Effect of Transplantation of Bone Marrow Monomuclears on Angiogenesis in Rats

T. Kh. Fatkhudinov[1,3,4], J. P. Baikova[1], G. B. Bolshakova[1], T. B. Buharova[2,3], T. K. Dubovaya[4], L. V. Kaktursky[1], and D. V. Goldshtein[1,2,3]

Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 3, pp. 132-135, September, 2011
Original article submitted February 22, 2011

We studied the effect of non-selective intracoronary transplantation of bone marrow mononuclears on day 30 after acute coronary infarction on angiogenesis in rats. On days 14 and 30 after transplantation of mononuclear cells, stable formation of new vessels was observed. The number of venules considerably increased after transplantation of mononuclear cells, which was seen from increased volume density of blood vessels and their caliber. Stable vascularization after transplantation of mononuclear cells improves blood supply, which is essential for reparation of the myocardium.

Key Words: *bone marrow mononuclear cells; transplantation; angiogenesis*

Drug therapy and surgical interventions in case of cardiovascular diseases, *e.g.* acute myocardial infarction, are not always possible and effective; therefore the therapy with SC is a promising trend in cardiology. Therapeutic angiogenesis based on the use of progenitor cells attracted much attention during the last decades [11,15]. Numerous experimental studies and clinical trials [4,5,10] confirmed the angiogenic effect of transplanted bone marrow (BM) mononuclear cells (MNC), but morphological aspects of this process are poorly studied.

Here we studied the effect of transplanted MNC on angiogenesis.

MATERIALS AND METHODS

Experiments were carried out on 36 male Sprague–Dawley rats. In all animals, transmural acute myocardial infarction followed by reperfusion was modeled. On day 30 after acute myocardial infarction, MNC were transplanted to experimental rats (n=20). The rats were sacrificed 14 and 30 days after cell transplantation as described elsewhere [2].

BM MNC were isolated routinely in a density gradient [5]. The isolated nucleated cells were resuspended in 1 ml physiological saline to a concentration of 5×10^6 cells/ml.

For evaluation of angiogenesis after cell transplantation, the myocardial sections were stained after Mallory (Fig. 1), the total number of blood vessels per 1 mm^2 and volume density of blood vessels in the cicatrix on days 14 and 30 after transplantation were determined. Arterioles, venules, and capillaries were counted separately and their areas were calculated by their outer diameters. The relative content of capillaries, arterioles, and venules and their number per 1 mm^2 were determined, which allowed evaluation of the dynamics of angiogenesis.

The means and standard errors of the means were calculated. Since in our experiments data distribution differed from normal, we used χ^2 test for comparison of different types of vessels and z test for compari-

[1]Laboratory of Growth and Development, Research Institute of Human Morphology, Russian Academy of Medical Sciences; [2]Laboratory of Stem Cell Genetics, Medical Genetic Research Center, Russian Academy of Medical Sciences; [3]ReMeTeks Company; [4]Department of Histology and Embryology, Russian State Medical University, Moscow, Russia. ***Address for correspondence:*** fatkhudinov@gmail.com. T. Kh. Fatkhudinov

0007-4888/11/1521-0120

son of independent groups of experimental data. The differences were significant at 5% significance level. Statistical processing of the results was performed using SigmaStat3.5 software.

RESULTS

We previously studied homing and differentiation of transplanted MNC and the processes of myocardial reparation after MNC transplantation [1,2]. MNC did not differentiate into vascular blood cells, but stimulation of angiogenesis did occur [1].

Two weeks after transplantation, the number of blood vessels per 1 mm^2 in the experimental group was lower than in the control, but the volume density of blood vessels per 1 mm^2 in experimental animals was significantly higher ($p \leq 0.001$), which attested to greater caliber of newly formed vessels after MNC transplantation. On day 30, both the number ($p \leq 0.001$) and volume density ($p=0.004$) of vessels in the experimental group was higher than in the control (Fig. 2). The number and density of vessels in the experimental group remained stable, whereas in the control these parameters decreased.

