Recovery of Contractile Function of Cryodamaged Rat Myocardium after Transplantation of Fetal Cardiomyocytes and Predifferentiated Bone Marrow Stromal Stem Cells

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The effect of cell transplantation into cryodamaged rat myocardium was studied on isolated hearts by increasing functional load to the left ventricle. Transplantation of allogeneic fetal cardiomyocytes improved the function of the left ventricle under conditions of considerably increased preload. Transplantation of autologous mesenchymal stem cells repaired left-ventricular function under conditions of increased pre- and afterload.

Key Words: *stem cells; transplantation; myocardium*

The potentialities of regeneration medicine based on cell technologies for repair of the contractile function of damaged myocardium are now intensely studied. Changes in the pumping function of the heart can be evaluated by measuring left-ventricular pressure [2,9, 10] or by noninvasive echocardiographic method in various modifications [4,10].

However, none of these method can evaluate changes in the functional reserve of the myocardium after intramyocardial transplantation of cells; these changes can be detected only by exposing the left ventricle to dosed increase in pre- and afterload.

We studied the reserve potentialities of the rat left-ventricular myocardium after heart failure induced by cryodestruction (CD) and after transplantation of fetal cardiomyocytes (FCMC) and predifferentiated bone marrow mesenchymal (stromal) stem cells (MSC) into the perinecrotic zone of these hearts.

MATERIALS AND METHODS

Experiments were carried out on 63 adult male Wistar rats (200-300 g) under intraperitoneal narcosis (ketamine 50 mg/kg, xylasolin 10 mg/kg) and intubation forced ventilation of the lungs.

The animals were divided into 4 groups: 1) sham-operated rats (n=21); 2) rats with cryodamaged myocardium, injected with 100 μl culture medium into the perinecrotic zone (control hearts; n=18); 3) rats with CD, injected with allogeneic FCMC (n=12); and 4) rats with CD, injected with autologous predifferentiated bone marrow MSC (n=12).

CD was inflicted with a metallic bar (6 mm in diameter) cooled in liquid nitrogen. FCMC for transplantation were obtained from the hearts of 18-day Wistar rat fetuses [7]. Two-day FCMC culture was used for transplantation. MSC for transplantation was obtained from autologous tibial bone marrow and cultured in IMDM medium with additives [6]. After 3-day culturing 5-azacitidine (6 μmol/liter) was added to the medium for 24 h in order to trigger differentiation of MSC into cardiomyocyte-like cells [3]. Three-week MSC culture was used for transplantation. FCMC ($1.15\pm0.59\times10^6$) and MSC ($1.49\pm0.93\times10^6$), resuspended in 100 μl Hanks' solution, were transplanted into 5 sites of the marginal zone of necrosis 7 days after CD. After transplantation of FCMC the animals were subcutaneously injected with cyclosporine A (5 mg/kg/day).

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Transplanted cells were identified in the myocardium histochemical by staining for β-galactosidase; the stain was delivered into the cell culture as a component of recombinant deleted adenovirus containing *E. coli* Lac-Z gene [1].

The pumping function of the hearts was evaluated 3 weeks after FCMC and bone marrow MSC transplantation in a testing unit by modified Neely's method [8], using a succession of protocols of left-ventricular loading (Table 1). For protocols 1-3, left-ventricular preload (volume load) was increased by elevating the atrial chamber. Then afterload was gradually increased (pressure load; protocols 4-5) by increasing the height of the perfusate level in the aortic tube. During testing according to protocol 6 afterload increased at the expense of decreased diameter of the

TABLE 1. Left-Ventricular Loading Protocols

Protocol	Perfusate height in tube, cm		Tube diameter, mm
	in left atrium	in aorta	
1	10	70	3
2	20	70	3
3	30	70	3
4	30	100	3
5	30	125	3
6	30	100	1.1
7	30	125	3
8	30	Clamped	0

