The incidence of coronary artery disease (CAD) is increasing in Japan and consequently, the morbidity of CAD has become a major concern of public health, with an increase in the morbidity and mortality caused by ischemic heart failure. The effectiveness of coronary artery bypass surgery or percutaneous coronary intervention (PCI) has been established in terms of the improvement in survival and the reduction of hospitalizations for the management of ischemic heart failure in patients with severe CAD. However, despite recent improvements in these therapeutic strategies, there are a number of patients who cannot receive complete revascularization because of severe or total arterial occlusion. Intramyocardial injection of autologous bone marrow mononuclear cells (ABMMCs) has been shown to induce neovascularization of ischemic myocardium.

Methods and Results The study will investigate the safety and feasibility of intramyocardial injections of ABMMCs and test the hypothesis that this treatment would promote neovascularization and improve left ventricular (LV) global and/or regional function in patients with severe CAD who have no other option. ABMMCs (~10^6 cells) will be injected into the area of ischemic myocardium where the coronary artery is not graftable, in combination with bypass surgery to the other coronary branches. Myocardial perfusion and LV global and regional function will be evaluated, based on the micromanometer-tipped catheter method, single-photon emission tomography, and myocardial enhanced and color tissue Doppler echocardiography at baseline and during 12 month follow-up.

Conclusions This project will demonstrate that intramyocardial injection of ABMMCs with or without coronary artery bypass surgery could be a safe and effective method for therapeutic neovascularization, resulting in an improvement of cardiac function in patients with severe CAD. (Circ J 2006; 70: 1180–1183)

Key Words: Angiogenesis; Autologous bone marrow mononuclear cell; Coronary artery disease; Left ventricular function

Background Despite recent improvements in the treatments of coronary artery disease (CAD), there are a considerable number of patients who can not receive complete revascularization because of severe or total arterial occlusion. Intramyocardial injection of autologous bone marrow mononuclear cells (ABMMCs) has been shown to induce neovascularization of ischemic myocardium.

Methods

Patients The study protocol was approved by the Committees on the Ethics of Human Research of Nagoya University Graduate School of Medicine, and informed consent will be obtained from each participant. This is a prospective study of 20 patients who have severe CAD and no other
option for standard revascularization therapy. Inclusion and exclusion criteria are presented in Table 1. To assess the safety and feasibility of this treatment strategy, an initial group will consist of 5 patients with preserved LV systolic function (LV ejection fraction (LVEF) >35%). After affirming the safety of the procedure after 3 months' follow-up, the remaining 15 patients will be enrolled.

**Catheterization**
An externally balanced and calibrated 6F pigtail angiographic micromanometer-tipped catheter will be advanced into the LV for the measurement of pressure. We will evaluate the maximum first derivative of LV pressure (LV dP/dt\(_{\text{max}}\)) as an index of contractility, and the pressure half-time (T1/2) as an index of relaxation during right atrial pacing as previously described. Left ventriculography will be carried out and the LVEF and volumes will be calculated by the area–length method.

**Scintigraphy**
Myocardial perfusion will be assessed by resting thallium-201 and exercise technetium-99-sestamibi single-photon emission computed tomography (SPECT) scan. The exercise protocol is outlined in the echocardiography section. Tracer distribution will be assessed semiquantitatively on the basis of analysis of the apical, midventricular, and basal short-axis and vertical long-axis tomograms. The LV myocardium will be divided into 20 segments (18 from the short-axis images and 2 from the vertical long-axis images). The defect score will be defined according to a 5-point scale (0=normal uptake, 1=equivocal uptake, 2=mildly reduced uptake, 3=severely reduced uptake, 4=absent uptake) used by 2 independent observers unaware of the clinical data. The summed stress score and summed rest score will be calculated as the sum of the scores for the 20 segments for the stress and rest images, respectively. The sum of the differences between each of the 20 segments on the stress and rest images will be the summed difference score.

**Echocardiography**
We will carry out color tissue Doppler echocardiography with a phased-array electronic ultrasound system (the Vivid seven system, GE VingMed Ultrasound, Norway) to assess LV regional function. Scanning will be performed longitudinally from the apex to acquire 4-chamber views. The LV myocardium will be divided into 8 segments (anterior base, anterior apex, inferior base, inferior apex, septal base, septal apex, lateral base and lateral apex), and longitudinal strain and strain-rates in each segments will be estimated by measuring the spatial velocity gradient over a

<table>
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<tr>
<th>Table 1 Inclusion and Exclusion Criteria for Intramyocardial Injection of ABMMCs</th>
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<td><strong>Patients will be eligible if they have:</strong></td>
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<tr>
<td>(1) Reversible myocardial ischemia on stress/rest single-photon emission computed tomography imaging</td>
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<td>(2) At least one major epicardial coronary artery with &gt;70% diameter stenosis</td>
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<td>(3) No standard revascularization therapy option such as coronary artery bypass surgery or catheter-based intervention as assessed by coronary angiography</td>
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<tr>
<td><strong>Patients will be excluded if they have:</strong></td>
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<tr>
<td>(1) Recent (&lt;3 month) myocardial infarction or unstable angina</td>
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<td>(2) History of malignant disease</td>
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<td>(3) Unexplained homologal or biochemical abnormalities</td>
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<td>(4) Active retinopathy</td>
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ABMMCs, autologous bone marrow mononuclear cells.
computation area of 8–10 mm. From the averaged strain and strain-rate, peak systolic strain-rate, peak early diastolic strain-rate, and systolic strain will be calculated.

