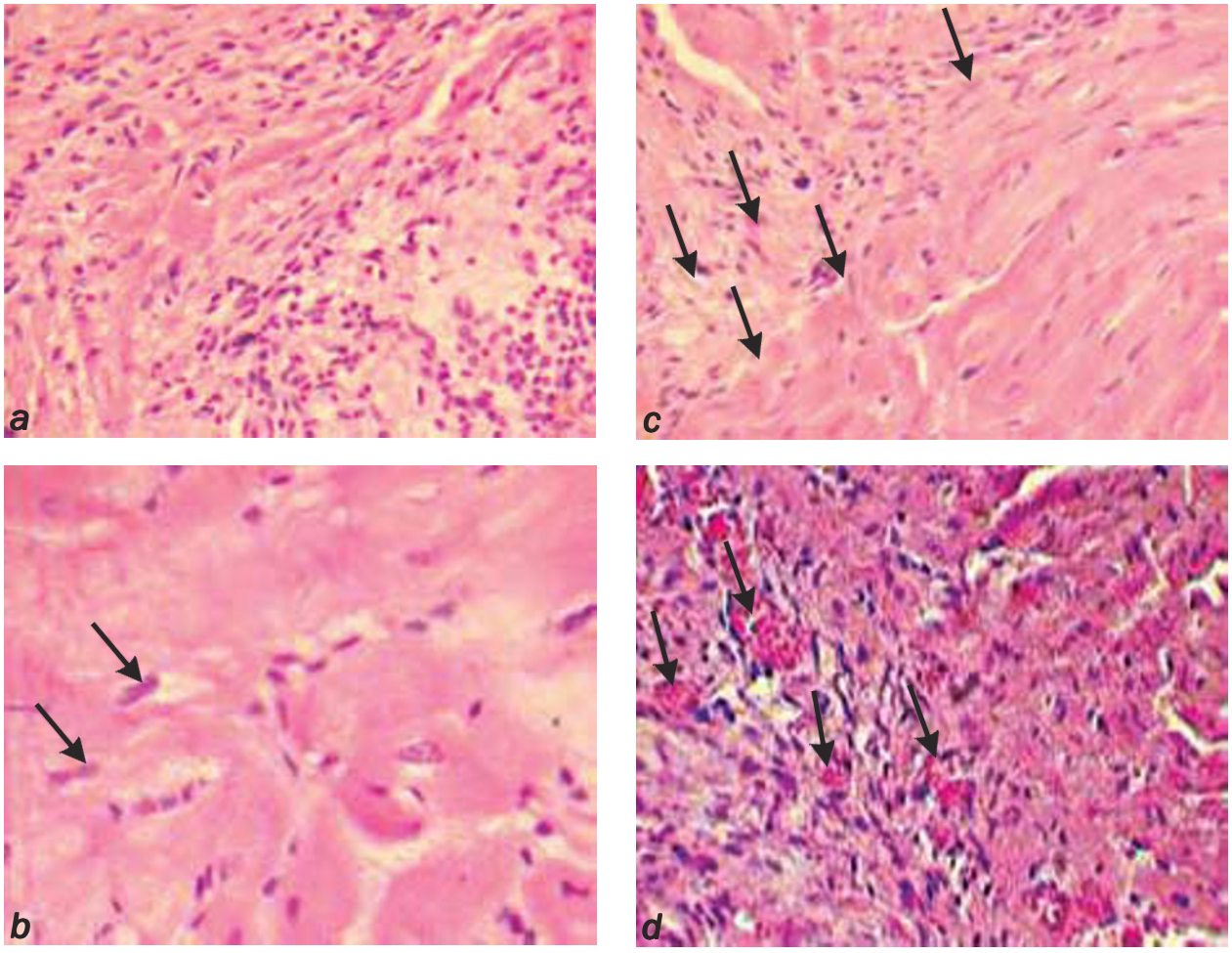

Fig. 2. Morphological picture of the rat left ventricular myocardium 40 days after cryodestruction and BM cell transplantation (hematoxylin and eosin staining). *a*) control (×200): concentrated uneven pronounced polymorphonuclear infiltration in demarcation zone; focus of destroyed cardiomyocytes left from the center; *b*) control injury (×400): pronounced interstitial edema, uneven cardiomyocyte hypertrophy, slight mononuclear infiltration, perinuclear vacuoles in the cytoplasm of some cardiomyocytes (arrows); *c*) cryodestruction with MSC transplantation (×200): connective tissue cicatrix at the site of cryodestruction in the upper left section, unevenly pronounced mononuclear infiltration. Arrows show new vessels; intact cardiomyocytes in the lower right part; *d*) cryodestruction with MSC transplantation (×100): left: connective tissue cicatrix at the site of cryodestruction, cell infiltration, blood vessels (arrows); upper right section: intact myocardium.

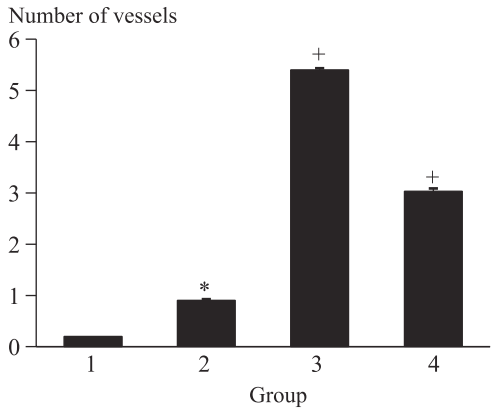

Fig. 3. Neoangiogenesis intensity in myocardial preparations 40 days after cryodestruction and BM cell transplantation. Ordinate: number of vessels per visual field. *1*) control cryodestruction; *2*) cryodestruction with transplantation of mononuclear cells; *3*) cryodestruction with MSC transplantation; *4*) cryodestruction with transplantation of predifferentiated MSC. $p<0.05$ comapred to *control cryodestruction, +transplantation of mononuclears.

endotheliocytes. It is known that 100% predifferentiation of stem cells in culture is impossible [4]. Some cells can change the direction of their differentiation; in our case these cells could initiate angiogenesis. Bone marrow MNF also contain not many stem cells [2]. Presumably, this fact was responsible for poor neoangiogenesis in animals of group 2. On the whole, intensive formation of additional capillary network promotes retention of a greater number of viable cardiomyocytes and eventually promotes the retention of heart function [3,10].

Hence, intramyocardial transplantation of BM cells inhibits the development of cardiac hypertrophy after destructive exposure. Transplantation of MSC and predifferentiated pool of these cells forms a more favorable morphological picture of the myocardium in the focus of injury. This effect is most likely realized at the expense of inflammation and neoangiogenesis stimulation.

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