Local Implantation of Autologous Bone Marrow Cells for Therapeutic Angiogenesis in Patients With Ischemic Heart Disease

Clinical Trial and Preliminary Results

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A new therapy for severe ischemic heart disease has been developed; therapeutic angiogenesis induced by the local implantation of autologous bone marrow cells (BMC). After confirming that no detrimental changes were induced by this treatment in a canine heart model, a clinical trial was commenced in 1999. Thus far, 5 patients have been given this new treatment concomitant with coronary artery bypass grafting and all have been followed up for at least 1 year. Autologous BMC were implanted into the ungraftable area and postoperative cardiac scintigraphy showed specific improvement in coronary perfusion in 3 of the 5 patients. Postoperative chest radiography, electrocardiography, echocardiography and blood tests did not reveal any detrimental changes. In conclusion, this new therapy appears to be safe and could provide a treatment option for patients with otherwise untreatable ischemic heart disease. (Jpn Circ J 2001; 65: 845–847)

Key Words: Angiogenesis; Bone marrow cells; Ischemic heart disease

Therapeutic angiogenesis has been the recent focus of attention as a new treatment for severe ischemic heart disease unable to be treated by medication, catheter intervention or coronary artery bypass grafting (CABG).1-2 We considered the possibility of achieving angiogenesis with bone marrow cells (BMC) because they contain various kinds of primitive cells that can differentiate into endothelial cells and secrete several growth factors.

We recently achieved angiogenesis by the local implantation of autologous BMC in vivo3 and subsequently reported angiogenesis in the ischemic rat heart and hindlimb induced by this method.4,5 Other groups have obtained similar results6,7 and the angiogenesis induced by local implantation of autologous BMC in rats has been shown to improve the loss of physical function related to ischemia.8 We investigated the safety of this treatment in a canine heart model. Echocardiographic evaluation of cardiac function, Holter electrocardiogram, blood tests and local histological findings of heart muscle did not demonstrate any evidence of systemic or local toxicity induced by this treatment in the acute or chronic phases. Therefore, we began a clinical trial in 5 patients in October 1999, referring to the new treatment as local BMC implantation (BMCI). All 5 have been followed up for 1 year and we present here the evidence for the efficacy and safety of this new treatment.

The clinical trial was approved by the Medical Ethics Committee of Yamaguchi University School of Medicine, and informed consent was obtained from all the patients enrolled. The Medical Ethics Committee stipulated that this therapy must only be performed concomitantly with CABG, and that the only approved candidates were those patients scheduled to undergo CABG who had at least one ischemic area unsuitable for the traditional treatments of percutaneous transluminal coronary angioplasty or bypass grafting to the stenotic coronary artery. Although an old myocardial infarction could be an indication, an infarcted area that involved wall thinning with little remaining myocardium was a contraindication.

The procedure was performed under general anesthesia as follows. Bone marrow cells were collected from the iliac bone, around the anterior superior spine, in a standard fashion, and the CABG procedure was begun simultaneously. The harvested BMC were diluted with RPMI 1640 (Nikken Bio Medical Laboratory, Kyoto, Japan) containing heparin and then 200–400 ml were saved in a sterile pack from the Bone Marrow Collection Kit (Baxter, IL, USA). During CABG, the mononuclear cell fraction was prepared with a COBE Spectra Apheresis System (Gambro, Stockholm, Sweden) and the cell count was adjusted from $5 \times 10^6$ to $1 \times 10^7$ cells/ml. When CABG was completed and the heart was still arrested under cardiopulmonary bypass, the autologous mononuclear cell-rich fraction of BMC was injected into the area of ischemic myocardium where no graft. The injection volume was 0.1 ml ($5 \times 10^7$ to $1 \times 10^8$ cells/point) and injections were spaced 1 cm apart, using a 1 ml syringe and a 26-gauge needle. After the final injection, the aortic clamp was released, the patient was weaned from cardiopulmonary bypass, and the chest wall was closed.

Blood tests, chest radiography, and electrocardiography
were carried out according to our standard clinical protocol. Echocardiography and scintigraphy were performed 1 month and 1 year after surgery. Cardiac catheterization was performed 1 month after surgery.

Table 1 shows the clinical and surgical details of the 5 patients in this series. The ischemic myocardium could not be grafted in any of these patients because no graftable coronary arteries existed, although the target myocardium remained viable. Postoperative coronary angiography was performed in all 5 patients and all the grafts were confirmed to be patent. However, it was difficult to clearly identify all vessels newly formed by BMCI on angiography. Three patients showed significant improvement on myocardial radionuclide images. The remaining 2 patients did not show specific ischemic changes on preoperative scintigrams, although there was significant stenosis in the coronary artery, and so the efficacy of BMCI could not be judged in those 2 patients. Brief reports of the 3 patients in whom improved blood flow was demonstrated are summarized.