Similar dynamics was revealed for the number of capillaries per 1 mm^2. On day 14, the number of capillaries in experimental animals was higher than in controls ($p \leq 0.001$), while on day 30 the number of capillaries per 1 mm^2 in the control and experimental groups was similar (Fig. 3). On days 14 and 30 after transplantation, the number of capillaries in the experimental group remained unchanged, while in the control group it considerably decreased. The number of arterioles per 1 mm^2 in both groups was stable (Fig. 3), but in the experimental group the number of arterioles in the cicatrix was lower than in the control ($p=0.901$).

The number of venules per 1 mm^2 also significantly differed between the groups on days 14 and 30 after transplantation. This parameter was stable in both groups and was higher in experimental animals (Fig. 4).

In the cicatrix of control animals, capillaries predominated and their relative content decreased by day 30 after transplantation ($p \leq 0.001$); the relative content of venules increased by day 30 ($p \leq 0.001$), while the relative content of arterioles remained unchanged ($p=0.501$). In the cicatrix of experimental animals, capillaries predominated and their relative content did not change on days 14 and 30 ($p=0.267$; Table 1).

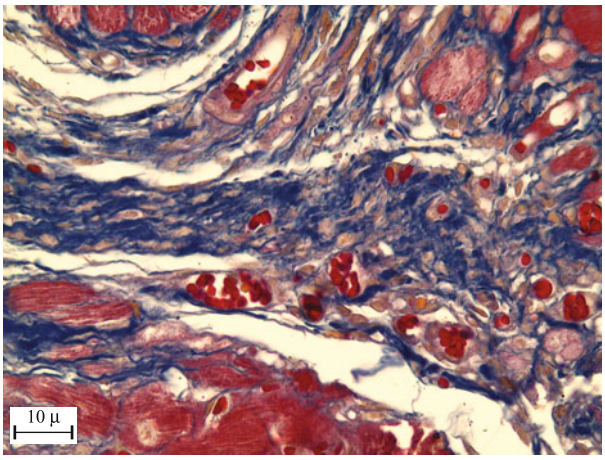

Fig. 1. Histological preparation, Mallory staining.

These findings agree with the data on angiogenesis processes in the damaged myocardium, because the rate of angiogenesis sharply increases by day 7 and then decreases by day 30 after myocardial infarction. The enhanced formation of capillaries is related to the development of granulation tissue enriched with sinusoid capillaries at the site of damage [6]. At early terms, the process of vascularization is characterized by proliferation of newly formed small blood vessels derived from preexisting vessels and fibroblasts [4]. By day 30, deceleration of angiogenesis processes was observed; no new vessels were formed and rearrangement of capillaries was started. Our results disagree with the data obtained in previous studies where the number of capillaries, venules, and arterioles increased after MNC transplantation [10,14]. In our study, the number of arterioles remained unchanged and the number of venules increased. This can be explained by the fact that transplanted cells did not differentiate into vascular smooth muscle cells, while division of smooth muscle cells in arterioles and migration of smooth muscle cells are insufficient for the formation of new arterioles. In our study, angiogenesis is pre-

TABLE 1. Relative Content of Capillaries, Arterioles, and Venules on Days 14 and 30 after MNC Transplantation to Rats

Group, time of observation		Capillaries	Arterioles	Venules	Total number of vessels
Control	day 14	0,853077197	0.00889	0.13803	2811
	day 30	0.558017632	0.00715	0.434834	4197
Experimental	day 14	0.733074601	0.00561	0.26132	2319
	day 30	0.718609865	0.00561	0.27578	2676

