Autologous Bone-Marrow Mononuclear Cell Implantation Improves Endothelium-Dependent Vasodilation in Patients With Limb Ischemia

Yukihito Higashi, MD; Masashi Kimura, MD; Keiko Harasawa, MD; Kensuke Noma, MD; Daisuke Jitsuiki, MD; Keigo Nakagawa, MD; Tetsuya Oshima, MD; Kazuaki Chayama, MD; Taijiro Sueda, MD; Chikara Goto, RTP, MS; Hiroaki Matsubara, MD; Toyoaki Murohara, MD; Masao Yoshizumi, MD

Background—Patients with limb ischemia were associated with endothelial dysfunction. The purpose of this study was to determine whether autologous bone-marrow mononuclear cell (BM-MNC) implantation improves endothelial dysfunction in patients with limb ischemia.

Methods and Results—We evaluated the leg blood flow (LBF) response to acetylcholine (ACh), an endothelium-dependent vasodilator, and sodium nitroprusside (SNP), an endothelium-independent vasodilator, before and after BM-MNC implantation in 7 patients with limb ischemia. LBF was measured with a mercury-filled Silastic strain-gauge plethysmograph. The number of BM-MNCs implanted into ischemic limbs was 1.6×10⁷±0.3×10⁷. The number of CD34⁺ cells included in the implanted BM-MNCs was 3.8×10⁷±1.6×10⁷. BM-MNC implantation improved the ankle-brachial pressure index (0.33±0.21 to 0.39±0.17, \( P = 0.06 \)), transcutaneous oxygen pressure (28.4±11.5 to 36.6±5.2 mm Hg, \( P = 0.03 \)), and pain-free walking time (0.8±0.6 to 2.9±2.2 minutes, \( P = 0.02 \)). After BM-MNC implantation, LBF response to ACh was enhanced (19.3±6.8 versus 29.6±7.1 mL/min per 100 mL; \( P = 0.002 \)). The vasodilatory effect of SNP was similar before and after BM-MNC implantation.

Conclusions—These findings suggest that BM-MNC implantation augments endothelium-dependent vasodilation in patients with limb ischemia. (Circulation. 2004;109:1215-1218.)

Key Words: angiogenesis • cells • endothelium • ischemia

Recent studies have shown that bone-marrow mononuclear cell (BM-MNC) implantation increases collateral vessel formation in both ischemic limb models and patients with limb ischemia.¹ ² However, it is not clear whether these collateral arteries have normal vascular function, especially endothelial function. Endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis and plays an important role in development and maintenance of atherosclerosis.³ Limb ischemia is generally associated with endothelial dysfunction.⁴ ⁵ Therefore, it is clinically important to evaluate the vascular function of collateral arteries induced by BM-MNC implantation. We hypothesized that BM-MNC implantation would improve impaired endothelial function in patients with limb ischemia.

To determine the effect of BM-MNC implantation on endothelial function in patients with limb ischemia, we evaluated endothelium-dependent vasodilation induced by acetylcholine (ACh) and endothelium-independent vasodilation induced by sodium nitroprusside (SNP) before and after BM-MNC implantation.

Methods

Subjects
Seven patients with peripheral arterial disease (6 men and 1 woman; mean age, 64±9 years) who had rest pain and nonhealing ulcers and who were not candidates for angioplasty or surgical revascularization were enrolled in this study. The diagnosis of limb ischemia was confirmed by angiography. Patients with diabetes mellitus, coronary artery disease, and history of malignant disorders were excluded. Four of the 7 patients had smoking habits, and those 4 patients stopped smoking 2 months before BM-MNC implantation. The drugs used were not changed throughout the study. Lifestyle also was regulated throughout the study. The study protocol was approved by the Ethics Committee of the Hiroshima University Graduate School of Medicine. Written informed consent for participation was obtained from all subjects.
BM-MNC Implantation

BM-MNCs were sorted and implanted in patients with limb ischemia as previously described.2

Effect of BM-MNC Implantation on Endothelial Function in Patients With Limb Ischemia

