Distribution of Mesenchymal Stem Cells in the Area of Tissue Inflammation after Transplantation of the Cell Material via Different Routes

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Translated from *Kletochnye Tehnologii v Biologii i Medicine*, No. 1, pp. 34-37, January, 2007
Original article submitted December 4, 2006

In experiments on male Wistar—Kyoto rats we studied the distribution of mesenchymal stem cells in intact body and in the presence of a focus of acute tissue inflammation. In healthy animals mesenchymal stem cells were transplanted intravenously. In the second case we used various routes of transplantation of mesenchymal stem cells: intravenous, into tissue adjacent to the inflammation focus, and into intact lobe of the damaged organ (prostate gland). Three weeks after transplantation, mesenchymal stem cells labeled with a fluorescent dye were detected in the bone marrow and intestine of intact animals. In case of inflammation focus, mesenchymal stem cells after transplantation migrated into the bone marrow, intestine, and prostate gland. After injection into the adjacent zone, these cells formed a compact agglomerate at the site of injection. After transplantation into the intact lobe of the prostate gland the cells migrated towards the inflammation focus. Thus, transplantation of mesenchymal stem cells into the venous blood is less traumatic and led to more uniform distribution of cells in the damaged tissue.

Key Words: *mesenchymal stem cells; fluorescent label; distribution; inflammation*

Approbation of cell therapy with mesenchymal stem cells (MSC) as the method of treatment of various pathologies is now in progress. One of practical questions is the choice of the site for cell transplantation. Various routes of cell transplantation were proposed: into venous blood [7], immediately into the damaged zone [8], or into the artery supplying the organ [3]. Transplantations into the damaged organ usually require complex surgical intervention. Injection of the cell material into the venous blood is a routine medical procedure. It was proved that MSC injected into the venous or arterial blood migrate into the target organ [5,6]. Migration of MSC into the zone of tissue necrosis can be explained by increased content of SDF-1 (stromal cell derived factor) associated with inflammatory and necrotic processes in tissues. At the same time, MSC are characterized by high level of expression of CXCR4 (SDF-1 receptor), which probably stimulates their migration from the bone marrow into the inflammatory focus [4,5,9].

In the present study we studied the distribution of MSC in intact rat organism after intravenous transplantation, evaluated the influence of the acute inflammatory focus on MSC distribution in the body after their intravenous transplantation, and analyzed the effect of transplantation mode on the distribution of these cells in the organ with pronounced inflammation focus.

MATERIALS AND METHODS

Experiments were carried out on 3-4-month-old male Wistar—Kyoto rats weighing 150-170 g (n=60).

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0007-4888/07/1431-0143

Bone marrow suspension was isolated from the femur immediately after decapitation. To this end, epiphyses were removed and diaphyses were washed with αMEM (HyClone) containing 20% FCS (Gibco) and 100 μg/ml penicillin and streptomycin (Gibco). The obtained suspension was plated onto plastic Petri dishes (Sarstedt). Forty-eight hours after bone marrow explantation, MSC were twice washed from blood cells with PBS (20 mM phosphate buffered saline, pH 7.4, 0.1 M NaCl). The cells were cultured in a monolayer in this medium at 37°C and 5% CO_2 for 6-7 days after explantation. Then, the culture was subcultured every 7 days at an initial density of 1.27×10^3 cells/cm^2 using trypsin EDTA (HyClone). The medium was replaced every 3 days.

Rat MSC were phenotyped by the method of flow cytoflyorometry on a FACSscan flow cytofuorometer (Becton Dickinson). MSC were stained with antibodies to negative marker CD45 and positive marker CD90 (Becton Dickinson). To this end, the cells were harvested from dishes with trypsin and EDTA (HyClone), washed twice with PBS, and incubated with fluorochrome-conjugated monoclonal antibodies diluted 1:20 for 1 h. Then, the cells were washed twice with PBS and fluorescence was measured. Phenotyping was performed after the first, second, and third subculturing.

After the second subculturing, rat MSC were stained with PKH26 fluorochrome. The cells were grown until the formation of a dense monolayer. PKH26 (1 μg/ml, Sigma) was added to the medium, after 48 h the cells were washed with PBS and cultured in a medium without PKH26 for at least 4 h. Stained MSC were harvested with trypsin and EDTA, centrifuged at 450*g* for 10 min, washed twice with PBS, and suspended in a serum-free medium (αMEM with 100 μg/ml penicillin/streptomycin) to a final concentration of 5×10^6 cells per 100 μl. The efficiency of staining was evaluated under a Leica fluorescent microscope (Leica).

