Bull Yamaguchi Med Sch 48(3-4):29-33, 2001

A Simple and Safe Method of Inducing Therapeutic Angiogenesis by the Implantation of Autologaus Bone Marrow Cells.

Toshiro Kobayashi

First Department of Surgery
Yamaguchi University School of Medicine, 1-1-1 Minamikogushi, Ube, Yamaguchi 755-8505, Japan.
(Received October 10, 2001, revised January 7, 2002)

Key words:angiogenesis, cell, ischemia

Introduction

Angiogenesis was induced by the proliferation and migration of endothelial cells from existing vessels in adults[1], and has recently been developed as a method of treating severe ischemic disease. Angiogenic growth factors such as vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF) play an important role in the angiogenic process and promote the proliferation and migration of endothelial cells.[2,3] They are utilized to promote collateral circulation and the various routes of administration, and forms of growth factors have been devised to induce a better angiogenic effect. Clinical trials have revealed good results following the direct injection of naked plasmid DNA encoding human VEGF or recombinant growth factors [4-7]; however, the effects of these treatments are unstable, and their performance is limited by high costs or difficult clinical techniques. Bone marrow contains many kinds of immature cells that can differentiate into hematopoietic cells and endothelial progenitor cells. A subset of CD34 positive cells derived from bone marrow has the capacity to differentiate into endothelial cells in vitro and be induced into the inner surface of small vessels *in vivo*.[8] Moreover, bone marrow cells have been shown to secrete an angiogenic growth factor such as bFGF and promote endothelialization of the luminal surface of a vascular prosthesis.[9,10] These reports indicate that bone marrow cells could be effective material for inducing angiogenesis. We selected bone marrow cells as the material for inducing angiogenesis because they contain endothelial progenitor cells that can participate in vascular formation in severe ischemic lesions, and are able to secrete a variety of growth factors such as VEGF and bFGF. We tried to develop a new form of therapy to induce angiogenesis by bone marrow cells implantation (BMCI).

First, we investigated whether bone marrow cells have the ability to secrete angiogenic growth factors and the capacity to induce angiogenesis.[11] These experiments proved that whole bone marrow cells without red blood cells have the ability to secrete growth factors such as VEGF and bFGF immunohistocemically, and the production of VEGF was observed by measurement of tissue culture supernatant, especially under hypoxic conditions. In a rat cornea model, the implantation of bone marrow cells created new vessels.

These findings indicated that bone marrow cells could be an effective material for inducing angiogenesis.

In the next experiments, we examined the angiogenic effect of bone marrow cells in animal ischemic experimental models, namely, a rat ischemic hindlimb model, and rat and canine myocardial infarction models.[12–14] The experiments using the rat ischemic hindlimb model [12] revealed that bone marrow cells implanted into ischemic muscle induced angiogenesis, which was confirmed by histoligical evaluation and microangiogram. Laser Doppler imaging and nonradioactive colored microspheres showed that ischemic tissue blood flow was significantly increased after BMCI. The BMCI treatment induced effective angiogenesis in the ischemic model by two mechanisms; one involved the local elevation of angiogenic growth factors derived from implanted bone marrow cells, and other was that he implanted bone marrow cells were differentiated in endothelial cells and incorporated into the vasculature. The angiogenic growth factors contained in the ischemic tissue were measured by enzyme-linked immunosorbent assay (ELISA), and the condition of ischemia caused an elevation in the level of bFGF in the ischemic muscle. Interleukine 1-beta from the im planted bone marrow cells also contributed to angiogenesis. Inflammatory cytokines like interleukine 1-beta and interleukine 8 caused effective angiogenesis *in vitro* and *in vivo*.[15–18] Our data showed that angiogenesis induced by BMCI treatment was mainly caused by inflammatory cytokines such as interleukine 1-beta derived from bone marrow cells. Some implanted bone marrow cells were positively immunostained with CD31 and vascular endothelial-cadherin (VE- cadherin); markers which are expressed on endothelial cells. These findings show that BMCI treatment induced effective angiogenesis, and can improve deteriorated physiological function. The running time on a motor-driven treadmill was used to represent exercise capacity.[13] Rats with ischemic hindlimbs treated with BMCI could run approximately 1.5 times longer than control rats, demonstrating that BMCI treatment can improve

