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Implantation of bone marrow mononuclear cells using injectable fibrin matrix enhances neovascularization in infarcted myocardium

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Abstract

Neovascularization may improve cardiac function and prevent further scar tissue formation in infarcted myocardium. A number of studies have demonstrated that bone marrow-derived cells have the potential to induce neovascularization in ischemic tissues. In this study, we hypothesized that implantation of bone marrow mononuclear cells (BMMNCs) using injectable fibrin matrix further enhances neovascularization in infarcted myocardium compared to BMMNC implantation without matrix. To test this hypothesis, infarction was induced in rat myocardium by cryoinjury. Three weeks later, rat BMMNCs were mixed with fibrin matrix and injected into the infarcted myocardium. Injection of either BMMNCs or medium alone into infarcted myocardium served as controls. Eight weeks after the treatments, histological analyses indicated that implantation of BMMNCs using fibrin matrix resulted in more extensive tissue regeneration in the infarcted myocardium compared to BMMNC implantation without matrix. Examination with fluorescence microscopy revealed that cells labeled with a fluorescent dye prior to implantation survived in the infarcted myocardium at 8 weeks of implantation. Importantly, implantation of BMMNCs using fibrin matrix resulted in much more extensive neovascularization in infarcted myocardium than BMMNC implantation without matrix. The microvessel density in infarcted myocardium was significantly higher ($p<0.05$) when BMMNCs were implanted using fibrin matrix (350 ± 22 microvessels/mm^2) compared to BMMNC implantation without matrix (262 ± 13 microvessels/mm^2) and medium injection (76 ± 9 microvessels/mm^2). In addition, average internal diameter of microvessels was significantly larger ($p<0.05$) in BMMNC implantation with fibrin matrix group ($14.6\pm1.2\,\mu m$) than BMMNC implantation without matrix group ($10.2\pm0.7\,\mu m$) and medium injection group ($7.3\pm0.5\,\mu m$). These results suggest that fibrin matrix could serve as a cell implantation matrix that enhances neovascularization efficacy for myocardial infarction treatment.

Keywords: Fibrin marix; Bone marrow mononuclear cells; Myocardial infarction; Neovascularization

1. Introduction

Myocardial infarction may result in left ventricle remodeling and subsequent heart failure. During the left ventricular remodeling process, injured cardiomyocytes are gradually replaced by fibrous tissue [1], the initial infarct area progressively expands, and the left ventricle dilates, which may lead to heart failure [2]. An effective method to reverse myocardial remodeling is to induce neovascularization within the infarcted myocardium [3,4]. Neovascularization may occur within the infarcted myocardium even under normal circumstances without any treatment. However, this may not be sufficient to support tissue growth required for contractile compensation and to satisfy the greater demands of the hypertrophied but viable myocardium [5]. The relative

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lack of oxygen and nutrients to the hypertrophied myocardium may result in the death of myocardium. Therefore, more extensive neovascularization is required to reverse myocardial remodeling and subsequent heart failure.

Neovascularization can be stimulated by angiogenic gene therapy [6], angiogenic cytokine administration [7], and transmyocardial laser revascularization [8]. However, these treatments have been applied in only a few clinical trials due to problems related to the unstable effect, the risk of systemic or local toxicity, and difficult techniques [9]. Implantation of bone marrow cells (BMCs) is attractive for the induction of neovascularization, since BMCs can differentiate into cardiomyocytes, endothelial cells and vascular smooth muscle cells [10] and secrete various angiogenic growth factors [11]. In addition, implantation of autologous BMCs avoids immunorejection. In clinical trials, implantation of bone marrow mononuclear cells (BMMNCs) into infarcted myocardium demonstrated myocardial regeneration, neovascularization, and treatment safety [12].

In the present study, we tested the hypothesis that implantation of BMMNCs using a cell implantation matrix enhances neovascularization in the infarcted myocardium compared to BMMNC implantation without matrix. The rationale of this hypothesis is that cell adhesion to matrix may be necessary for the differentiation of BMCs into somatic mesenchymal cells [13] and for the survival of the differentiated adherent cells, including endothelial cells [14]. Fibrin matrix was utilized as a cell implantation matrix in this study because fibrin is easily injectable and autologous fibrin avoids the potential risk of foreign body reactions. Rat BMMNCs were mixed with fibrin matrix and injected into infarcted myocardium in rat myocardium 3 weeks after cryoinjury. Injection of either BMMNCs or medium alone into infarcted myocardium served as controls. Neovascularization in each group was evaluated by determining the density and average internal diameter of microvessels in the infarcted myocardium 8 weeks after the treatments. Tissue regeneration and implanted cell survival in the infarcted myocardium were also examined.

2. Materials and methods

2.1. Rat myocardial infarction model

Sprague-Dawley rats (200–250 g, SLC, Tokyo, Japan) were anesthetized with an intramuscular administration of ketamin hydrochloride (90 mg/kg) and xylazine hydrochloride (5 mg/kg). The anesthetized rats were incubated and placed on a ventilator (model 683, Harvard Apparatus, South Natick, MA, USA). The rat heart was exposed through a 2-cm left lateral thoracotomy. Cryoinjury was made with a metal probe (8 mm in diameter) cooled by immersion in liquid nitrogen. The cooled metal probe was applied to the left ventricle free wall for 10 s twice, afterwards for 60 s six times. The muscle layer and the skin incision were closed with sutures. All care and handling of animals were performed according to the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH publication 85–23, revised 1985).

2.2. BMMNC isolation

BMCs were flushed from the femurs and tibias of Sprague-Dawley rats (200–250 g) into culture medium (Medium 199; Gibco BRL, Gaithersburg, MD, USA). The cell suspension was loaded on Ficoll-Paque density gradient (specific gravity = 1.077, Amersham Biosciences, Arlington Heights, IL, USA), and centrifuged for 20 min at 230 g. BMMNCs were isolated from the layer between the Ficoll-Paque reagent and blood plasma, and washed three times in phosphate-buffered saline (PBS; Sigma, St. Louis, MO, USA).

2.3. BMMNC labeling and detection

Prior to implantation, BMMNCs were labeled with the fluorescent probe Cell Tracker™ chloromethyl-1,1-dioactadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (CM-DiI; Molecular Probes, Eugene, OR, USA) that incorporates into cell membranes. BMMNCs were incubated serially in Hank's balanced salt solution (Sigma) containing 1 μg/ml CM-DiI dye at 37°C for 5 min and at 4°C for 15 min. The labeled cells were washed three times in PBS and used immediately for implantation. Eight weeks after implantation, fluorescently labeled BMMNCs were detected using a fluorescence microscope (Eclipse E800, Nikon, Tokyo, Japan).

2.4. Fibrin matrix preparation

Fibrin matrix was prepared from a commercially available fibrin gel kit (Greenplast®, GreencrossPD Co., Yongin, Korea). Plasminogen-free fibrinogen (100 mg) and fibrin-stabilizing factor XIII (66 units) were dissolved in 1 ml of plasmin inhibitor aprotinin solution (1100 kIU/ml) for fibrinogen solution. Thrombin (500 IU) was dissolved in 1 ml of calcium chloride solution (5.9 mg/ml) for thrombin solution. The fibrinogen solution and thrombin solution containing cells were mixed at a 1:1 volume ratio and injected into infarcted myocardium.

2.5. Cell implantation

Thrombin solution (100 μl) containing BMMNCs (2×10^7 cells) and fibrinogen solution (100 μl) were