Leg vascular responses to ACh (Daiichi Pharmaceutical Co) and SNP (Malushi Pharmaceutical Co) were evaluated by use of a mercury-filled Silastic strain-gauge plethysmograph (EC-5R, D.E. Hokanson, Inc) before and at 4 weeks after BM-MNC implantation in all subjects and at 24 weeks after BM-MNC implantation in 6 of the 7 subjects. Subjects fasted for at least 12 hours before cell implantation. They were kept in the supine position in a quiet, dark, air-conditioned room (temperature, 22°C to 5°C) throughout the study. A 23-gauge polyethylene catheter was inserted into the BM-MNC–implanted femoral artery for the infusion of ACh and SNP under local anesthesia. After each patient had spent 30 minutes in the supine position, we measured leg blood flow (LBF) and arterial blood pressure. Then, the effects of the ACh and SNP infusion on leg hemodynamics were measured. ACh (7.5, 15, and 30 μg/min) and SNP (0.75, 1.5, and 3.0 μg/min) were infused intra-arterially for 5 minutes at each dose. The infusions of ACh and SNP were performed in random order. Each study proceeded after the LBF had returned to baseline.

To evaluate the drug-related effect on endothelium-dependent vasodilation, the infusion of ACh and SNP was performed using a protocol identical to that used for the study of limb ischemic patients with implanted MN-MNCs before and after 4 weeks of follow-up in 5 patients with limb ischemia (4 men and 1 woman; mean age, 65±7 years) as a control group. Five patients were taking ACE inhibitors and antiplatelet agents; 3 of those 5 patients were taking calcium antagonists, and 2 were taking statins. The patients were subjected to 4 weeks of follow-up without any drug treatment or lifestyle modification.

Measurement of LBF

The blood flow was measured using a mercury-filled Silastic strain-gauge plethysmograph (EC-5R, D.E. Hokanson, Inc) as previously described.6,7

Statistical Analysis

Results are presented as the mean±SD. All reported probability values were 2-tailed. Values of P<0.05 were considered significant. Comparisons of parameters before and after BM-MNC implantation were performed with adjusted means by ANCOVA using baseline data as covariates. Comparisons of time-course curves of parameters during the infusions of ACh and SNP were analyzed by 2-way ANOVA for repeated measures on 1 factor followed by the Bonferroni correction for multiple-paired comparisons.
Results

Clinical Characteristics
The baseline clinical characteristics before and at 4 weeks and 24 weeks after BM-MNC implantation of patients with limb ischemia are summarized in the Table. The number of BM-MNCs implanted into ischemic limbs was $1.6 \times 10^9 \pm 0.3 \times 10^9$. The number of CD34+ cells included in the implanted BM-MNCs was $3.8 \times 10^8 \pm 1.6 \times 10^7$. BM-MNC implantation improved the ankle-brachial pressure index from $0.33 \pm 0.21$ to $0.39 \pm 0.17$ after 4 weeks ($P = 0.06$) and to $0.35 \pm 0.38$ after 24 weeks ($P = 0.16$), transcutaneous oxygen pressure from $28.4 \pm 11.5$ to $36.6 \pm 5.2$ mm Hg after 4 weeks ($P = 0.03$) and to $33.7 \pm 3.8$ mm Hg after 24 weeks ($P = 0.06$), pain-free walking time from $0.8 \pm 0.6$ to $2.9 \pm 2.2$ minutes after 4 weeks ($P = 0.02$) and to $2.5 \pm 1.6$ minutes after 24 weeks ($P = 0.03$), and basal LBF from $1.7 \pm 1.2$ to $2.4 \pm 1.4$ mL/min per 100 mL tissue after 4 weeks ($P = 0.04$) and to $2.0 \pm 1.2$ mL/min per 100 mL tissue after 24 weeks ($P = 0.05$).

