ISCHEMIC HEART DISEASE

Ischemic heart disease causes reversible or irreversible myocardial injury when the blood flow to myocardium is decreased or stopped by stenosis or blockade and may be acute or chronic. Treatment has evolved to include various methods, such as percutaneous catheter intervention (PCI) and coronary artery bypass grafting (CABG) with good results. However, these techniques also have their limitations and many patients with ischemic heart disease continue to have serious ischemia despite optimal therapy (Table 1).

Review

Angiogenic Strategy for Human Ischemic Heart Disease

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Abstract: The number of severe ischemic heart disease or idiopathic dilated cardiomyopathy patients who need heart transplant is expected to increase every year. However, the shortage of heart donors is severe, and alternatives to organ transplant are urgently needed. A variety of therapeutic approaches have been attempted, and one of the most promising is regenerative angiogenic therapy. Angiogenic therapy is the stimulation of blood vessel growth in ischemic disease. The therapy for ischemic disease has developed rapidly in the 10 years. I review Angiogenic strategies for ischemic disease include angiogenic protein or gene administration, bone marrow mononuclear cells implantation, peripheral blood endothelial progenitor cells implantation, and hematogenous cytokine administration. And as conclusions, I think that the use of angiogenic therapy for ischemic heart disease using gene therapy, cell transplantation, and hematogenous cytokine administration will benefit increasing numbers of patients. However, long-term studies monitoring side-effects to establish safety and effectiveness are required. The angiogenic therapy is a promising medical development in the treatment of ischemia that will likely improve the quality of life in patients who have severe ischemic heart disease.

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ischemic heart disease or idiopathic dilated cardiomyopathy (DCM), for which PCI and CABG are effective, and the only treatment is a heart transplant. The number of these patients is expected to increase by 50,000 or more per year. However, the shortage of heart donors is severe, and alternatives to organ transplant are urgently needed. A variety of therapeutic approaches have been attempted, and one of the most promising is regenerative angiogenesis.

**Regenerative Therapy**

Regenerative therapy using human embryonic stem cells (ESCs) was first developed at the University of Wisconsin in 1998. Almost simultaneously, it was reported that endothelial progenitor cells (EPCs) existed in peripheral blood, and the proteins and genes for vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and hepatocyte growth factor (HGF) which stimulate vascular endothelial cells proliferation were identified. Moreover, it was reported that a myocardial cell can specialize not only from interstitial cells in bone marrow but from hematogenous stem cells. Angiogenic treatment using the protein, gene, and cells for no-option ischemic disease has already been tried in some centers.

**Neoangiogenesis**

The word neovascularization is often used in the same general sense as angiogenesis. Neovascularization encompasses two types of new vessels: one from vasculogenesis, in which completely new vessels are generated from angioblasts or endothelial progenitor cells (Fig. 1), and another, more narrow sense, by angiogenesis, in which endothelial cells migrate from existing vessels to areas with few vessels and then multiply (Fig. 2). Usually, when ischemia develops gradually, both angiogenesis and vasculogenesis contribute to collateralization. Factors, such as VEGF, HGF, bFGF, and Angiopoietin-1 help to guide the supply of ischemic tissue with new vessels.

**Angiogenic Therapy**

Angiogenic therapy is the stimulation of blood vessel growth in ischemic disease. Experiment research on angiogenesis for ischemia started in the 1960s. In 1992, Yanagisawa-Miwa *et al.* infused bFGF into the coronary arteries of dogs and improved cardiac function after experimental myocardial infarction. Takeshita *et al.* reported in 1994 that VEGF infused into an iliac artery promoted the development of collateral vessels in an ischemic rab-

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Table 1  Characteristics of therapy, pharmacotherapy, PCI, and CABG were shown.

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<thead>
<tr>
<th>therapy</th>
<th>pharmacotherapy</th>
<th>PCI</th>
<th>CABG</th>
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<tbody>
<tr>
<td>benefits</td>
<td>* non invasive</td>
<td>* less invasive, * helpful to reduce symptom, * repeatable</td>
<td>* high patency rate, * complete revascularization</td>
</tr>
<tr>
<td>defect</td>
<td>* inadequate effect</td>
<td>* unsuitable lesion, * re-stenosis, * difficulty of complete revascularization of multi vessel disease</td>
<td>* invasive, * unrepeatable</td>
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bit hind limb. Moreover, in 1999 Morishita et al.\textsuperscript{13} reported human HGF administered into an iliac artery improved collateral vessel formation using an ischemic rabbit hind limb. Shin-tani et al.\textsuperscript{14} found in 2001 that mononuclear cells (MNCs), separated from autologous bone marrow, injected into muscle increased collateral vessel flow in a similar model.

