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Review Article

Bone marrow stem cell injection for the treatment of critical limb ischemia

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Background

Vascular reconstruction remains a treatment of choice for critical limb ischemia. Bypass surgery has been accepted as the most effective therapy to achieve increase of blood flow and cure the ischemic symptoms. On the other hand, continuous increase of the patients suffering diabetes mellitus enhances the increase of the number of the patients with peripheral arterial disease (PAD). This tendency is also observed in Japan and expected to continue from now on. The reports of the Ministry of Health, Labor and Welfare of Japan indicated that more than 6% of population was treated for diabetes mellitus and more than 12% of population was supposed to be diabetic in 2002.[1] This report also gave a warning for rapid and continuous increase of the diabetic patients at present and in the future. As is well known, diabetes mellitus is one of the strong risk factors to cause an arteriosclerosis obliterans (ASO). Therefore, the patients with PAD will increase continuously and more severe ischemic limb will need to be treated. Moreover, diabetic macroangiopathy is characterized as showing diffuse stenosis and occlusion down to the foot arteries[2.] Monckeberg's medial calcification is also frequently observed in the ASO with diabetes mellitus[3].Therefore, along with the increase of the patients with PAD, the vascular reconstruction becomes technically demanding due to co-morbid diabetes with diffuse lesion and severe calcification. The patients, who show critical limb ischemia but are excluded from the operative candidate, are eventually performed major amputation. To prevent this disastrous sequel, therapeutic angiogenesis has been investigated.

In 1996, Isner reported a new therapy to treat ischemic limb by using angiogenetic gene; vascular endothelial growth factor (VEGF) [4]. Since then, angiogenetic therapy started to be investigated by using gene transfer with VEGF, fibroblast growth factor (FGF)[5] or hepatic growth factor (HGF)[6].Animal study showed increase of the vascular network, disappearance of local coldness and recover of limb function.[7,8] Human study also started by using these genes. Major concern to use gene transfer is possible infection or carcinogenic effect via adenovirus which is used as a vector. To avoid this problem, plasmid is also used although the efficacy of transfer is less. The human application revealed that skin temperature increased, pain was cured and vascular network seemed to increase in

angiography. However, the long-term effect remains undetermined.

Bone marrow monocyte injection

In place of gene transfer, bone marrow stem cell has been studied whether causing angiogenesis or vasculogenesis. Angiogenesis, which means development from capillary endothelial cells, has been considered as a main cause to increase blood vessels in the adults. On the other hand, angioblast or endothelial progenitor cells (EPC), which differentiates to the endothelial cells, enhances vasculogenesis in the embryogenic stage. These cells surround hematopoietic cells and form a blood island, then develop to blood vessels in the embryo[9]. Bone marrow monocytes were reported to include these cells even in the adults. Therefore, vasculogenesis, the development of vascular network independent to capillaries, could occur in the adults[10]. They also secrete angiogenetic cytokines such as VEGF or FGF[11].From these observations, injection of the monocytes collected from the bone marrow was expected to cause vasculogenesis and angiogenesis in the ischemic limb. The animal experiment using EPC demonstrated occurrence of angiogenesis and development of collateral flow in the ischemic hind limb of the rats[12]. The advantages using bone marrow monocytes are that infection or immune reaction can not occur different from the technique using gene transfer. The disadvantage is necessity to collect bone marrow blood with a volume of 500 to 600ml under general anesthesia. The EPC and angioblast also exists in the peripheral blood, so they could be collected by using apheresis[13]. However, the numbers of these cells are much lower than in the bone marrow and the efficacy of collection is less. To investigate the effects to human ischemic limb by bone marrow monocyte injection, Japan Trial for Therapeutic Angiogenesis Using Cell Transplantation (J-TACT) started since 2000[14]. Our institute also started therapeutic angiogenesis to use bone marrow monocytes since 2004, independently.

Methods

The patients who are scheduled to be treated by angiogenetic therapy using bone marrow monocyte are fully consent before treatment. Those who show critical ischemia in the extremities and are diagnosed as inoperable are candidates for this therapy. The angiography is performed to clarify the vascular network in the ischemic limb. The patients are also examined for cancer or diabetic retinopathy to exclude adverse side effects. Those who have these diseases or other severe morbidity are excluded because this treatment may worsen the disease. After the ethical committee of each institute agrees, angiogenetic therapy starts. The bone marrow blood is collected from the iliac bone of the patient under general anesthesia. Usually the necessary volume of the collected blood is from 500ml to 600ml. Then this blood is transferred to the laboratory, and monocytes are collected and purified from the bone marrow blood. Usually 1.0x109 of monocytes is obtained. These monocytes are divided to small syringes, and then injected to the muscles of ischemic limb.