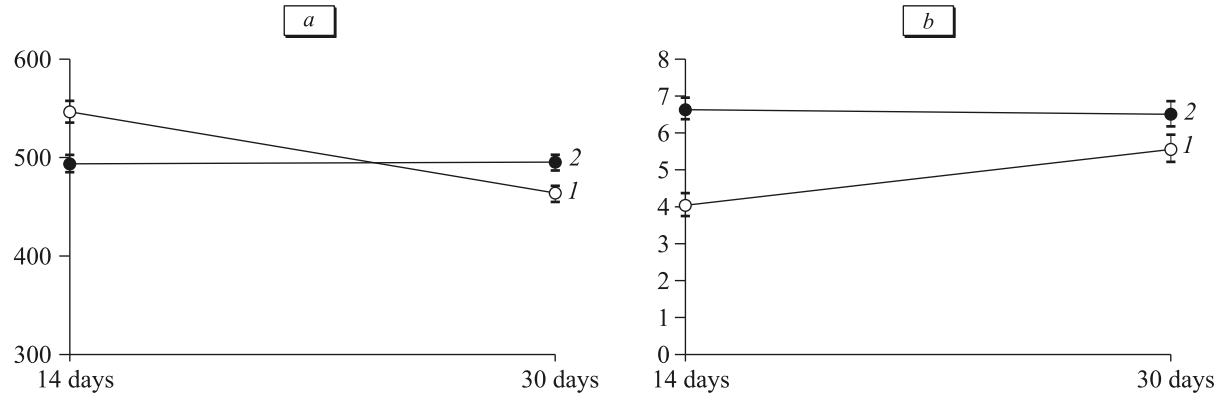

Fig. 2. Number of vessels (*a*) and their volume density (*b*) per 1 mm² on days 14 and 30 after transplantation. Here and in Fig. 3-4: *1*) control group; *2*) experimental group.

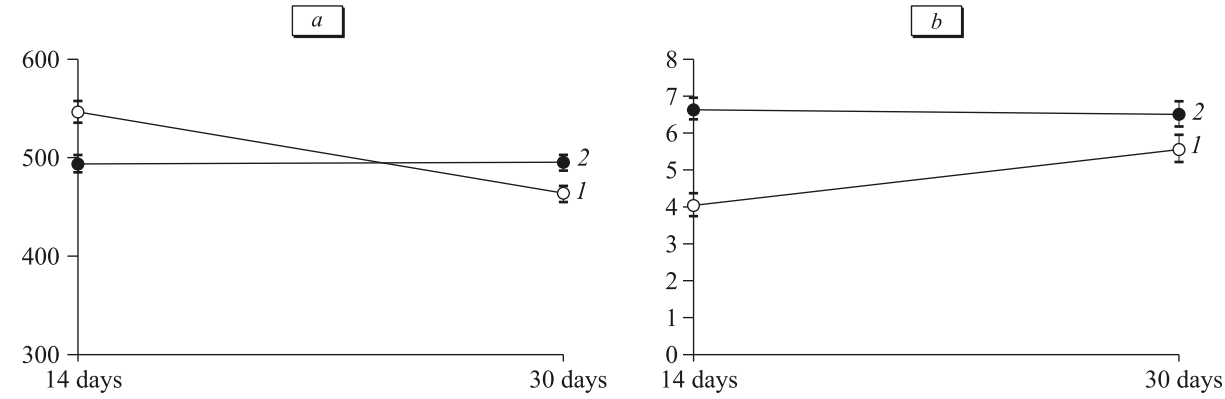

Fig. 3. Number of capillaries (*a*) and arterioles (*b*) per 1 mm² on days 14 and 30 after transplantation.

sumably confined to transformation of preexisting arterioles into small muscular-type arteries, which does not lead to the increase in the number of these vessels.

According to published data, some factors, e.g. vascular endothelium growth factor (VEGF), stimulate angiogenesis in the zone of damage [12]. MNC can produce growth factors, including SCF and VEGF [3,7], which determine their therapeutic effects on angiogenesis. However, the number of vessels (except venules) in the experimental group in our study did not exceed the corresponding parameter in the control. Transplantation of MNC stabilizes vascularization processes by maintaining angiogenesis at a constant level in contrast to negative dynamics of angiogenesis in the control group. In many studies, replacement mechanism of the action of progenitor cells is discussed, due to which transplanted MNC differentiate into intima and media cells [8,9,13]. According to our findings, the replacement mechanism is not realized, because no new arterioles are formed. We believe that the effect of MNC is mediated by the induction mechanism.

