Life Sciences 83 (2008) 511–515

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Mesenchymal stem cell administration at coronary artery reperfusion in the rat by two delivery routes: A quantitative assessment

Sharon L. Hale *, Wangde Dai, Joan S. Dow, Robert A. Kloner

The Heart Institute of Good Samaritan Hospital, 1225 Wilshire Blvd., Los Angeles, CA 90017, United States
Keck School of Medicine, Division of Cardiovascular Medicine, University of Southern California, Los Angeles, CA 90017, United States

ARTICLE INFO

Article history:
Received 14 April 2008
Accepted 31 July 2008

Keywords:
Cell therapy
Infarction
Reperfusion
Rat mesenchymal stem cell
Stem cell administration
Nanoparticle
Neutron activation

ABSTRACT

Aims: Ideally, mesenchymal stem cells (MSC) home to and/or remain at the site of damaged myocardium when administered after myocardial infarction. However, MSC may not remain in the heart, but instead relocate to other areas. We investigated quantitatively the distribution of labeled rat MSC, given by two routes after coronary artery occlusion/reperfusion in rats.
Main methods: Rats were subjected to 45 min of coronary artery occlusion and 7 days of reperfusion. Before reperfusion rats received 2×10^6 MSC, labeled with europium, injected directly into the ischemic region of the heart ($n=9$) or intravenously ($n=8$). After 1 week tissues were analyzed for label content together with a standard curve of known quantities of labeled MSC.
Key findings: In rats receiving cells injected directly into the myocardium, 15% of labeled cells were retained in the heart. When the cells were administered intravenously, no MSC were detected in the heart. The route of administration did not affect distribution to other organs, as the number of MSC in liver, spleen and lung was similar with both routes of delivery.
Significance: Even with direct intramyocardial injection, only a small proportion of the cells are retained in the heart, instead traveling to other organs. With intravenous injection there was no evidence that cells "homed" to the damaged heart. Although cell delivery to the heart was significantly affected by the route of administration, the distribution of cells to other organs was similar with both routes of administration.

Introduction

Stem cell therapy for the treatment of myocardial infarction has gained a wide acceptance in the medical community, and human clinical trials are being performed in an attempt to regenerate heart tissue and to improve function in the infarcted heart. These early clinical studies have shown only modest effect on cardiac function, negative effect or transient effect only, indicating that despite the promise of cell therapy, factors such as cell type, dose (number of injected cells) and the precise timing and route of the injection still need to be determined.

Although many types of cells have been tested, including unpurified bone marrow (Orlic et al., 2001), neonatal cardiomyocytes (Muller-Ehmsen et al., 2002), fetal cardiomyocytes (Yao et al., 2003), skeletal myoblasts (Menashe, 2004) and endothelial progenitor cells (Kawamoto et al., 2001), mesenchymal stem cells (MSC) show promise since they can be expanded in large numbers, can be used allogeneically without immune suppression, can express cardiac markers, and have been shown to improve cardiac function, possibly through a paracrine effect. Whether MSC truly differentiate into adult-type cardiomyocytes remains controversial (Makino et al., 1999; Toma et al., 2002; Murry et al., 2004; Dai et al., 2005).

Some studies have shown that MSC may have the ability to home to the site of injury when administered intravenously after myocardial infarction (Price et al., 2006; Krause et al., 2007; Nagaya et al., 2004; Barbash et al., 2003). This route of delivery is appealing since it is not invasive, large numbers of cells can be administered, and it has been shown to be safe in human trials (Hare et al., 2007). However, potential disadvantages of systemic delivery can be low uptake/retention in the infarcted heart and cell relocation to organs other than the heart. This relocation phenomenon is a problem even when cells are injected directly into the muscle or scar of the heart, as cells may move out of the injected area and relocate to other sites in the body (Dow et al., 2005).

There are ~40 million cardiomyocytes in 1 g of normal rat heart tissue (Van der Laarse et al., 1987). In a rat heart that weighs 1 g, an infarct comprising 20% of the left ventricle would require about 8 million cells for one-to-one replacement of dead myocytes; the human hearts average 200 to 300 g, so if direct replacement of dead myocytes were required to restore normal geometry of the heart, a very large dose of cells would be required. However many preclinical

* Corresponding author. The Heart Institute, Good Samaritan Hospital, 1225 Wilshire Blvd, Los Angeles, CA 90017, United States. Tel.: +1 213 977 4045; fax: +1 213 977 4107.
E-mail address: sharon.hale@netscape.com (S.L. Hale).

0024-3205/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2008.07.020

studies suggest that only a small number of stem cells may provide a functional benefit and enhance angiogenesis (Gnecchi et al., 2006; Dai et al., 2007), although the mechanisms have yet to be determined. In addition, the proliferative nature of immature cells may allow fewer to be injected.

Both the timing and route of administration will be important variables for successful application of cell therapy. Early in the evolving infarct the inflammatory process begins and cytokines that may be related to the homing process of stem cells are released. The appropriate window of treatment for either reducing infarct size or improving remodeling is unknown at this time.