For modeling acute inflammation in the parenchymatous tissue, 200 μl turpentine in olive oil (1:1) was injected into the left lobe of the prostate gland.

The animals were divided into 4 groups. Group 1 rats (intact) received 5 mln MSC in 100 μl medium. In group 2 animals intravenous transplantation of MSC was performed on day 3 after acute inflammation modeling; group 3 rats received 0.2 mln MSC in 100 μl medium into the zone adjacent to the inflammation focus, and group 4 rats received 0.2 mln MSC in 100 μl medium into the intact lobe of the prostate gland.

All animals were decapitated 3 weeks after MSC transplantation.

Immediately after decapitation, samples of the bone marrow, intestine, liver, and lungs and the whole prostate gland were taken from animals receiving intravenous MSC transplantation (groups 1 and 2). In animals receiving MSC transplantation into the organ (groups 3 and 4), only the prostate gland was fixed. The samples were cooled in nitrogen vapors for 10 sec, then 1 h in liquid nitrogen, and then stored at -70°C.

Fluorescence of labeled MSC in tissues was detected under a fluorescent microscope (Leica) on histological sections (7 μ) prepared on a cryostat microtome (Leica).

RESULTS

In group 1 rats 3 weeks after transplantation, fluorescing cells were present in the bone marrow and intestine, we also saw minor number of these cell in the lung and liver of these animals (Fig. 1). In group 2 animals, labeled MSC were detected in the bone marrow, intestine, and in the damaged (left) lobe of the prostate gland. They surrounded the turpentine drop and lay diffusely in the inflammation zone (Fig. 2, *a*). In group 3 animals, the labeled cells were seen as compact agglomerate at the site of injection (Fig. 2, *b*). In group 4 rats, a tendency to cell migration towards the damaged zone was observed: the fluorescent zone was seen as a bar between the site of injection and the damaged zone of the prostate gland (Fig. 2, *c*). In the focus of inflammation no labeled MSC were detected.

Our findings suggest that in the intact organism MSC 3 weeks after intravenous transplantation were detected only in the bone marrow and intestine. The existence of an acute inflammatory focus had no effect on MSC distribution in the body, but under these conditions labeled cells were always detected in the damaged organ. These findings were confirmed by the results of our experiments on rats with modeled myocardium infarction. MSC were injected intravenously 1-14 days before and 1-14 days after infarction modeling. After 6 weeks, MSC were detected in the zone of ischemic damage and in the bone marrow and intestine [1,2].

After injection into the adjacent zone, these cells did not migrate from the site of injection into the zone of tissue damage. At the same time, MSC transplanted into the intact lobe of the prostate gland tended to migrate towards the inflammation area, but migration in the parenchymatous tissue was practically impossible. These findings suggest that injection into the organ can be little effective, at least in case of prostate gland inflammation.

