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Original article

Molecular imaging, biodistribution and efficacy of mesenchymal bone marrow cell therapy in a mouse model of Chagas disease

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Abstract

Chagasic cardiomyopathy, resulting from infection with the parasite *Trypanosoma cruzi*, was discovered more than a century ago and remains an incurable disease. Due to the unique properties of mesenchymal stem cells (MSC) we hypothesized that these cells could have therapeutic potential for chagasic cardiomyopathy. Recently, our group pioneered use of nanoparticle-labeled MSC to correlate migration with its effect in an acute Chagas disease model. We expanded our investigation into a chronic model and performed more comprehensive assays. Infected mice were treated with nanoparticle-labeled MSC and their migration was correlated with alterations in heart morphology, metalloproteinase activity, and expression of several proteins. The vast majority of labeled MSC migrated to liver, lungs and spleen whereas a small number of cells migrated to chagasic hearts. Magnetic resonance imaging demonstrated that MSC therapy reduced heart dilatation. Additionally metalloproteinase activity was higher in heart and other organs of infected mice. Protein expression analyses revealed that connexin 43, laminin γ1, IL-10 and INF-γ were affected by the disease and recovered after cell therapy. Interestingly, MSC therapy led to upregulation of SDF-1 and c-kit in the hearts. The beneficial effect of MSC therapy in Chagas disease is likely due to an indirect action of the cells of the heart, rather than the incorporation of large numbers of stem cells into working myocardium.

Keywords: Chagas disease; Cardiomyopathy; Cellular therapy; Mesenchymal stem cells; Cells tracking

1. Introduction

The protozoan parasite that causes Chagas disease, *Trypanosoma cruzi*, is naturally transmitted by hematophagous triatomine insects and affects approximately 15–16 million people in Latin American countries [1]. It is a serious public health problem due to the impact on worker productivity, premature disability and death. The parasite can also be transmitted by blood transfusion, organ transplantation or congenitally [2,3]. Thus, although originally limited to Latin America there is an increased concern about Chagas disease in the United States and Europe due to the large number of immigrants from endemic areas [3–6]. The acute phase of the disease is difficult to diagnose [7] and the clinical acute manifestations disappear in weeks to months. The disease then enters the chronic phase, generally starting with a long

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period of clinical latency called the indeterminate form. During the indeterminate form approximately 30% of infected individuals develop a symptomatic chronic phase, of which 10% display gastrointestinal diseases and 90% develop heart disease [8]; there is no consensus regarding the efficacy of anti-parasitic drugs benznidazole and nifurtimox during the chronic phase [9].

Chronic Chagas heart disease is a progressive, fibrotic inflammatory cardiomyopathy that results in irreversible heart damage [10] which leads to dilation and arrhythmias, and ultimately to congestive heart failure, the primary cause of death in these patients [11,12]. As the disease progresses, few therapeutic options are left and they are identical to those for congestive heart failure of other etiologies and often include β-blockers, diuretics, angiotensin-enzyme inhibitors angiotensin receptor blockers and amiodarone. These treatments are often not satisfactory and the last therapeutic option is often heart transplantation [13]. In that complex scenario, cell therapy appears as an alternative for Chagas disease therapy.

Mesenchymal stem cells (MSC) are a rare subset of stem cells residing in the bone marrow where they closely interact with hematopoietic stem cells and support their growth and differentiation. MSC can differentiate into multiple mesenchymal cell types providing a promising tool for tissue repair [14]. In addition, MSC suppress many T cell, B cell and natural killer cell functions and may also affect dendritic cell activities. Therefore, given the established role of the immune system in the physiopathology of Chagas disease [15] and the immune modulatory properties of MSC we hypothesized that MSC could be an optimal cell type for therapy in chagasic cardiomyopathy.

In a previous study that we pioneered, we investigated the migration of transplanted MSC in an acute model of Chagas disease, and correlated MSC biodistribution with glucose metabolism and morphology of heart in chagasic mice by small animal positron emission tomography (microPET). It was observed that a small but significant number of transplanted labeled MSC migrated to chagasic hearts, whereas the vast majority of labeled MSC migrated to liver, lungs and spleen. Additionally, using the radioactive tracer [^{18}F] fluoro-2-deoxyglucose, it was possible to demonstrate by microPET that therapy with MSC reduced right ventricular dilation and increased heart glucose metabolism [16].

In the present study, we extend our studies of MSC transplantation to a chronic murine model. Furthermore, we labeled the cells with two types of nanoparticles to correlate MSC migration with heart morphology and protein expression and global distribution of metalloproteinase activity.

2. Material and methods

2.1. Mice

All experiments were initiated in 8–10 week old adult male mice CD-1 in accordance with the U.S. NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23), approved by the Institutional Animal Care and Use Committee of the Albert Einstein College of Medicine. The animal protocol numbers are 20130202 and 20110307.

2.2. Obtaining mesenchymal cells from bone marrow

Bone marrow cells were obtained from mouse tibias and femurs. The bones were isolated, epiphyses were removed and individually inserted in 1 mL automatic pipette tips inside 15 mL tubes. The bones were centrifuged at 300 × g for 1 min and the pellets suspended in Dulbecco's modified Eagle's high glucose medium (DMEM; Invitrogen Inc., Carlsbad,CA), supplemented with 10% fetal bovine serum (Invitrogen Inc.), 2 mM L-glutamine (Invitrogen Inc.), 100 U/mL penicillin (Sigma–Aldrich Co., St. Louis, MO), and 100 μg/mL streptomycin (Sigma–Aldrich). The cells were plated in culture dishes with supplemented DMEM and maintained in 5% CO_2 atmosphere at 37 °C. After 48–72 h of culture the medium was replaced to remove non adherent cells and the adherent cells were grown to confluence before each passage. Medium was replaced three times a week and all experiments were performed on second or third passage cells.

2.3. Mesenchymal cell labeling

Since each imaging modality has its inherent limitations, we used two different nanoparticle approaches to label and track MSC. Thus, the cells were labeled with magnetic and fluorescent nanoparticles which can be visualized by magnetic resonance imaging (MRI) and in vivo imaging system (IVIS), respectively.

2.3.1. Magnetic nanoparticles

Feridex IV (Advanced Magnetics Inc., Cambridge) is a superparamagnetic iron oxide nanoparticle coated with dextran; it and another, dextran-coated ferumoxide, are clinically used as intravenous MRI contrast agents for analyzing liver pathology. Although dextran-coated SPION do not show sufficient cellular uptake by MSC, cationic compounds, such as protamine, facilitate the interaction with the negatively charged cell surface and subsequent enable endosomal uptake [17,18]. Feridex was combined with Protamine chlorhydrate (Valeant Pharmaceuticals International, SP, Brazil) in culture medium and shaken for 30 min. The solution containing Feridex and protamine was added to MSC cultures at a proportion of 1:1 with supplemented DMEM in 5% CO_2 atmosphere at 37 °C for 4 h. The final concentration of protamine was 5 μg/mL and of Feridex was 50 μg/mL. The labeled cells were washed three times with phosphate-buffered saline (PBS), trypsinized and transplanted in infected and not infected animals for MRI tracking.

2.3.2. Fluorescent nanoparticles

For fluorescence tracking we used X-Sight761 nanospheres (Carestream Health Inc., Rochester, NY) which are fluorescent nanoparticles (17 nm in diameter) with a near infrared (NIR) wavelength 761 nm excitation and 789 nm emission. MSCs were incubated with a solution of 0.3 mg/mL X-Sight with