Homing of bone marrow derived mononuclears after intracoronary transventricular transplantation

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INTRODUCTION

The safety and therapeutic benefits of intracoronary infusion of bone marrow derived mononuclear cells (BMMCs) have now been demonstrated in a number of clinical trials. Although previous animal studies have shown that BMMCs can positively affect the postinfarction remodelling process, the underlying mechanisms remain unclear. It is thought that stem cells migrate into the infarct zone and differentiate into cardiac myocytes or blood vessel cells, however this idea has been challenged. Our aim was to study engraftment and differentiation of BMMCs after transventricular intracoronary transplantation.

MATERIALS AND METHODS

BMMCs isolation and labeling

Bone marrow sampling was performed from femur and tibia under general anesthesia. BMMCs were isolated by density gradient centrifugation. The cell suspension consists of heterogeneous cell populations including hematopoietic progenitor cells, multipotent stromal cells and other. Finally, BMMCs were labeled with PKH26 according to its manufacturer's instructions (Sigma).

Rat acute myocardial infarct model

A left thoracotomy was performed with the anesthesia induced with Ketamine and Xsilasin (80-100 mg/kg, 10 mg/kg, IM). Acute myocardial ischemia was produced by transient occlusion (total of 20 min) of the proximal LAD coronary artery and followed by reperfusion.

Transventricular intracoronary cells administration

The animals were randomly assigned to 2 groups: (1) control group (n=16), (2) BMMCs delivery (n=20). Thirty days after AIM the left external carotid artery was catheterized. After the catheter with a pressure sensor was positioned in the cavity of LV, a total of 5×10^6 BMMCs in 1 ml saline solution or only the saline solution were infused. BMMCs were infused during 1-min periods with 10-sec intervals between infusions to allow for normal LV and coronary flow. At the same time and intervals simultaneous cross-clamping of aorta was performed. So the larger portion of the cells reached right and left coronary arteries.

Fig. 1. Transventricular intracororonary cells delivery

Histological and morphometric studies

Rats were humanely killed 14 and 30 days after BMMCs therapy. Their hearts were exposed by median sternotomy and quickly removed. The hearts were sliced into 2 transverse sections from apex to base. The hearts were immersion fixed in 4% paraformaldehyde and embedded in paraffin. Serial transverse sections of 5 μm were cut on 10 levels from apex to base of LV and subsequently mounted on slides. The sections were then stained with Sirius red. Scar size was determined as the percentage of Sirius red-stained collagen area and total LV area. The infarcted wall thickness was determined as the ratio of the thickness of the infarcted wall to that of the noninfarcted the most thick wall. The left ventricular cavity area and total left ventricular area were measured and the dilatation index was calculated as follows: Dilatation index=LV cavity area/Total LV area.

Detection of PKH26-labeled cells

Samples of hearts, spleens, lungs and livers of animals that received labeled BMMCs were cryofixed. Frozen specimens were cut on positively charged slides at 4 μm. PKH26-labeled cells were detected and counted using fluorescent microscopy.

Statistical Analysis

Nonparametric values were expressed as means±SE for percentage or medians and percentiles. Comparisons between the groups were made using Kruskal-Wallis one-way ANOVA on Ranks. P value <0.05 was considered significant.

RESULTS

BMMCs Homing

A large portion of the cells delivered into LV cavity reached right and left coronary arteries. In 15 min after transplantation BMMCs were found both in myocardial tissue and in the lumen of coronary vessels. At this stage, the transplanted cells were evenly distributed in the heart tissue and were not present in other organs.

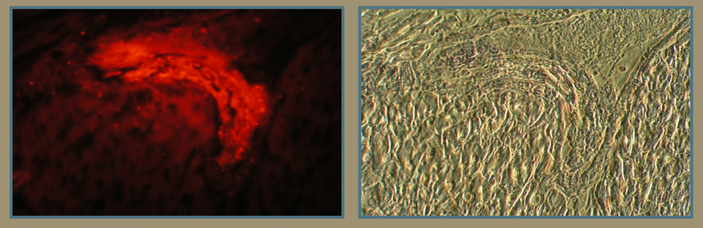


Fig. 2. BMMCs homing at 15 min after transplantation

At day 14, labeled cells were found in the scar tissue – 55.5±17.7 cells in 37070 μm sq the field of view, or 49.5% of all cells. There was a considerable number of the cells in spleen – 52.3±9.5 (46.7% of all cells), whereas only single cells were detected in the liver 2.9±1.9 (2.6%) and lungs 1.4±1.7 (1.2%).

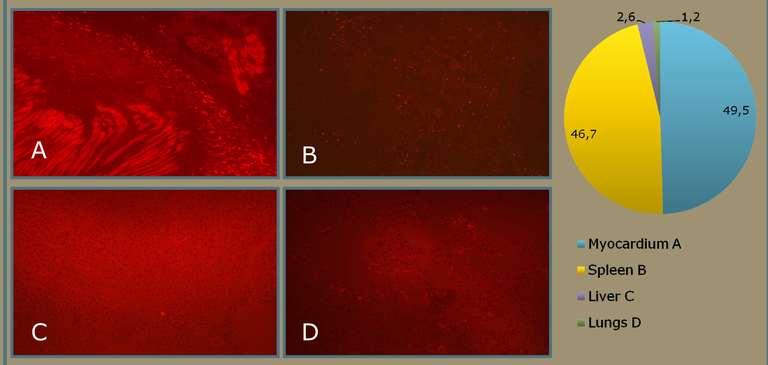


Fig. 3. BMMCs distribution at day 14 after transplantation

At day 30, the proportion of engrafted labeled cells in the myocardium averaged at 34.5±13.4 cells in 37070 μm sq (38.7%), while the highest concentration of labeled cells was in spleen: 43.8±13.1 cells (49.2%). Cells manly located in the red pulp of spleen. As before, single cells were present in liver and lungs: 7.6±3.1 (8.6%) and 3.1±4.0 (3.5%) cells in the field of view, respectively.