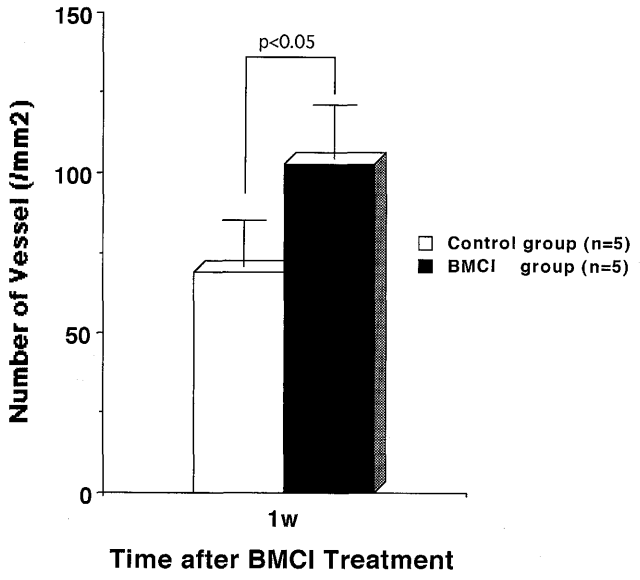

Fig. 1 Effect of the implantation of bone marrow cells in the rat myocardial infarction model. Light microscopic analysis of the vessel count in the restricted infarcted myocardium per 1 mm2 is shown in this figure. The number of vessels in the BMCI group was significantly greater than that in the control group, 1 week after BMCI treatment.
(□) control group, (■) BMCI group

deteriorated exercise capacity in a rat ischemic hindlimb model.

In the rat and canine myocardial infarction models, BMCI treatment improved angiogenesis[14] and improved local cardiac wall motion (unpublished data). In the rat model, BMCI treatment was found to have induced effective angiogenesis according to histological evaluation of the microvessel count. The mechanism responsible was local elevation of the inflammatory cytokines, interleukine 1-beta and interleukine 8 like chemokine cytokine-induced neutrophil chemoattractant (CINC), but not of angiogenic growth factors such as VEGF and bFGF. These cytokines were released from implanted bone marrow cells because these cells were positively stained with interleukine 1-beta. In the canine model, locally implanted bone marrow cells induced angiogenesis and improved percentage of local wall thickening (%WT) in marginal areas which exist between the normal and infarction areas. A hemodynamic study evaluated by echocardiography did not show any significant improvement by BMCI treatment. Systemic and local toxicity following local bone marrow cell implantation was evaluated by electrocardiogram, echocardiography, blood serum examinations and histological observation. There were no significant changes in electrocardiogram, cardiac function, or local wall motion observed by echocardiography, or in the serum levels of WBC, GPT, GOT, CRE, BUN, LDH, CK, or CK-MB. No significant histological changes were observed in the hematoxilin-eosin stained specimen from local myocardium after BMCI treatment, such as fibrosis, calcification, blood cell production, or inflammatory cell migrations.

These data obtained from experimental animal models indicated that BMCI treatment induces effective therapeutic angiogenesis. On the basis of our these data, a clinical trial was commenced in 1999.[19] Patients with severe ischemic heart disease have been given this new treatment concomitant with coronary artery bypass grafting. Autologous BMC were implanted into ungraftable areas. Five patients underwent this treatment, these of whom showed specific improvement in coronary perfusion postoperatively, by cardiac scintigraphy. Postoperative chest radiography, electrocardiography, echocardiography, and blood tests did not reveal any detrimental changes.

The implantation of self-bone marrow cells is a simple method for inducing angiogenesis without causing toxicity or immunological rejection, in comparison with other therapeutic methods of angiogenesis such as by using human recombinant protein, naked plasmid DNA, and viruses. Further investigations on BMCI treatment are required to clarify the optimal populations of whole bone marrow cells that will give the most forceful angiogenic potency, and to determine whether BMCI will induce stronger angiogenesis in an ischemic environment.

In summary, the results of the present study demonstrated that bone marrow cell implantation (BMCI) improved angiogenesis in animal experimental models and clinical trials, the mechanism of which seem to be related to the increase in some inflammatory cytokines, and the differentiation of bone marrow cells into endothelial cells. Our results clearly demonstrated that BMCI is a novel and simple method of inducing therapeutic angiogenesis for ischemic diseases.

References

1) Risau, W. : Mechanisms of angiogenesis. *Nature*, **386**:671-674, 1997.
2) Ferrara, N., and Davis-Smyth, T. : The biology of vascular endothelial growth factor. *Endocr.Rev*, **18**:4-25, 1997.
3) Bikfalvi, A., Klein, S., Pintucci, G., and Rifkin, D. B. : Biological roles of fibroblast growth factor-2. *Endocr Rev*, **18**:26-45, 1997.
4) Schumacher, B., Pecher, P., von Specht, B. U., and Stegman, T. : Induction of neoangiogenesis in ischemic

myocardium by human growth factors: first clinical results of a new treatment of coronary heart disease. *Circulation*, **97**:645-650, 1998.

