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Original article

Transplantation of mesenchymal stem cells attenuates myocardial injury and dysfunction in a rat model of acute myocarditis

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Abstract

Acute myocarditis is a non-ischemic inflammatory disease of the myocardium for which there is currently no specific treatment. We have previously shown that mesenchymal stem cells (MSC) can ameliorate heart injury during acute ischemia and in dilated cardiomyopathy; however, the therapeutic potential in acute myocarditis is unclear. In this study, we investigated the ability of MSC to attenuate myocardial injury and dysfunction during the acute phase of experimental myocarditis. Ten-week-old male Lewis rats were injected with porcine myosin to induce myocarditis. Cultured MSC (3×10^6 cells/rat) were injected intravenously 7 days after myosin injection. At 3 weeks, myosin injection resulted in severe inflammation and significant deterioration of cardiac function. MSC transplantation attenuated increases in CD68-positive inflammatory cells and monocyte chemoattractant protein-1 (MCP-1) expression in myocardium, and improved cardiac function in this model. Furthermore, myocardial capillary density was higher in myocarditis tissue, and was further increased by MSC transplantation. *In vitro*, cultured adult rat cardiomyocytes were injured in response to MCP-1, whereas this effect was attenuated by MSC-derived conditioned medium, suggesting cardioprotective effects of MSC acting in a paracrine manner. MSC transplantation attenuated myocardial injury and dysfunction in a rat model of acute myocarditis, at least in part through paracrine effects of MSC.

Keywords: Acute myocarditis; Mesenchymal stem cell; Paracrine effect; Cytokine; Cell death

1. Introduction

Acute myocarditis is a non-ischemic heart disease characterized by myocardial inflammation and edema. This disease is associated with rapidly progressive heart failure, arrhythmias and sudden death [1,2]. Although the early evidence for efficacy of immunoglobulin and interferon therapy appears promising, these results have yet to be demonstrated in randomized or controlled clinical trials. The current options are restricted to supportive care for heart failure or arrhythmias. The lack of specific treatment and the potential severity of the illness emphasize the importance of novel and effective therapeutic strategies for myocarditis.

Mesenchymal stem cells (MSC) are multipotent stem cells present in adult tissues, and have the ability to differentiate into a variety of lineages, including vascular smooth muscle cells, endothelial cells and cardiomyocytes [3,4]. We have previously reported that bone marrow-derived MSC engrafted in experimental myocardial infarction expressed both cardiac and endothelial phenotypes in the heart, and further increased capillary density and decreased the infarct size [5]. Moreover, we have recently demonstrated that monolayered MSC derived from adipose tissue reversed wall thinning in the scar area and improved cardiac function in rats with myocardial infarction [6]. The cardioprotective effects of MSC are known to be mediated

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not only by their differentiation into vascular cells and cardiomyocytes, but also by their ability to supply large amounts of angiogenic, anti-apoptotic and mitogenic factors [5–7]. These findings suggest the therapeutic potential of MSC for heart failure. However, whether intravenously transplanted MSC attenuate myocardial inflammation and cardiac dysfunction in acute myocarditis remains unknown.

In the present study, we used porcine myosin-induced acute myocarditis in Lewis rats. This model closely resembles human giant cell myocarditis, a frequently fatal disorder characterized by multinucleated giant cells in the myocardium [8]. To examine the therapeutic potential of MSC in the acute phase of myocarditis, MSC were intravenously injected into rats 7 days after myosin injection.

Thus, the purposes of this study were 1) to investigate whether intravenous transplantation of MSC improves cardiac function and pathological findings including myocardial inflammation in rats with myosin-induced myocarditis, and 2) to investigate the underlying mechanisms responsible for the effects of MSC.

2. Materials and methods

2.1. Animals

Ten-week-old male Lewis rats (Japan SLC, Hamamatsu, Japan) were used in all experiments, and were maintained in our animal facilities. The experimental protocols were approved by The Animal Care Committee of the National Cardiovascular Center.