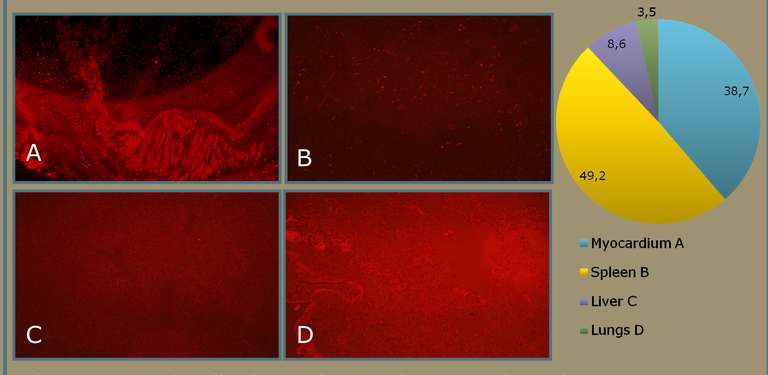


Fig. 4. BMMCs distribution at day 14 after transplantation

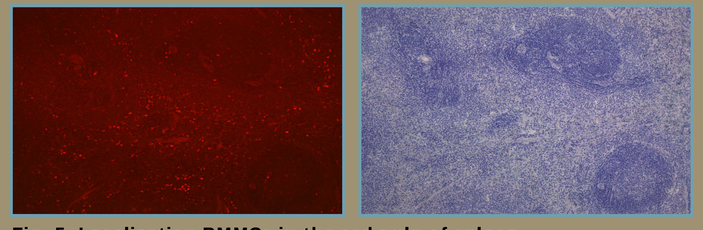


Fig. 5. Localization BMMCs in the red pulp of spleen

At all follow-up points, the transplanted cells were detected only in the scar tissue and had fibroblast-like appearance and were embedded in collagen fibers. The labeled cells were not seen within intima or media of blood vessels. Nor were they ever observed in vicinity of intact cardiac myocytes adjacent to the zone of infarct.

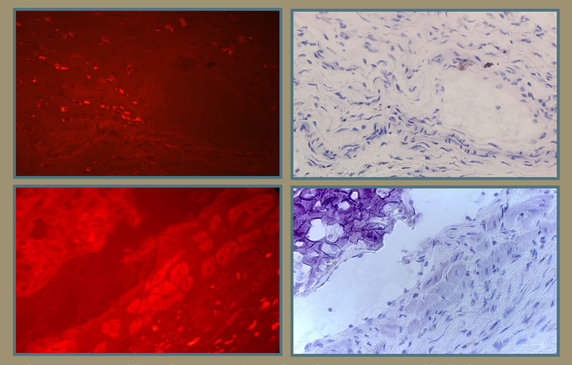
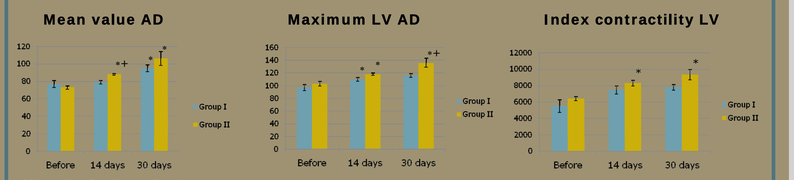


Fig. 5. Localization BMMCs in the myocardial tissue

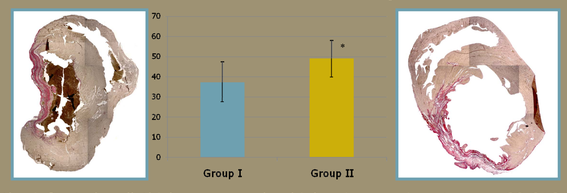
Functional assessment

Exercise stress tests were performed prior to cell transplantation and before animals were killed. There were no significant differences between the control and experiment groups. Other indicators of LV function, such as systolic blood pressure, LV maximum pressure and LV global contractility index (+dP/dt) improved after BMMCs transplantation.

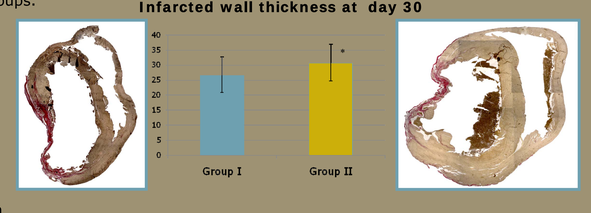


Histological analysis

In both groups at day 14 after myocardial infarction there were features of remodeling process. The BMMCs transplantation had no effect on scar size and dilatation index. The infracted wall thickness was significantly higher in the group with cell transplantation 48.9±4.7% compared with the control group 37.2±5.1% (p<0.001).



At day 30, the infarcted wall thickness was also higher in experiment group 30.6±3.2% than in control group 26.5±3.1% (p=0.002). There were no significant differences among others indexes between groups.



Conclusion

In this study we show that after migration into the area of infarct, BMMCs differentiate into fibroblast-like cells and localize exclusively within the thickened scar tissue. Improvement of LV function after cell transplantation in this study was not paralleled by LV reverse remodeling.