Case 1
A 67-year-old man underwent a preoperative coronary angiogram, which showed significant stenosis in the left anterior descending artery (LAD), diagonal branch, and the circumflex artery (Cx) (nos. 11 and 15). CABG to the LAD, diagonal branch, and posterolateral (no. 14PL) branch was carried out. The no. 15PL branch could not be grafted because the coronary artery was too small and so we performed BMCI. Postoperative exercise thallium (201Tl) scintigraphy showed no ischemic changes in the area 1 month after the treatment.

Case 3
A 59-year-old man underwent a preoperative coronary angiogram, which showed significant stenosis in the left main trunk, Cx (no. 11), posterolateral branch (no. 15PL), and right coronary artery (RCA). CABG to the LAD, RCA, and no. 14PL branch was performed, but the no. 15PL branch could not be grafted so we performed BMCI. Postoperative technetium (99mTc) scintigraphy showed an improved perfusion image in the posterolateral region 1 month after the treatment.

Case 5
A 73-year-old woman underwent a preoperative coronary angiogram, which showed significant stenosis in the LAD, Cx, and RCA. There was no graftable coronary artery in the Cx area (Fig 1A), so we performed BMCI to the entire Cx. Postoperative 201Tl scintigraphy showed improved perfusion image in the Cx area 1 month after the treatment (Fig 1B).

The scintigraphic improvements of blood flow in the BMCI-treated areas were confirmed 1 year after surgery in all 3 patients.

The mechanism of BMCI in combination with CABG is extremely complex. Based on our studies of animal ischemic models, the improvement of blood flow in the BMCI-treated area can be attributed partly to the endothelial differentiation and the production of angiogenic factors in the local implanted BMC.

All the patients given this treatment had an uneventful postoperative course without any complications. The white blood cell counts, and serum C-reactive protein, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, blood urea nitrogen, creatinine, creatine kinase (CK), and CK-MB levels in the 5 BMCI patients did not significantly differ from those in 30 control patients who underwent standard CABG alone during the same period. There was no new-onset arrhythmia or any significant bleeding in the BMCI patients. Moreover, no significant changes were observed in the postoperative ECG recorded during the acute phase, and the Holter electrocardiogram showed a normal pattern in these patients 1 year after treatment. Echocardiography did not reveal any evidence of

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**Table 1** Bone Marrow Cell Implantation (BMCI) in the 5 Patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Sites of CABG</th>
<th>Area of BMCI</th>
<th>No. of cells/point (points)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>67</td>
<td>LAD, D1, 14PL</td>
<td>15PL</td>
<td>5x10^9 (6 points)</td>
<td>Improvement</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>68</td>
<td>LAD, OM1, OM2</td>
<td>14PL</td>
<td>5.6x10^9 (11 points)</td>
<td>Inestimable</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>59</td>
<td>LAD, 14PL, 4PD</td>
<td>15PL</td>
<td>1x10^9 (5 points)</td>
<td>Improvement</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>61</td>
<td>LAD, OM, 4PD, 4PL</td>
<td>D1</td>
<td>1x10^9 (10 points)</td>
<td>Inestimable</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>73</td>
<td>LAD, D1, 4PD, 4PL</td>
<td>Cx</td>
<td>1x10^9 (22 points)</td>
<td>Improvement</td>
</tr>
</tbody>
</table>

CABG, coronary artery bypass grafting; LAD, left anterior descending artery; Cx, circumflex artery; D1, first diagonal branch; PL, posterolateral branch; PD, posterorderscending branch; OM, obtuse marginal branch.

**Fig 1.** Coronary angiogram (A) and 201Tl scintigram (B) of case 5. BMCI was carried out for the entire circumflex artery. Improved perfusion can be seen on the postoperative 201Tl scintigram, done 1 month after surgery. Arrows indicate the area of bone marrow cell implantation (A) and the area of improved perfusion (B). Ant, anterior; Sep, septal; Lat, lateral; Post, posterior.
calcification or teratogenic tumours in the BMCI area 1 month or 1 year after treatment. Computed tomography examination was also performed in 4 of the 5 patients 1 year after treatment, and no local calcification was found in the treated myocardium.

Based on these clinical outcomes and our safety analysis in dog hearts, we believe that BMCI is a safe and effective new treatment for ischemic heart disease. Although BMCI induces angiogenesis and increases collateral blood flow, the amount of blood flow to the tissue would be less than that achieved by bypass grafting; however, until now there have not been any treatment options for ungraftable areas and BMCI provides a viable treatment option for such patients. We will continue our clinical trial, which will include 5–10 more patients, and will re-evaluate our data.

Acknowledgment

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References