Angiogenic therapy for ischemic disease was first performed at Tufts University in the United States in 1994. Isner et al.\textsuperscript{15} reported that administration of a plasmid vector incorporating VEGF\textsubscript{165} into arteries supplying a ischemic limb of patients suffering from arterio sclerosis.
Angiogenic therapy for ischemic disease has developed rapidly in the 10 years since its introduction. However, although in vivo experiments produced collateral vessels, some of the procedures used were not clinically appropriate. Recently angiogenic therapy for patients with ischemic disease has just been introduced. Angiogenic strategies for ischemic disease include: (I) angiogenic protein or gene administration, (II) bone marrow MNCs implantation, (III) peripheral blood EPCs implantation, and (IV) hematogenous cytokine administration.

(I) Angiogenic therapy using angiogenic protein or gene administration for ischemic heart disease

Basic research using a porcine model of chronic ischemic heart showed that the injection of the phVEGF165 gene or VEGF-2 gene into stunned (or hibernated) myocardium improved collateral vessel blood flow, and the injection of an adenovirus coded with the VEGF121 gene into stunned myocardium also increased collateral vessel flow and improved cardiac function. Although the administration of the FGF-2 protein into the coronary arteries improves myocardial ischemia in dogs and pigs, recombinant FGF-1 had no effect in a canine chronic myocardial infarction model. In the pig model, the injection of both DNA coded with FGF into stunned myocardium and adenovirus coded with FGF-5 into the coronary artery increased collateral blood flow.

Animal experiments suggest the administration of VEGF or FGF protein improves collateral blood flow in the ischemic myocardium. However, in clinical cases, these proteins had no effect. Asahara et al. administered a plasmid vector including the phVEGF165 gene to patients with severe ischemic heart disease who were not candidates for PCI or CABG into their myocardium through a small left anterior thoracotomy under transesophageal echocardiographic guidance as a phase I clinical trial. This therapy improved patients’ exercise tolerance and reduced their symptoms, demonstrating the safety and usefulness of this approach. To minimize invasions, cardiac catheterization was used to administer the VEGF-2 DNA gene into stunned myocardium in patients with severe ischemic disease, and reduced the frequency of angina attacks compared with a placebo group. A few patients have received the bFGF gene for ischemic heart disease by injection into epicardial fat during CABG in areas where no graft could be placed. The results were not unequivocal since the clinical situation was complex and not easily categorized. Still, data suggested that angina symptoms improved and myocardial blood flow increased.

Further study to enhance effectiveness is needed, particularly to prolong its effect prior to its catabolism.

Angiogenic gene therapy offers the additional benefit of avoiding the need for surgery in this group of very sick patients.

(II) Angiogenic therapy using bone marrow MNC implantation for ischemic heart disease

A few vascular endothelial stem cells exist in the bone marrow. However, MNCs and hematogenous stem cells other than vascular endothelial stem cells can synthesize and release VEGF, bFGF, and angiopoietin-1 as factors which induce the multiplication and maturation of endothelial cells. Furthermore, it has been reported that synthesis and release are enhanced under hypoxic conditions. Noishiki et al. reported in 1996 that endothelialization of an artificial blood vessel occurred after implantation.
ing bone marrow MNCs in a canine abdominal aortic replacement model. Shi et al.\textsuperscript{37} reported in 1998 that endothelial stem cells (CD\textsuperscript{34+} cells), implanted from other animals, homogenously colonized endothelial flow surfaces of vascular prostheses of the descending thoracic aorta in dogs which following whole body irradiation. It was suggested that bone marrow MNCs have the potential to differentiate into vascular endothelial cells. When using large animals, Kamihata et al.\textsuperscript{38} pointed in 2001 that autologous bone marrow MNCs were introduced into stunned myocardium through the epicardium by the open chest method, collateral blood flow was significantly increased without arrhythmia.