Results

The results of J-TACT demonstrated the increase of ankle brachial pressure index (ABPI), increase of walking distance with tread-mill and relief from pain with significant difference in comparison with the control group.14) ABPI increased 0.097 in the injection group and 0.024 in the control group. Walking distance increased to 2.6 times and pain relief was observed in 90% of the study group. However, in the midterm at 2 years, these parameters decreased again to the pretreatment value. Therefore, the effects may discontinue at least in 2 years and the long-term results remained undetermined. In our institute, 4 patients were treated with bone marrow-derived monocyte injection as a therapeutic angiogenesis; 3 patients are effective and 1 patient is not effective in the short term. The all effective cases were operated for inflow reconstruction and

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received concomitantly monocyte injection to the area of non-operative below-knee lesion. As the increase of ABPI after therapeutic angiogenesis was slight, the combination of the angiogenetic therapy and concomitant inflow reconstruction seemed to be effective from these clinical experiences.

At present and in the future

As the results showed, therapeutic angiogenesis using bone marrow monocytes seemed to be effective in the short-term. The pain relief and prolongation of walking distance are impressive, while increase of ABPI is slight. Angiography after monocyte injection seemed to show the increase of the vascularity in the ischemic leg, but it is unclear whether it is significant or not because of the difficulties of quantification of angiographic demonstration. With regard to pain, relief does not necessarily mean the increase of blood flow. Cytokines secreted from monocytes may lower the threshold of the pain through direct effect to the nerve. The continuation of the effects by bone marrow monocyte injection remains unclear especially in the long-term. Theoretically, the vasculogenesis around the injection field *does* occur. However, the conjunction from the newly formed vessels to the inflow artery is necessary for the certain increase of the blood flow. It has not been proven yet whether this phenomenon occurs regularly or not by angiogenetic therapy. Otherwise, the formation of the new vessels may be temporary phenomena, similar to inflammation.

The increase of the blood flow after bypass surgery has been recognized as the most effective treatment for the ischemic leg. The vascluogenetic therapy by the bone marrow monocyte injection could not achieve as much effects as vascular reconstructive surgery. Therefore, the vasculogenetic therapy alone is considered not to increase the blood flow enough to cure the severe ischemia completely. The combination of inflow reconstruction by the bypass surgery and therapeutic angiogenesis to the inoperative distal area would be a realistic application at present. The future investigation should focus on how to connect the new vessels formed by vasculogenesis to the inflow artery; the development from vasculogenesis to arteriogenesis. Otherwise, the long-term effect of the present vasculogenetic treatment could not prove the clinical value, whether the gene transfer or bone marrow stem cell injection is applied.

References

1. Reports of surveillance of diabetes mellitus in Japan in 2002. The reports of the Ministry of Health, Labor and Welfare of Japan, 2004.

2. Joseph WS, LeFrock JL. The pathogenesis of diabetic foot infections-immunopathy, angiopathy, and neuropathy. J Foot Surg 26;S7-11, 1987.

3. Ballota E, Renon L, Toffano M, et al. Patency and limb salvage rates after distal revascularization to unclampable calcified out flow arteries.? J Vasc Surg 39;539-546, 2004.

4. Isner JM, Pieczek A, Schainfeld R, et al. Clinical evidence of angiogenesis after arterial gene transfer of phVEGF165 in patients with ischaemic limb. Lancet 348;370374, 1996.

5. Masaki I, Yonemitsu Y, Yamashita A, et al. Angiogenetic gene therapy for experimental critical lomb ischemia; acceleration of limb loss by overexpression of vascular endothelial growth factor 165 but not of fibroblast growth factor-2. Circ Res 90;966-973, 2002.

6. Morishita R, Aoki M, Hashiya N, et al. Safety evaluation of clinical gene therapy using hepatocyte growth factor to treat peripheral arterial disease. Hypertension 44;203-209, 2004.

7.Takeshita S, Zheng LP, Brogi E, et al. Therapeutic angiogenesis. A single intraarterial bolus of vascular endothelial growth factor augments revascularization in a rabbit ischemic hind limb model. J Clin Invst 93;662-670, 1994.

8. Banai S, Jaklitsch MT, Shou M, et al. Angiogenic-induced enhancement of collateral blood flow to ischemic myocardium by vascular endothelial growth factor in dogs. Circulation 89;2183-2189, 1994.

9. Risau W.Mechanism of angiogenesis. Nature 386;671-674, 1997.

JSRM/ 003010200004/Nov 14, 2007.

10.Shintani S, Murohara T, Ikeda H, et al. Augmentation of postnatal neovascularization with autologous bone marrow transplantation. Circulation 103;897-903, 2001.

11.Rehman J, Li J, Orschell CM, et al. Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. Circulation 107;1164-1169, 2003.

12. Murohara T, Ikeda H, Duan J, et al. Transplanted cord blood-derived endothelial precursor cells augment postnatal neovascularization. J Clin Invest 105;1527-1536, 2000.

13. Minamino T, Toko H, Tateno K, et al. Peropheral-blood or bone-marrow mononuclear cells for therapeutic angiogenesis Lancet 360;427-435, 2002.

14. Tateishi-Yuyama E, Matsubara H, Murohara T, et al. Therapeutic angiogenesis for patients with limb ischemia by autologous transplantation of bone-marrow cells; a pilot study and a randomized controlled trial. Lancet 360;427-435, 2002.

JSRM/ 003010200004/Nov 14, 2007.