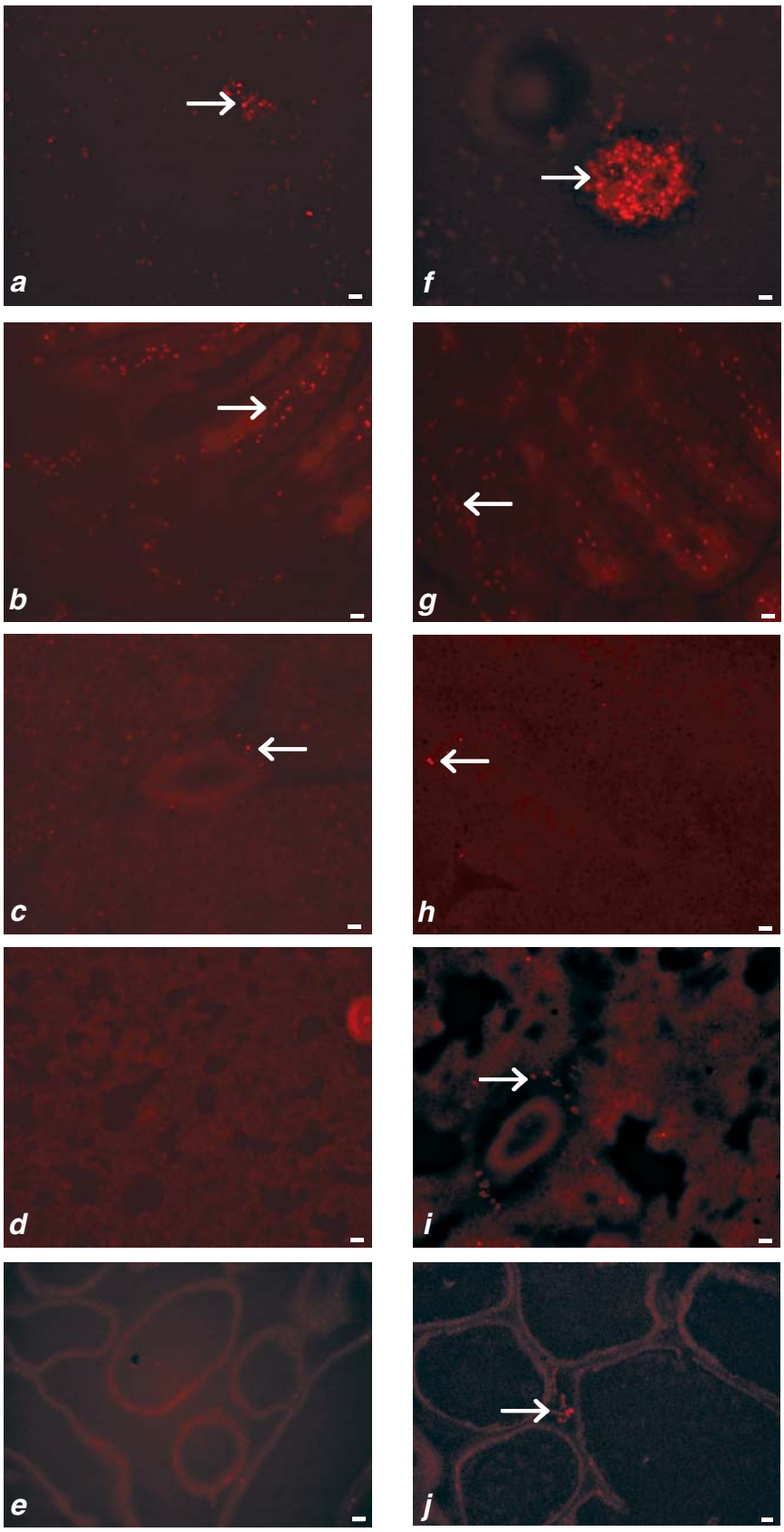

Fig. 1. Distribution of MSC (intravenous injection) in an intact animal (*a-e*) and in an animal with modeled acute local inflammation in the prostate gland (*f-j*). *a*, *f*) bone marrow, *b*, *g*) intestine, *c*, *h*) liver, *d*, *i*) lungs, *e*, *j)* prostate gland.