The purpose of this study was to determine whether MSC can survive and target the myocardium when transplanted immediately after myocardial infarction in a model of coronary artery occlusion followed by reperfusion. In addition, we tested whether the route of delivery, i.e. intravenous administration or direct intramyocardial injection alters localization in the jeopardized region of the heart. To establish cell distribution, we labeled the MSC with nanoparticles containing europium. The advantages of this technique are that 1) the label is non-radioactive (does not expose either cells or recipient to radiation); 2) the nanoparticles are taken up and entrapped within cytoplasmic vesicles and therefore do not readily diffuse from the cell, and 3) unlike fluorescent labeling, this technique is quantitative. Because neutrons are not attenuated in a sample, measurements can be made in tissues without the need for extraction (Vaccaro et al., 2006). This technique of neutron activation, using the isotope iridium, has been used previously to track cells in a pig model (Freyman et al., 2006). Nanoparticles have been used for many years to track labeled cells using Magnetic Resonance Imaging (for example see Hill et al., 2003). Although the MRI technique can localize implanted cells, it does not allow quantification of the cells.

Materials and methods

This study was approved by the Institutional Animal Care and Use Committee of Good Samaritan Hospital, and the investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996.

Cell labeling and counting

Adult bone marrow-derived stem cells derived from Lewis rats, at passage 6, were obtained from the Tulane University stem cell bank. The cells were expanded in culture in complete culture medium (CCM) containing α-Minimal Essential Medium Eagle with L-glutamate (400 ml), fetal bovine serum (100 ml), antibiotic/antimycotic (5 ml), and L-glutamate (10 ml). On the last day of expansion the cells were labeled with the europium-containing nanoparticles (Biophysics Assay Laboratory, Worcester, MA) at a 1:100 concentration in medium for 21 h. This concentration and timing was shown to be suitable for our cells by a previous pilot study performed in our laboratory. The cells were then washed, placed in tubes with medium composed of CCM +5% DMSO. The tubes were put in a cryo-container containing isopropanol and frozen overnight in a −70 °C freezer. The tubes were then transferred to storage in liquid nitrogen until use. Two expansions were used in this study.

The tracer used in this study was composed of a colloidal material in which a lanthanide metal, in this case europium, was embedded in a nanoparticle of approximately 20 nm (BioPAL, Biophysics Laboratory, Worcester, MA). The cells were incubated in medium containing the tracer. The nanoparticles containing the europium have been shown to incorporate into cytoplasmic vacuoles within the cells (Vaccaro et al., 2006). A standard curve of various cell numbers was prepared. Cells were administered to the rats as described below. At the end of the study, tissue samples were obtained from the animal and desiccated. The samples were sent to Biophysics Laboratory and were subjected to neutron activation. The assay for europium was performed by exposing the samples containing the nanoparticles to a neutron beam. The neutrons combine with the atoms of the metallic element of the probe causing these atoms to become radioactive. These unstable atoms release energy in several forms including high-energy photons which can be quantified in an automatic spectroscopic instrument. The disintegrations per minute (dpm) were reported for each sample, and the number of cells present in the tissue was then calculated using the standard curve.

Surgical preparation and cell injection

Adult Fischer female rats (130–180 g) were anesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (5 mg/kg), intubated, and ventilated with room air. The chest was shaved and swabbed with betadine and then alcohol. The chest wall was infiltrated with bupivicane (0.1 mg/kg). The thorax was opened under sterile procedure (at the fourth intercostal space) and the pericardium excised. A stitch was taken around the left coronary artery (4-0 suture with an atraumatic needle) entering the myocardium below the atrio-ventricular groove and exiting close to the pulmonary cone just beneath the lower edge of the left atrial appendage. The ends of the suture were threaded through a piece of plastic tubing forming a snare that could be used to occlude and reperfuse the artery. The rats were subjected to 45 min of coronary artery occlusion, and 5 min before reperfusion, rats randomly received 2×10^6 labeled MSC, injected either directly into the ischemic anterior free wall of the heart using a 0.5 ml insulin syringe with attached 28-gauge, 1/2 in. needle bent at a 45 degree angle (70 µl, $n=9$) or through a PE-50 catheter placed in the jugular vein (1 ml, $n=8$). Incisions in the neck and chest were closed, buprenorphine (0.02 mg/kg subcutaneously) and saline 10 ml/kg (subcutaneously) were administered, and after weaning from the respirator, the rats were placed on a heating pad while recovering from anesthesia. After 1 week, the rats were re-anesthetized and euthanized, and tissues were obtained from the heart, lung, kidney, spleen, and liver. In the heart, the right ventricle was removed and the left ventricle was cut into two sections: 1. the anterior free wall (site of infarction) and 2. posterior wall plus septum (remote from the site of infarction). Samples were taken from lung, kidney, spleen and liver and were weighed. All samples were then desiccated and sent for counting.

Statistical analysis

Wilcoxon's non-parametric statistic was used to compare the distribution by the two routes of administration to lung, kidney, liver and spleen.

Results

Relationship between europium content and cell number

Standard curves of known quantities of labeled cells were prepared in vitro and analyzed by the neutron activation method. Fig. 1 shows the relationship between the number of cells and the disintegrations per minute for each sample in one expansion. The variables were linearly related and showed a good correlation in both expansions ($r=0.99$). The limit of detection was 10,000 cells.

Animals and treatment

Nineteen rats were assigned to the study. One rat died during coronary artery occlusion prior to cell injection and one rat (assigned to direct injection) died at 10′ after reperfusion. Data are reported in 9