After completion of the strain and strain-rate measurements, myocardial contrast echocardiography with intravenous bolus injections of perfluorocarbon contrast agents will be performed to assess myocardial perfusion at rest and immediately after ergometer exercise. The ergometer exercise will be initiated at 25 W for 3 min and then increased by 25 W at 3-min intervals until leg fatigue or severe chest pain occurred. Myocardial contrast echocardiography allows for higher-resolution myocardial perfusion images to be repeatedly compared with SPECT.\(^{11}\)

**Bone Marrow Aspiration and Injection**

We will aspirate 200 ml of bone marrow from the posterior iliac crest under general anesthesia, ABMMCs will be isolated by centrifugation using AS104-Plus blood-cell separator (Baxter, Deerfield, USA), and concentrated to a final volume of 4–5 ml. The bone marrow cell population will be analyzed by a fluorescence-activated cell sorter using anti-CD34, anti-CD45, anti-CD-133, and vascular endothelial growth factor 2 receptor (KDR) antibodies. If the patient has an ischemic area where there are graftable vessels, coronary artery bypass grafting (CABG) will be carried out at first. Then, 0.2 ml of ABMMCs containing approximately 10^6 cells will be injected into each of approximately 20 sites in the ischemic area, excluding scar tissue, where there is no possibility of grafting or eligibility for PCI. If the entire ischemic area is unsuitable for revascularization, only intramyocardial injection of ABMMCs will be performed. Red blood cells separated from the bone marrow will be returned to the patient.

**Follow-up Examination**

Clinical evaluation (Canadian Cardiovascular Society angina class and physical), laboratory data (complete blood count, blood chemistry, brain natriuretic peptide), echocardiography and treadmill test will be performed at baseline, and at 1, 6 and 12 months’ follow-up (Fig 1). The SPECT scan and cardiac catheterization will be repeated at baseline, and at 1 and 12 months’ follow-up.

**Statistical Analysis**

Statistical comparisons between baseline and follow-up data will be performed using a repeated measure of variance. We will judge a p-value of less than 0.05 as statistically significant.

**Discussion**

Based on the observation that both bone marrow mononuclear cells and endothelial progenitor cells enhance neovascularization in ischemic tissue, recent studies performed in patients with limb ischemia demonstrated that injection of bone marrow-derived progenitor cells into the gastrocnemius resulted in significant improvement in limb perfusion and clinical symptoms. Moreover, intramyocardial implantation of ABMMCs has been shown to increase myocardial perfusion. To develop this strategy as a simple and effective therapy for CAD, the present study will evaluate its safety, feasibility and effectiveness. There remain several questions about the technique’s safety and feasibility, including myocardial damage caused by inflammatory cytokines released from implanted bone marrow cells, development of intramyocardial tumor, and long-term prognosis, which will be assessed by the study.

**Comparison With Previous Studies**

Previous studies have assessed the LVEF as an index of systolic function based on echocardiography, angiography, scintigraphy or magnetic resonance imaging. One of the limitations of the LVEF is its load dependence which confounds accurate assessment of LV systolic function. In the present study, we will assess LV dP/dtmax as an index of contractility and T\,1/2 as an index of relaxation. These indices are assumed to be relatively load independent and an impairment of relaxation is known to precede contractile abnormality in myocardial ischemia. In addition, we will obtain strain and strain-rate images, which will allow quantitative assessment of regional myocardial wall motion and may be more sensitive to regional myocardial ischemia to our knowledge, this will be the first study to demonstrate an improvement of LV regional myocardial diastolic function, as a result of increasing regional myocardial perfusion after intramyocardial injection of ABMMCs.

Several studies performed in Europe reported that intracoronary infusion of ABMMCs may enhance recovery of LV systolic function after acute myocardial infarction. An 18-month follow-up after intracoronary infusion of ABMMCs reported that ABMMC implantation is safe and not associated with adverse clinical events. However, it also reported that intracoronary infusion of ABMMCs could not promote sustained improvements of regional LV function and global LVEF compared with a randomized control group. It is possible that intramyocardial injection of ABMMCs, not intracoronary infusion, might improve the long-term results in patients with CAD.

**Study Limitations**

We cannot have a placebo group because of ethical issues. Also, when a patient has an ischemic area for which standard revascularization therapy, such as CABG or PCI, can be performed, the standard therapy will be carried out initially, and then the ABMMCs will be injected into the ischemic area that is unsuitable for the standard therapy. Accordingly, it may be difficult to elucidate the exact efficacy of intramyocardial injections of ABMMCs on LV function. However, sole therapy of intramyocardial injection of ABMMCs will also be performed if none of the coronary branches to the ischemic area are suitable for grafting or PCI. Based on analysis of this subgroup of patients who have the sole therapy, we will be able to evaluate the effectiveness of intramyocardial injection of ABMMCs on LV function.

In conclusion, this study will demonstrate the safety and feasibility of intramyocardial injection of ABMMCs, and elucidate that mechanism by which the therapy may promote neovascularization in patients with CAD and thus contribute to improved LV global and regional functions.

**References**


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