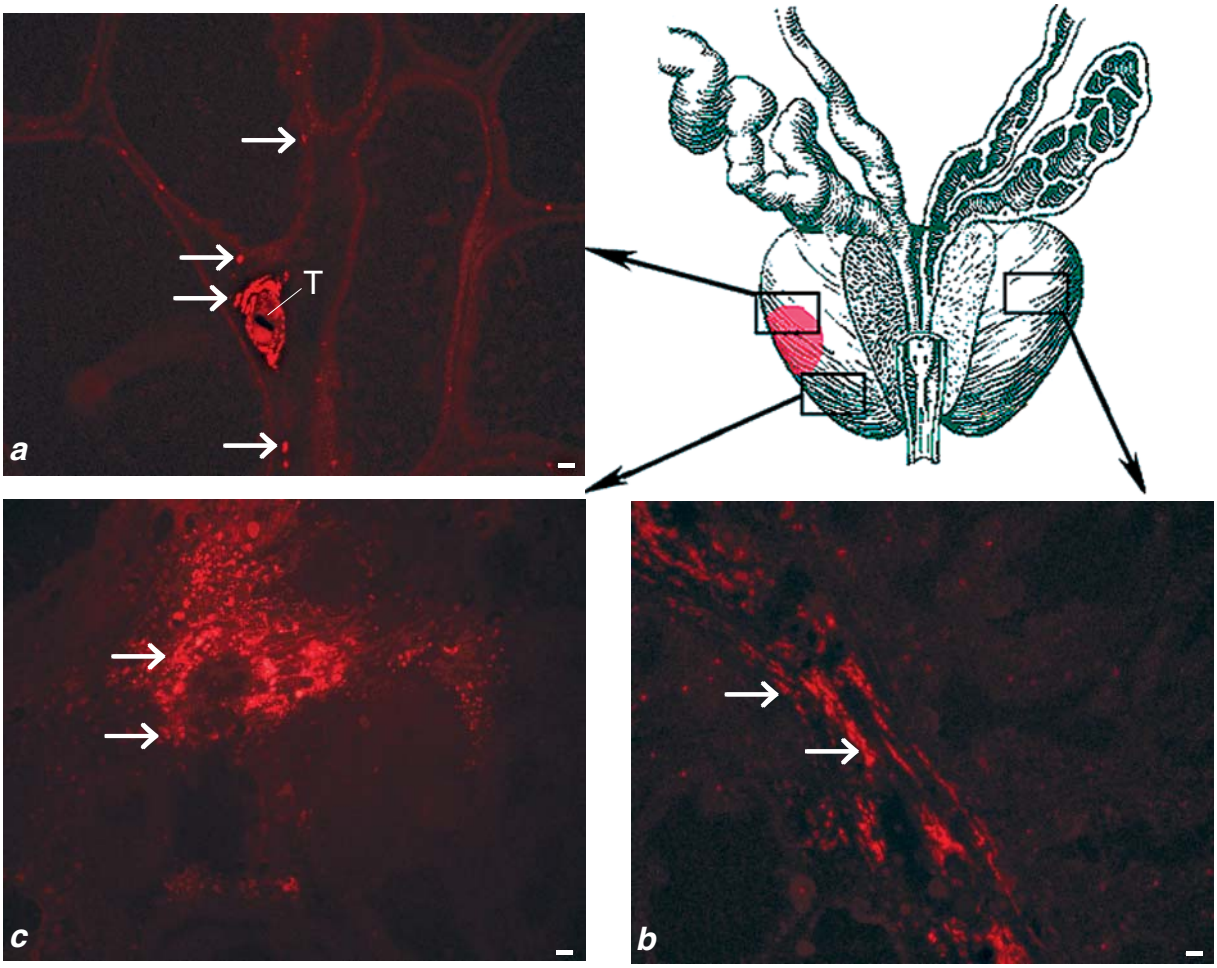

Fig. 2. MSC distribution in the prostate gland. *a*) intravenous injection; *b*) injection into the intact lobe of the prostate gland; *c*) injection into the prostate gland zone adjacent to the inflammation focus. Arrows show labeled MSC, T: turpentine drop.

Thus, intravenous injection is the most safe and easy way of MSC transplantation for all pathologies, because it leads to uniform and diffuse distribution of transplanted cells in the damaged area.

REFERENCES

1. P. V. Kruglyakov, I. B. Sokolova, Kh. K. Amineva, *et al., Thitologiya*, **12**, 1043-1054 (2005).
2. *P. V. Kruglyakov, I. B. Sokolova, Kh. K. Amineva, et al., Ibid*., **5**, 404-416 (2005).
3. B. Assmus, V. Schachinger, C. Teupe, *et al. Circulation*, **106**, No. 24, 3009-3017 (2002).
4. S. Bhakta, P. Hong, and O. Koc, *Cardiovasc. Revasc. Med*., **7**, 19-24 (2006).
5. J. F. Ji, B. P. He, S. T. Dheen, and S. S. Tay, *Stem Cells*, **22**, No. 3, 415-427 (2004).
6. Y. Li, J. Chen, L. Wang, *et al., Neurology*, **56**, No. 12, 1666-1672 (2001).
7. D. Orlic, *Ann. N. Y. Acad. Sci*., **996**, 152-157 (2003).
8. S. Tomita, D. A. Mickle, R. D.Weisel, *et al., J. Thorac. Cardiovasc. Surg*., **123**, 1132-1140 (2002).
9. R. F. Wynn, C. A. Hart, C. Corradi-Perini, *et al., Blood*, **104**, No. 9, 2643-2645 (2004).