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Original article

Effective engraftment but poor mid-term persistence of mononuclear and mesenchymal bone marrow cells in acute and chronic rat myocardial infarction

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Abstract

Bone marrow cells are used with promising results for cell therapy after myocardial infarction (MI). We determined the survival and organ distribution of transplanted mononuclear (MNC) or mesenchymal (MSC) bone marrow cells, and the influence of cell type, cell number and application time. MNC and MSC (male Fischer 344 rats) were injected into the border zone of MI (syngeneic females) immediately or 7 days after LAD ligation (10^5 or 10^6 cells, 50 μl). After 0 h, 48 h, 5 days, 3 weeks and 6 weeks, DNA of heart, lung, liver, spleen, kidney, blood, bone marrow, brain and skeletal muscle was isolated and the number of donor cells determined by quantitative real-time PCR with Y-chromosome specific primers (each $n \geq 4$). The percentage of donor-cells in the heart decreased rapidly from 34–80% of injected cells (0 h) to 0.3–3.5% (6 weeks) independent from cell type, number and application time. The absolute number increased after increasing injected cell number (10^6 vs. 10^5). In the lung, MNC and MSC were found at 0 h (126±48 and 140±3 per million organ cells), but in liver and kidney, only few. At 48 h and 6 weeks, an increasing number of MNC, but not MSC, were detected in the spleen (6 weeks, 602±173 per million organ cells vs. 95±50 in the heart, $P=0.02$). In all other organs, only few or no grafted cells of either cell type were detected at these times. Organ distribution was independent from injection time. The low survival of grafted cells may limit their therapeutic impact, while their distribution to other organs must be considered in all cell therapy applications.

Keywords: Myocardial infarction; Cell therapy; Stem cells; Transplantation; Cell differentiation; Bone marrow cells; Mononuclear; Mesenchymal

1. Introduction

Bone marrow cell transfer has been under investigation for the treatment of myocardial infarction and heart failure for several years. Most functional analyses and many clinical studies that used bone marrow cells for the treatment of myocardial infarction showed beneficial results on cardiac function, perfusion and remodeling [1,2]. Initially, the therapeutic goal was replacement of lost cardiomyocytes which seemed possible by early results showing the potential of bone marrow cells to transdifferentiate into cardiac muscle cells [3,4]. These results were repeated recently in a somewhat different setting [5]; however, there is other evidence suggesting that transdifferentiation of bone marrow cells into cardiomyocytes occurs at very low numbers if at all [6–8]. Besides transdifferentiation and direct support of cardiac function, paracrine mechanisms may play a role for the beneficial effects of bone marrow cell therapy [9,10].

Independent from the mode of action, the survival of the grafted cells may play a major role for long-term therapeutic effects. Interestingly, up to date there is little or no data existing

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on the number of cell survival after cell therapy, although the assessment of overall cell survival will add further important information to understand the biological processes after such intervention. Similarly, it is not known whether grafted cells can be found in organs other than the heart for longer term. Therefore, in the present study, a quantitative analysis of cell survival and organ distribution of gender mismatched mononuclear and mesenchymal bone marrow cells grafted into rat myocardial infarction was performed using quantitative real-time PCR with Y-chromosome specific primers. While initial engraftment of both cell types was good, mid-term cell survival was poor, independent of cell type, injected cell number (100,000 vs. 1,000,000) and time of injection after infarct (immediately vs. 7 days later). In addition, donor cells were detected also in other organs, such as lung, liver and spleen.

2. Materials and methods

Protocols were approved by the regional government's Animal Care and Use Committee (Bezirksregierung Köln, No. 50.203.2-K47, 34/03, and 50.203.2-K47, 2/03), and conformed with the "Guide for the Care and Use of Laboratory Animals" (NIH publication No. 85-23, National Academy Press, Washington, DC, revised 1996).

2.1. Bone marrow cell isolation from male rats

After dissecting femurs, humeral and tibial bones (male Fischer 344 rats, 2 months old, Charles River, Sulzfeld, Germany) from surrounding muscle and connective tissue, the bone marrow was eluted with a 19G needle and DMEM, high Glucose, 10% FCS, 1% penicillin/streptomycin. The cell suspension was filtered through 70 μm filters centrifuged and resuspended in PBS.