2.2. Preparation of cardiac myosin

Purified cardiac myosin from the ventricular muscle of pig hearts was prepared according to a procedure described previously [8]. The antigen was dissolved at a concentration of 20 mg/ml in phosphate-buffered saline (PBS) containing 0.3 M KCl, mixed with an equal volume of complete Freund's adjuvant containing 11 mg/ml *Mycobacterium tuberculosis* (Difco Laboratories, Sparks, MD, USA). Rats were anesthetized with an intraperitoneal injection of 20 mg/kg sodium pentobarbital, and 0.1 ml of the antigen-adjuvant emulsion was injected into the each footpad.

2.3. Acute myocarditis model

Forty-five rats were randomly divided into three groups and received the following treatment: 1) 0.2 ml saline and sham surgery (Sham group, $n=15$), 2) 0.2 ml cardiac myosin antigen and sham surgery (MyoC group, $n=15$), and 3) 0.2 ml cardiac myosin followed by MSC transplantation 7 days post-myosin injection (MyoC+MSC group, $n=15$). Rats were weighed and observed daily for signs of morbidity and for death.

2.4. Preparation and transplantation of bone marrow-derived MSC

MSC were prepared as described previously [5]. Briefly, bone marrow cells were isolated by flushing out the femoral and tibial cavities with PBS, and plated onto 10-cm dishes in complete culture medium: Dulbecco's Modified Eagle's Medium (DMEM), 15% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. Five days after plating, non-adherent cells were removed, and adherent cells were further propagated for 4 to 5 passages.

Seven days after myosin injection, MSC (3×10^6 cells) or vehicle (0.9% saline) was intravenously administered via the jugular vein. Sham rats also received saline administration but without myosin injection.

2.5. Hemodynamic studies

Hemodynamic studies were performed on day 21 post-myosin injection. Anesthesia was maintained with an intraperitoneal injection of 20 mg/kg sodium pentobarbital, and a 1.5 Fr micromanometer-tipped catheter was placed in the left ventricle through the right carotid artery (Millar Instruments, Houston, TX, USA). Heart rate (HR) was also monitored by electrocardiography. HR, mean arterial pressure (MAP), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), maximum dP/dt (Max dP/dt) and minimum dP/dt (Min dP/dt) were used as indices of hemodynamics, and recorded simultaneously during ventilation after a minimum equilibration period of 20 min.

2.6. Echocardiographic studies

Echocardiography was performed on day 21 post-myosin injection. Rats were anesthetized with an intraperitoneal injection of 20 mg/kg sodium pentobarbital. A 12 MHz probe was placed at the left 4th intercostal space for M-mode imaging using 2D echocardiography (Sonos 5500, Philips, Bothell, WA, USA). Left ventricular systolic dimension (LVDs), left ventricular diastolic dimension (LVDd), anterior wall thickness (AWT), posterior wall thickness (PWT) and ejection fraction (EF) were measured, and taken as an average of three beats. Fractional shortening (%FS) was calculated as $(\mathrm{LVDd}-\mathrm{LVDs})/\mathrm{LVDd}\times100$.

2.7. Histological examination

The heart was excised above the origin of the great vessels, and heart weight and body weight were recorded on day 21 post-myosin injection. Portions of the midventricular heart, spleen, pancreas, kidney and liver were fixed with 4% paraformaldehyde, embedded in paraffin, sectioned at 4-μm thickness, stained with either hematoxylin and eosin (H & E) or Masson's trichrome, and subjected to immunohistochemical staining. H & E-stained sections were evaluated by a cardiovascular pathologist (H.I.-U.) for the characterization of myocardial injury and inflammation without knowledge of the experimental groups, on the following scale: 0, absent or questionable presence; 1, limited focal distribution; 2–3, intermediate severity; and 4, coalescent and extensive foci throughout the entire transversely sectioned ventricular tissue.