Based on these studies, Matsubara et al.\textsuperscript{37} reported in 2002, in a randomized double-blind control study of patients with severe ischemic limb disease (Fontaine grade III or IV) resistant to medical or surgical therapy. MNCs were separated and synthesized from autologous bone marrow and injected into the ischemic limb muscle, leading to improved ankle brachial pressure index (ABI), treadmill exercise tolerance, reduced symptoms and collateral blood flow on angiography. Moreover, there were no complications, the change to cells other than endothelial cells, inflammation, edema, or new bone formation. This is the first clinical report on angiogenic therapy for ischemic disease using cell transplantation.

In our institution, Kugiyama et al. have treated no-option severe ischemic limb ASO patients with autologous bone marrow MNCs implantation with significant improvement in symptoms and in collateral blood flow on angiography.

Fig. 3. Angiographies to the patient with ASO underwent BMCs implantation were shown. (ASO: arterio sclerosis obliterans, BMCs; bone marrow cells)
The initial clinical successes in conjunction with good results in basic animal studies, warrants further investigation into the bone marrow MNC transplantation for clinical ischemic heart disease. Hamano et al. performed autologous bone marrow MNCs transplantation to the domain inaccessible to surgical revascularization concomitantly with CABG. Although it is difficult to isolate the effect of angiogenic therapy because cell transplantation was performed simultaneously with CABG, left ventricular wall motion improved in distributions that received cell transplantation but no graft. There was no complication of angiogenic therapy in 18 months of follow-up. Matsubara et al. performed autologous bone marrow MNC transplantation via small left thoracotomy in patients who previously underwent five PCIs and two CABGs, and reported decreased angina, improved left ventricular contraction, better exercise tolerance, and no arrhythmic complications.

At present, angiogenic therapy using bone marrow MNCs is performed world-wide in patients with treatment-resistant ischemic limbs. Moreover, angiogenic therapy using autologous bone marrow MNCs injected into stunned (hibernated) myocardium is performed in several institutions, and the number of centers is increasing.

(III) Angiogenic therapy using peripheral blood EPCs

The EPC of peripheral blood origin is the CD34+, which differentiates into a vascular endothelial cell under the influence of VEGF, bFGF, and insulin-like growth factor (IGF). However, since the number of peripheral CD34+ EPCs is only 10% of that in bone marrow, angiogenic therapy is more effective when bone marrow MNCs are used rather than peripheral blood EPCs.

(IV) Angiogenic therapy using hematogenous cytokine

Takahashi et al. reported that granulocyte macrophage colony stimulating factor (GM-CSF) as one of the hematogenous cytokines that causes EPCs to migrate bone marrow into the peripheral blood. Seiler et al. infused GM-CSF into the coronary arteries of 21 patients with ischemic heart disease who were not candidates for CABG due to severity of disease. The authors reported increased collateral blood flow in a randomized double blind study. Another hematogenous cytokine, granulocyte colony stimulating factor (G-CSF) was examined for its angiogenic potential, but no beneficial effect was achieved.

SAFETY OF ANGIOGENIC THERAPY

Theoretically angiogenic factors may not only increase the number of collateral vessels (neovascularization) in ischemic areas but may also promote the development of neoplasm in other tissues. We have been unable to find any data showing that VEGF promotes neoplasm growth in in-vivo or in-vitro studies. Although the multi-potential cells used in angiogenic therapy with cell implantation may differentiate in unexpected ways, no report of ectopic tissue formation, such as bone, has been reported in observation for several years.

Nevertheless, at this point in its development, clinicians should remain highly alert to the possibility of untoward cell differentiation, and close long-term follow-up is essential.

CONCLUSION

Angiogenesis has been studied for over 40 years. In the past 5 years since the discovery of ESCs, basic science and clinical studies concerning the application of angiogenesis have been
published. Accelerating interest in the clinical application of angiogenic therapy reflects the increase in treatment-resistant severe ischemic heart disease and the practical and economic limitations of heart transplantation. These factors make it reasonable to expect continued progress in angiogenic therapy and expanded clinical use.

I think that the use of angiogenic therapy for ischemic heart disease using gene therapy, cell transplantation, and hematogenous cytokine administration will benefit increasing numbers of patients. However, long-term studies monitoring side-effects to establish safety and effectiveness are required. Finally a randomized double blind study is needed to compare the efficacy of angiogenic therapy and conventional therapy with PCI and CABG.

The angiogenic therapy is a promising medical development in the treatment of ischemia that will likely improve the quality of life in patients who have severe ischemic heart disease.

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REFERENCES