BM-MNC implantation did not alter blood pressures (mean blood pressure, from $86.2 \pm 10.3$ to $88.1 \pm 11.2$ mm Hg after 4 weeks and to $87.3 \pm 12.1$ mm Hg after 24 weeks) or serum concentrations of total cholesterol (from $5.28 \pm 1.24$ to $5.22 \pm 1.06$ mmol/L after 4 weeks and to $5.23 \pm 1.18$ mmol/L after 24 weeks), LDL cholesterol (from $3.88 \pm 0.78$ to $3.78 \pm 0.72$ mmol/L after 4 weeks and to $3.72 \pm 0.81$ mmol/L after 24 weeks), glucose (from $4.6 \pm 0.4$ to $4.5 \pm 0.5$ mmol/L after 4 weeks and to $4.6 \pm 0.6$ mmol/L after 24 weeks), and insulin (from $41.8 \pm 9.8$ to $42.3 \pm 10.1$ pmol/L after 4 weeks and to $43.6 \pm 11.4$ pmol/L after 24 weeks).

Effect of BM-MNC Implantation on Endothelial Function in Patients With Limb Ischemia
The intra-arterial infusion of ACh increased LBF in a dose-dependent manner. After BM-MNC implantation, LBF responses to ACh were enhanced in patients with limb ischemia (Figure, top). There was no significant difference in LBF response to ACh after 4 weeks and 24 weeks of follow-up (Figure, top). The intra-arterial infusion of SNP also increased LBF in a dose-dependent manner. The LBF response to SNP was unaffected by BM-MNC implantation (Figure, bottom). In the control group, there was no significant difference in LBF responses to ACh and SNP before and those after 4 weeks and 24 weeks of follow-up (Figure). No significant change was observed in arterial blood pressure or heart rate in response to intra-arterial infusion of either ACh or SNP before or after BM-MNC implantation and after 4 weeks of follow-up.

Discussion
In the present study, BM-MNC implantation improved not only limb ischemic symptoms and findings of angiography but also endothelium-dependent vasodilation in patients with limb ischemia. This beneficial effect of BM-MNC implantation on vascular function may be selective in endothelium-dependent vasodilation (endothelial cell function) but not in endothelium-independent vasodilation (smooth muscle cell function).

Our results showed that BM-MNC implantation increased the ankle-brachial pressure index, transcutaneous oxygen pressure, and basal LBF per se. Therefore, one possible mechanism by which BM-MNC implantation augments endothelium-dependent vasodilation is by increasing shear stress results from blood flow. Acute or chronic increases in shear stress stimulate the release of nitric oxide in isolated vessels and cultured cells through the enhanced expression of endothelial nitric oxide synthase gene.8,9

BM-MNCs (CD34+ fraction) include endothelial progenitor cells and various angiogenic growth factors, such as the vascular endothelial growth factor (VEGF) and angiopeptin families. Supplementation of the progenitor endothelial cells results in augmentation of neovascularization of ischemic tissue and repair of mature endothelial cells that release nitric oxide.10 VEGF induces the formation of collateral vessels and increases collateral blood flow, leading to improvement in endothelium-dependent vasodilation.11 In addition, VEGF directly upregulates endothelial nitric oxide synthase expression and increases subsequent nitric oxide release.12 Rajagopalan et al13 recently reported that gene therapy using an adenoviral vector encoding a 121-amino-acid isoform of VEGF augmented ACh-induced vasodilation in lower-leg circulation in patients with peripheral arterial disease. Although the mechanism by which BM-MNC implantation improves endothelial function in patients with limb ischemia is not clear, the multiplier effect of progenitor endothelial cells and VEGF may contribute to the angiogenesis-induced improvement in endothelium-dependent vasodilation.
We have recently shown that antihypertensive agents, such as ACE inhibitors, restore endothelial function in patients with mild to moderate hypertension but not in patients with severe hypertension. It is clinically important that endothelial dysfunction is reversible by BM-MNC implantation in patients with severe atherosclerosis. BM-MNC implantation is expected to prevent the development of atherosclerosis through improvement in endothelial function.

Although a drastic change in endothelial function was observed after BM-MNC implantation, the number of subjects in this study was small, and the observation period is relatively short. In addition, this phase 1 clinical trial was not placebo-controlled. Controlled studies using a large population of patients and with long observation periods are needed to determine the role of BM-MNC implantation in endothelial function in patients with severe atherosclerosis.

Although the effectiveness of therapeutic angiogenesis with VEGF gene therapy in patients with peripheral arterial diseases has been established, BM-MNC implantation therapy may provide a new aspect of therapeutic angiogenesis in such patients.

References

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