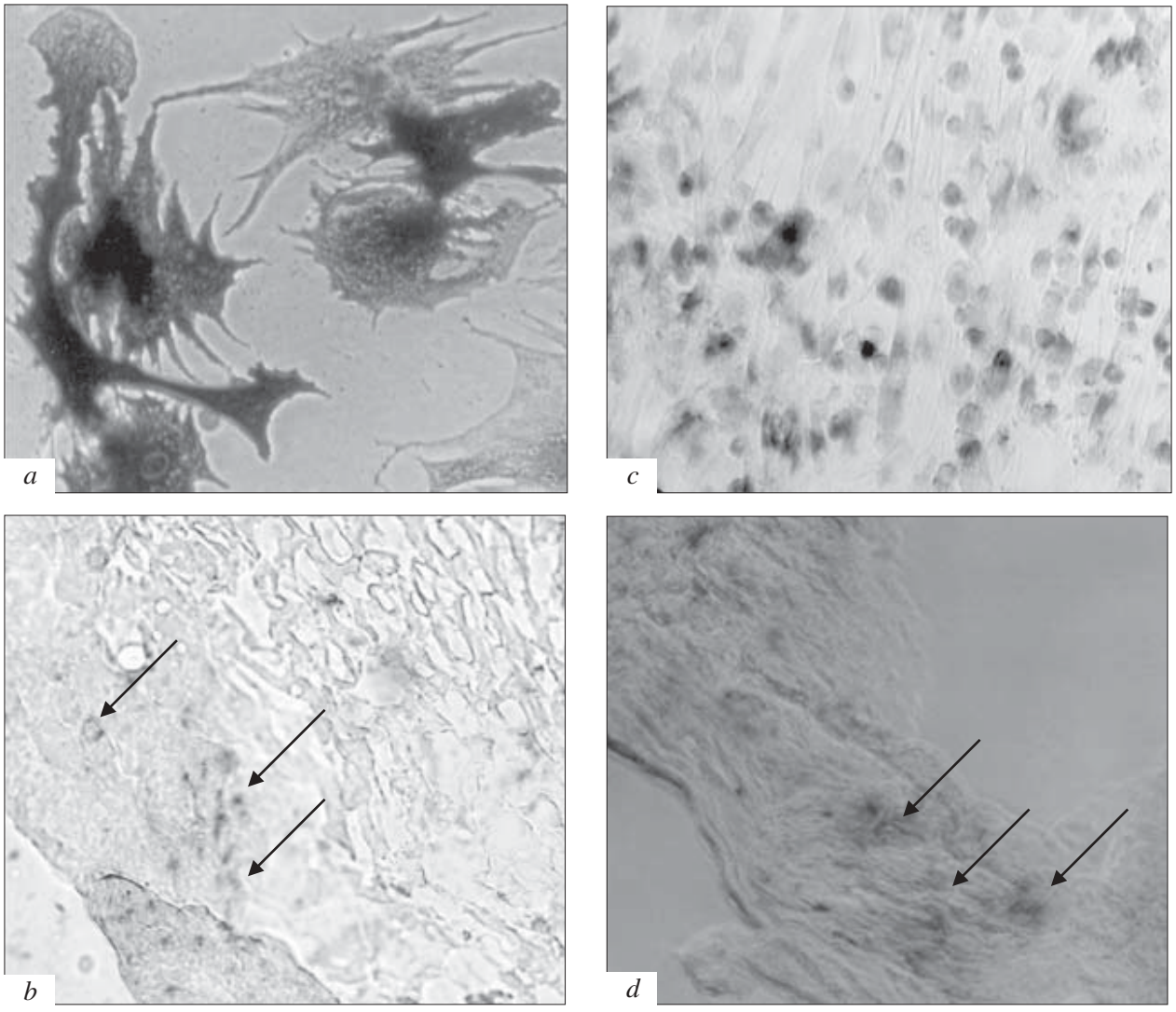

Fig. 1. FCMC and bone marrow MSC labeled with Lac-Z gene in culture and rat myocardium. Histochemical staining for β-galactosidase. *a*) 2-day FCMC culture 24 h after addition of adenovirus with Lac-Z gene into culture medium, ×200; *2*) cryosection of rat myocardium 3 weeks after transplantation of labeled FCMC, ×40; *c*) 3-week MSC culture 24 h after addition of adenovirus with Lac-Z gene into culture medium, ×100; *d*) cryosection of rat myocardium 3 weeks after transplantation of labeled MSC, ×40. Arrows show cells survived after transplantation.

TABLE 2. Functional Parameters of Isolated Hearts in Animals of Different Groups during Exposure to Successive Loading Protocols (*M*±*m*)