For recovery of mesenchymal bone marrow cells (MSC), 50×10^6 bone marrow cells were plated on 10 cm dishes, selected by adherence to the polystyrene surface of the culture dishes and kept in culture for 10 days. Cultured cells were characterized using differentiation media (MSC Bulletkit©, Cambrex, Vervien, Belgium) confirming their ability to differentiate into osteocytes and adipocytes (Fig. 1). At day 10 MSC were detached with trypsin/EDTA, washed and resuspended (DMEM, no FCS) at a concentration of 100,000 or 1,000,000 cells/50 μl.

For isolation of mononuclear cells, the crude bone marrow cell suspension was subjected to a ficoll gradient centrifugation (NycoPrep, Progen GmbH, Heidelberg, Germany). The interphase cells were collected, washed and kept in suspension (DMEM, no FCS) until use (12 h at 4 °C).

2.2. Labeling of MNC and MSC with DAPI and histological cell tracking

Some separate experiments were performed with DAPI labeled MNC and MSC for histological cell tracking. Labeling of cells with DAPI (1 μg/ml) was done according to the manufacturer's protocol (Molecular Probes Inc., USA).

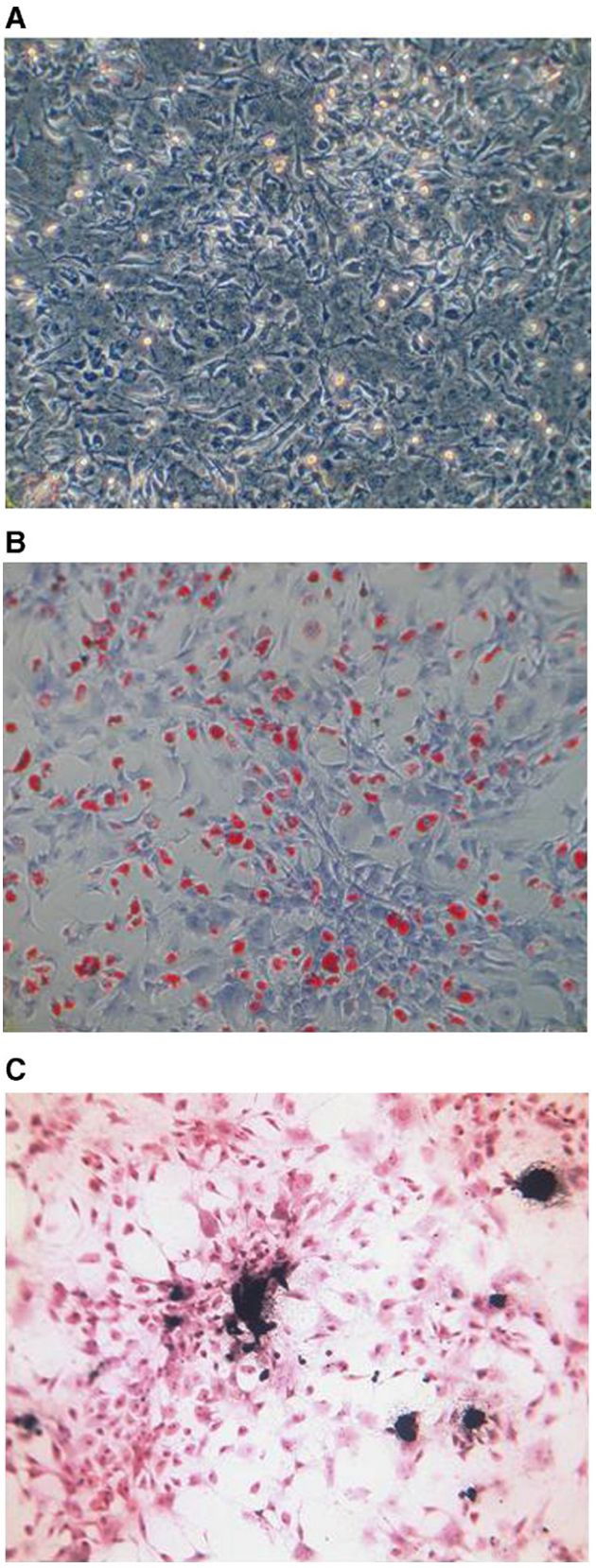

Fig. 1. Differentiation assay proving the potential of MSC for multi-lineage differentiation. (A) Culture of MSC without induction of differentiation (×10 objective). (B) Adipogenic differentiation of MSC as visualized by Oil Red O-staining (×10 objective). (C) Osteogenic differentiation as visualized by von Kossa-staining (×10 objective).