In our study we observed stabilization of the vascularization process manifesting in unchanged number of capillaries, venules, and arterioles on days 14 and

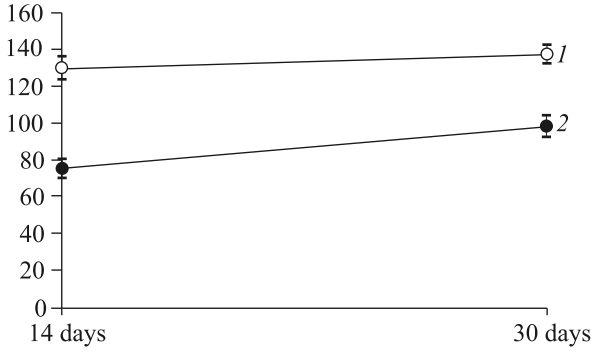

Fig. 4. Number of venules per 1 mm² on days 14 and 30 after transplantation.

30 after BM MNC transplantation. This stability of angiogenesis maintains blood supply, which is essential to reparation of damaged myocardium. We believe that angiogenesis was stimulated by paracrine factors released by transplanted cells [3,7,11]. We did not observe the increase in the number of capillaries and arterioles after MNC transplantation reported by other investigators [8,9,12]. This agrees with our previous data that transplanted MNC do not differentiate into vascular wall cells. We did not observe the decrease

in angiogenic activity on days 14 and 30 after transplantation in the experimental group, whereas in the control the number and volume density of vessels decreased by day 30 after transplantation. Our findings suggest that despite the absence of MNC differentiation into vascular cells, transplantation of MNC produces a positive therapeutic effect on angiogenesis by maintaining and stabilizing vascularization.

REFERENCES

1. J. P. Baikova, T. Kh. Fatkhudinov, G. B. Bolshakova, *et al., Kletochn. Tekhnol. Biol. Med*., No. 4, 203-211 (2010).
2. T. Kh. Fatkhudinov, G. A. Slashcheva, G. B. Bolshakova, *et al., Klet. Tekhnol. Biol. Med*., No. 4, 222-228 (2009).
3. J. Chen, Z. Zhang, Y. Li, *et al., Circ. Res*., **92**, No. 6, 692-699 (2003).
4. K. L. Dishart, L. M. Work, L. Denby, and A. H. Baker, *J. Biomed. Biotechnol.,* No. 2, 138-148 (2003).
5. T. Egeland and J. E. Brinchmann, *Eur. Heart. J.,* **28**, No. 17, 2174-2175 (2007).
6. *S. Fukuda, J. Mol. Cell. Cardiology*., **36**, No. 4, 547-559 (2004).
7. M. K. Majumdar, M. A. Thiede, J. D. Mosca, *et al., J. Cell Physiol*., **176**, No. 1, 57-66 (1998).
8. M. Pesce, A. Orlandi, M. G. Iachininoto, *et al., Circ. Res*., **93**, No. 5, e51-e62 (1983).
9. V. Schächinger, B. Assmus, M. B. Britten, *et al., J. Am. Coll Cardiol*., **44**, No. 8, 1690-1699 (2004).
10. S. Shintani, T. Murohara, H. Ikeda, *et al., Circulation*, **103**, No. 6, 897-903 (2001).
11. D. M. Smadja, A. Cornet, J. Emmerich, *et al., Cell. Biol. Toxicol*., **23**, No. 4, 223-239 (2007).
12. M. L. Springer, G. Hortelano, D. M. Bouley, *et al., J. Gene Med.,* **2**, 279-288 (2000).
13. B. E. Strauer, M. Brehm, T. Zeus, *et al., Circulation*, **106**, No. 15, 1913-1918 (2002).
14. A. Taguchi, T. Soma, H. Tanaka, *et al., J. Clin. Invest*., **114**, No. 3, 330-338 (2004).
15. S. Tomita, D. A. Mickle, R. D. Weisel, *et al., J. Thorac. Cardiovasc. Surg*., **123**, No. 6, 1132-1140 (2002).