Protocol		Volume, ml/min×g		Aortic volume/minute volume, %	Heart rate, min^{-1}	Stroke volume, ml/g	Pressure, mm Hg			dP/dt, mm Hg
		aortic	coronary				systolic	diastolic	developing	
1	group 1	111.0±32.4	51.8±9.1+	67.0±7.6	282.2±23.6+	0.40±0.12	88.3±21.2	47.2±15.4	41.0±13.2	644.8±180.4
	group 2	127.4±20.9	50.9±5.1	71.1±4.5	278.3±28.9	0.89±0.16	82.2±24.5	39.9±13.5	42.3±17.3	545.4±123.9
	group 3	124.2±31.0	71.1±12.7*	63.1±7.3	307.8±22.2*	0.97±0.13	76.3±17.5	44.3±13.2	32.1±11.1	583.4±185.8
	group 4	110.3±19.2	48.6±4.5+	69.1±3.4	275.5±27.5+	0.32±0.09	83.0±13.4	43.3±13.9	39.8±7.3	734.3±174.0*
2	group 1	243.7±76.2*	60.9±20.3+	79.4±4.6	280.9±30.2+	0.46±0.09	106.9±25.0	39.7±17.2	67.2±16.7	987.1±260.7
	group 2	203.7±42.9	58.7±6.4	77.0±5.1	272.3±26.4	1.07±0.28	103.4±22.3	35.8±12.2	67.5±21.7	895.9±209.3
	group 3	289.7±41.7*	81.5±21.5*	78.1±4.0	312.8±17.4*	0.94±0.23*	88.9±31.4	39.2±18.9	49.8±21.9*	911.1±381.4
	group 4	240.3±43.3*	53.8±7.1+	81.5±2.0*	266.3±24.0+	0.34±0.05	96.8±13.4	36.3±13.3	60.6±11.6	1038.2±218.3
3	group 1	283.9±69.4*+	61.34±20.6+	82.1±3.8*	268.5±28.6+	0.40±0.09*	104.5±22.6+	35.3±16.5	69.2±16.2+	1036.7±274.8
	group 2	232.3±43.9	72.4±32.5	76.6±7.4	263.6±28.7	0.83±0.17	98.6±18.5	36.0±13.2	62.5±16.3	870.0±207.4
	group 3	357.7±44.7*	96.0±44.0	79.4±6.7	300.9±12.4*	0.77±0.23*	83.5±18.0	33.9±15.4	49.6±15.1	913.7±330.6
	group 4	271.4±53.0+	52.0±8.4+	83.8±1.4*	258.8±25.5+	0.36±0.10	95.6±17.5	34.8±14.5	60.8±16.0	1016.1±250.9
4	group 1	258.2±64.0	80.4±31.5+	76.4±5.4	273.3±27.7	0.40±0.08	118.7±20.6	53.9±16.4	64.8±17.99	87.9±231.9
	group 2	211.7±63.6	81.1±15.2	70.9±9.1	262.3±30.6	1.18±0.19	113.2±20.9	55.9±16.4	57.3±19.0	818.5±252.2
	group 3	292.2±48.1*	123.3±56.4*	71.2±7.9	299.0±19.0*	0.67±0.21	102.2±19.6	52.8±16.1	49.4±15.5	848.0±294.4
	group 4	252.5±61.0	71.3±9.9+	77.5±3.5	269.0±28.5	0.67±0.28	119.9±15.3	53.0±14.0	66.9±17.1	1037.8±204.1
5	group 1	210.8±59.3	92.2±43.2	70.0±7.4	276.1±25.3	0.86±0.24	131.8±19.3	73.1±17.8	58.7±18.5	802.3±207.0
	group 2	161.0±70.0	94.9±30.0	64.7±6.9	264.3±31.0	1.04±0.21	125.6±22.6	75.1±17.0	50.5±20.6	667.3±280.6
	group 3	208.4±32.6	133.5±57.3	62.2±9.3	292.2±23.5*	0.72±0.09	116.3±20.9	74.0±15.1	42.3±14.1	675.5±232.7
	group 4	203.0±60.8	81.6±11.6+	70.2±6.8	267.4±26.2	0.54±0.23	136.6±15.1	73.3±15.0	63.3±15.4+	920.8±210.7*
6	group 1	101.7±25.2*	105.3±59.4	51.3±11.1	276.6±26.6	0.74±0.15	147.4±20.2	115.8±17.8	31.6±9.3	466.7±121.9
	group 2	76.9±30.4	108.6±48.2	45.1±9.4	265.2±35.1	0.96±0.27	133.5±27.7	108.3±21.9	25.3±11.9	357.7±159.3
	group 3	95.7±13.9	159.0±90.5	41.1±12.2	285.2±17.8	0.76±0.22	126.4±27.0	100.6±21.5	25.8±10.1	411.0±166.3
	group 4	98.4±24.9	97.2±17.2	49.9±7.1	269.6±25.2	0.51±0.17	157.5±17.1*+	120.3±15.0	37.3±9.6*	551.5±110.1*
7	group 1	181.2±76.3	77.6±21.1+	68.5±6.9*+	274.9±28.1	0.93±0.13	124.5±16.5	75.1±14.5	49.4±15.1+	681.4±156.3
	group 2	128.4±65.4	99.5±41.2	58.4±9.3	265.2±38.4	0.80±0.24	124.1±22.7	79.7±19.4	44.4±14.8	627.3±214.7
	group 3	146.2±49.3	130.5±68.1	54.1±14.7	289.2±16.9	0.37±0.11	106.9±25.0	74.5±21.9	32.4±14.0	494.5±194.4
	group 4	208.9±64.7*	86.2±15.1	69.4±9.5*+	271.3±27.6	0.76±0.23	135.3±16.2+	75.3±16.4	60.1±17.6*+	897.1±221.6*+
8	group 1	—	—	—	—	—	181.3±30.2+	156.8±29.6+	24.6±7.7*	—
	group 2	—	—	—	—	—	151.8±32.2	136.2±28.3	15.5±7.9	—
	group 3	—	—	—	—	—	137.9±28.8	121.5±25.7	16.5±8.8	—
	group 4	—	—	—	—	—	175.6±13.2+	146.7±11.3	28.9±8.2*+	—