5) Losordo, D. W., Vale, P. R., Symes, J. F., Dunnington, C. H., Esakof D. D., Maysky, M., Ashare A. B., Lathi, K., and Isner, J. M. : Gene therapy for myocardial angiogenesis: initial clinical results with direct myocardial injection of phVEGF165 as sole therapy for myocardial ischemia. *Circulation*, **98**:2800-2804, 1998.

6) Sellke, F. W., Laham, R. J., Edelman, E. R., Pearlman, J. D., and Simons, M. : Therapeutic angiogenesis with basic fibroblast growth factor: technique and early results. *Ann Thorac Surg*, **65**:1540-1544, 1998.

7) Baumgartner, I., Pieczek, A., Manor, O., Blair, R., Kearney, M., Walsh, K., and Isner, J. M. : Constitutive expression of phVEGF165 after intramuscular gene transfer promotes collateral vessel development in patient with critical limb ischemia. *Circulation*, **97**:1114-1123, 1998.

8) Asahara, T., Murohara, T., Sullivan, A., Silver, M., van der Zee, R., Li, T., Witzenbichler, B., Schatteman, G., and Isner, J. M. : Isolation of putative progenitor endothelial cells for angiogenesis. *Science*, **275**:964-967, 1997.

9) Shi, Q., Rafii, S., Wu, M. H., Wijelath, E. S., Yu, C., Ishida, A., Fujita, Y., Kothari, S., Mohle, R., Sauvage, L. R., Moore, M. A., Storb, R. F., and Hammond, W. P. : Evidence for circulating bone marrow-derived endothelial cells. *Blood*, **92**:362-367, 1998.

10) Noishiki, Y., Tomizawa, Y., Yamane, Y., and Matsumoto, A. Autocrine angiogenic vascular prosthesis with bone marrow transplantation. *Nat Med*, **2**:90-93, 1996.

11) Hamano, K., Li, T. S., Kobayashi, T., Kobayashi, S., Matsuzaki, M., and Esato, K. : Angiogenesis induced by the implantation of self-bone marrow cells: a new material for therapeutic angiogenesis. *Cell Transplant*, **9**:439-443, 2000.

12) Hamano, K., Li, T. S., Kobayashi, T., Tanaka, N., Kobayashi, S., Matsuzaki, M., and Esato, K. : The induction of angiogenesis by the implantation of autologous bone marrow cell: a novel and simple therapeutic method. *Surgery*, **30**:22-54, 2001.

13) Ikenaga, S., Hamano, K., Nishida, M., Kobayashi, T., Li, T. S., Kobayashi, S., Matsuzaki, M., Zempo, N., and Esato, K. : Autologous bone marrow implantation induced angiogenesis and improved deteriorated exercise capacity in a rat ischemic hindlimb model. *J Surg Res*, **96**:277-283, 2001.

14) Kobayashi, T., Hamano, K., Li, T. S., Katoh, T., Kobayashi, S., Matsuzaki, M., and Esato, K. : Enhancement of angiogenesis by the implantation of self bone marrow cells in a rat ischemicheart model. *J Surg Res*, **89**:189-195, 2000.

15) Prendergast, R. A., Lutty, G. A., and Dinarello, C. A. : Interleukin-1 induces corneal vascularization. *Invest Opthalmol Vis Sci*, **28**:100. Abstract, 1987.

16) Giulian, D., Woodward, J., Youg, D. G., Krebs, J. F., and Lachman, L. B. : Interleukin-1 injected into mammalian brain stimulates astrogliosis and neovascularization. *J Neurosci*, **8**: 2485-2490, 1998.

17) Nakagawa, H., Komorita, N., Shibata, F., Ikesue, A., Konishi, K., Fujioka, M., and Kato, H. : Identification cytokine-induced neutrophil chemoattractants (CINC), rat GRO/CINC-2 alpha and CINC-2 beta, produced by granulation tissue in culture: purification, complete amino acid sequences and characterization. *Biochem J*, **301**: 545-550, 1994.

18) Yoshida, A., Yoshida, S., Hata, Y., Khalil, A. K., Ishibashi, T., and Inomata, H. The role of NF-KappaB in retinal neovascularization in the rat. Possible involvement of cytokine-induced neutrophil chemoat-

tractant (CINC), a member of the interleukin-8 family. *J Histochem Cytochem*, **46**:429-436, 1998.

19) Hamano, K., Nishida, M., Hirata, K., Mikamo, A., Li, T. S., Harada, M., Miura, T., Matsuzaki, M., and Esato, K. : Local implantation of autologous bone marrow cells for therapeutic angiogenesis in patients with ischemic heart disease: clinical trial and preliminary results. *Jpn Circ* J, **65**:845-7, 2001.