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Incremental benefits of repeated mesenchymal stromal cell administration compared with solitary intervention after myocardial infarction

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Abstract

Background aims. Traditionally, stem cell therapy for myocardial infarction (MI) has been administered as a single treatment in the acute or subacute period after MI. These time intervals coincide with marked differences in the post-infarct myocardial environment, raising the prospect that repeat cell dosing could provide incremental benefit beyond a solitary intervention. This prospect was evaluated with the use of mesenchymal stromal cells (MSCs). *Methods.* Three groups of rats were studied. Single-therapy and dual-therapy groups received allogeneic, prospectively isolated MSCs (1×10^6 cells) by trans-epicardial injection immediately after MI, with additional dosing 1 week later in the dual-therapy cohort. Control animals received cryopreservant solution only. Left ventricular (LV) dimensions and ejection fraction (EF) were assessed by cardiac magnetic resonance immediately before MI and at 1, 2 and 4 weeks after MI. *Results.* Immediate MSC treatment attenuated early myocardial damage with EF of 35.3 ± 3.1% (dual group, n = 12) and 35.2 ± 2.2% (single group, n = 15) at 1 week after MI compared with 22.1 ± 1.9% in controls (n = 17, $P < 0.01$). In animals receiving a second dose of MSCs, EF increased to 40.7 ± 3.1% by week 4, which was significantly higher than in the single-therapy group (EF 35.9 ± 1.8%, $P < 0.05$). Dual MSC treatment was also associated with greater myocardial mass and arteriolar density, with trends toward reduced myocardial fibrosis. These incremental benefits were especially observed in remote (non-infarct) segments of LV myocardium. *Conclusions.* Repeated stem cell intervention in both the acute and the sub-acute period after MI provides additional improvement in ventricular function beyond solitary cell dosing, largely owing to beneficial changes remote to the area of infarction.

Key Words: *cardiac magnetic resonance, hypoxic conditioning, mesenchymal stromal cells, multiple intervention, myocardial infarction, optimization, prospective isolation, repair, timing*

Introduction

Novel therapies are needed for the treatment of left ventricular (LV) dysfunction after acute myocardial infarction (MI). Therapy with mesenchymal stromal cells (MSCs) has shown significant promise in pre-clinical experiments; however, only modest benefits have been observed in clinical trials (1). MSCs have a relatively immuno-privileged phenotype that permits their allogeneic use, offering the prospect of cell optimization and immediate off-the-shelf treatment, which was previously not achievable with autologous cell therapy. Of cell optimization strategies currently evaluated for MSCs (2), prospective isolation and hypoxic pre-conditioning have been shown to enhance their *in vitro* (3,4) and *in vivo* capacity for repair (5,6) and could be readily applied to clinical use.

Almost all studies evaluating stem cell treatment have used a solitary dose intervention, often applied either acutely or deferred to the sub-acute period,

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1−2 weeks after MI (7). These two time points represent greatly differing myocardial environments; early after MI, the myocardium is highly inflamed and consists of hypoxic, metabolically disordered tissue undergoing a mixture of necrosis and apoptosis that may be particularly noxious to transplanted stem cells (8,9). In contrast, 1 week later, the infarcted myocardium has already undergone considerable cardiomyocyte death and completed the early wave of acute inflammatory infiltration, with commencement of collagen deposition and remote myocardial remodeling (10). So far, the therapeutic potential of MSCs after MI has largely been attributed to their paracrine properties (3,11,12), and their secretome is known to be modulated by the local milieu in which they find themselves (13). We reported that timing of MSC intervention in a rat model of MI influenced the reparative effects of MSCs, such that immediate cell administration had a greater relative impact on the infarct territory, whereas deferral of treatment for 1 week caused more beneficial change in remote (non-infarct) LV myocardium (14). Because mechanisms appear to differ, we hypothesized that repeated cell dosing delivered at both these times could provide incremental benefit to ventricular function compared with a single early intervention. In this study, we evaluated whether a second, late delivery of MSCs (optimized by prospective isolation and hypoxic conditioning) in animals already treated with MSCs immediately after MI would enhance myocardial repair.

Methods

Study protocol

This study was approved by the Animal Ethics Committees of the Central Adelaide Local Health Network (No. 104/10) and the University of Adelaide (No. M-2010-105), South Australia. Animal handling was carried out in accordance with the guidelines outlined in the "Position of the American Heart Association on Research Animal Use" (15).

The experimental protocol employed 44 male Sprague-Dawley rats (350−370 g). Animals were randomly assigned at baseline to one of three investigation arms: single-treatment group, dual-treatment group, or control (Figure 1). Baseline evaluation of ejection fraction (EF) was undertaken using cardiac magnetic resonance (CMR) followed by thoracotomy and permanent surgical ligation of the left anterior descending artery (LAD). Allogeneic MSCs were delivered trans-epicardially immediately after MI; control animals received injections of ProFreeze (Lonza, Walkersville, MD, USA) cryopreservation vehicle. At repeat thoracotomy 1 week later, a second MSC dose was transplanted in the dual-therapy group, whereas the single-therapy and control groups received ProFreeze injections. Reassessment of EF with CMR was conducted at 1, 2 and 4 weeks after MI. Euthanasia was performed at week 4 after final imaging for tissue harvesting and histologic evaluation.

Prospective isolation of rat MSCs

Six donor male Sprague-Dawley rats were euthanized (by CO_2 inhalation), and the femora and tibiae were excised, epiphyses removed and bones flushed. The resultant cell suspension was centrifuged, supernatant was removed and the cell pellet was re-suspended for density gradient separation. The long bones, now depleted of bone marrow, were crushed into small fragments using sterile scissors and washed. Digestion of the bone fragments, using collagenase type I and 0.2% DNAse solution in phosphate-buffered saline (PBS), was performed on a shaking platform for 45 min at 37°C. The supernatant containing compact bone cells was collected and processed for mononuclear cells (MNCs) by centrifugation using Lymphoprep (Axis Shield, Rodeløkka, Oslo, Norway) as previously described (3).

Magnetic activated cell sorting (MACS) was used to deplete any contaminating $CD45^+$ hematopoietic cells from the compact bone-derived MNC population. Briefly, compact bone MNCs were pelleted and blocked before incubation with purified anti-rat CD45 (10 μg/mL; BD Biosciences, San Diego, CA, USA). After repeated washes in Hank's balanced salt solution 10% fetal calf serum (FCS), secondary incubation was performed with goat anti-mouse immunoglobulin G (IgG)-biotin (10 μg/mL; Southern Biotech, Birmingham, AL, USA) followed by two washes in MACS buffer (5 mmol/L ethylenediamine tetraacetic acid, 1% bovine serum albumin and 0.01% sodium azide in PBS) and incubation with Anti-Biotin MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany). The cell suspension was passed through a MACS MS Separation Column (Miltenyi Biotec). The $CD45^-$ fraction was collected as the target cell population harboring the MSCs to establish primary cultures.

Hypoxic conditioning

$CD45^-$ cells were initially seeded in plastic culture flasks at $3 \times 10^4/cm^2$ in Alpha Modification of Eagle's Medium (Sigma-Aldrich, St. Louis, MO, USA; supplemented with 20% [v/v] FCS, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 100 μmol/L L-ascorbate-2-phosphate and penicillin/streptomycin sulfate) in a humidified hypoxic chamber in the