Note. $p<0.05$ compared to *group 2, +group 3.

aortic tube. Protocol 7 repeated protocol 5: the capacity of the left ventricle to restore the pumping function parameters after protocol 6 testing was evaluated. During protocol 8 testing the aortic mouth was clamped for 10 sec.

The results were processed using Student's *t* test with consideration for Bonferroni's correction for multiple comparisons at $p=0.05$. The data are presented as $M \pm m$.

RESULTS

Three weeks after transplantation of FCMC and predifferentiated MSC they were detected in the recipient myocardial perinecrotic zone; the cells were functionally active, because they produced β-galactosidase (Fig 1).

The pumping function of the myocardium during protocol 1 testing was virtually the same in all groups; differences in the functional parameters of the myocardium manifested only during load tests (Table 2).

All hearts adequately reacted to increase of preload and hence, their functioning conformed to Frank—Starling law. Group 3 hearts functioned better during testing by protocols 2 and 3, than groups 2 and 4 hearts. The study of the contractile characteristics of the hearts under conditions of increased pre- and afterload (protocols 4-7) showed that group 4 hearts better tolerated volume and pressure overload than group 3 hearts. Testing of groups 1 and 4 hearts showed no difference between them, that is, hearts of these animals similarly reacted to changes in pre- and afterload; moreover, some parameters were better than in group 2. These data indicate that transplantation of predifferentiated MSC virtually normalized the contractile function of the hearts after CD, which was not observed after transplantation of FCMC (group 3 values differed from those in groups 2 and 1). Presumably, higher coronary volume and heart rate, lower systolic, diastolic, and developed pressures, velocity of pressure increment in the aortia (dP/dt) under conditions of high afterload in group 3 animals can be due to the cytotoxic effect of cyclosporine A [5] on the myocardium; this drug used after transplantation is known to reduce the compensatory reserve and adaptation potential of the cardiac muscle.

Hence, the study of the pumping function of intact and damaged hearts on a testing setup under conditions of dosed increase in left-ventricular pre- and afterload revealed signs of latent left-ventricular insufficiency in cryodamaged hearts and its compensation after transplantation of FCMC and predifferentiated bone marrow MSC into the myocardium. FCMC and predifferentiated bone marrow MSC increased the systolic function of the left ventricle during step-by-step increase in preload. Transplantation of autologous bone marrow MSC also improved myocardial resistance to simultaneous increase in afterload. Improvement of cardiac function after CD was due to the presence of viable FCMC and autologous bone marrow predifferentiated MSC (identified by β-galactosidase staining) in the myocardium.

REFERENCES

1. B. Bittira, J. Q. Kuang, A. Al-Khaldi, *et al.*, *Ann. Thorac. Surg.*, **74**, No. 4, 1154-1160 (2002).
2. F. R. Eberli, F. Sam, S. Ngoy, *et al.*, *J. Mol. Cell. Cardiol.*, **30**, 1443-1447 (1998).
3. K. Fukuda, *Artificial Organs*, **25**, 187-193 (2001).
4. H. Kamihata, H. Matsubara, T. Nishiue, *et al.*, *Circulation*, **104**, 1046-1052 (2001).
5. I. Kingma, E. Harmesen, and H. E. ter Keurs, *Int. J. Cardiol.*, **31**, No. 1, 15-22 (1991).
6. T. Kobayashi, K. Hamano, T. S. Li, *et al.*, *J. Surg. Res.*, **89**, 189-195 (2000).
7. R. K. Li, L. C. Tumiaki, R. G. Weifel, and J. A. G. Mickli, *J. Tissue Cult. Methods*, **15**, 147-154 (1993).
8. J. R. Neely, H. Liebermeister, and H. E. Morgan, *Am. J. Physiol.*, **212**, No. 4, 804-814 (1967).
9. T. Sakai, R. K. Li, and R. D. Weisel, *Ann. Thorac. Surg.*, **68**, No. 6, 2074-2081 (1999).
10. M. Scorsin, A. A. Hagege, I. Dolizy, *et al.*, *Circulation*, **98**, II151-II156 (1998).
11. N. Tran, N. Bertrand, Y. Li, *et al.*, *Transplant. Proc.*, **34**, 3262-3264 (2002).