Introduction

The incidence of ischemic heart disease and arteriosclerosis obliterans, basically caused by atherosclerosis, is rising with the Westernization of eating habits and the aging of society. Though treatments with advanced drugs and such devices as stents have been developed, there are still many patients who must undergo lower-limb amputations in existing treatments, and post-angioplastial restenosis remains a major problem. Attention has been focused in recent years on the possibilities of gene therapy for these vascular diseases. Gene therapy got its start as a treatment for such congenital diseases as adenosine dianimase (ADA) deficit and has been spreading as a treatment for cardiovascular diseases and multifactor diseases. Therapeutic angiogenesis therapy using VEGF (vascular endothelial growth factor) gene is already being performed in Europe and North America for arteriosclerosis obliterans and ischemic heart disease, and it is reported that their effectiveness exceeds expectations.

Therapeutic Angiogenesis and Their Practice

Endothelial cell growth factors such as VEGF play an extremely important role and are placed at the center of therapeutic aging. Gene therapy for ischemic heart disease and chronic arteriosclerosis obliterans using VEGF genes has already begun in the United States led by Isner et al. at Tufts University, and has shown favorable results. They are introducing VEGF genes into muscle in plasmid form by means of intramuscular injection without using virus or other vectors. (Fig. 1)

Rabbit lower-limb ischemic models showed that the intramuscular administration of VEGF or other endothelial cell growth factor plasmid DNA results in the production of vascularization factor and regeneration of blood vessels. Isner et al. administered intramuscular injections of VEGF gene plasmid to the lower limbs of arteriosclerosis obliterans patients and reported marked improvement in blood flow. They observed marked increases in ABI (ankle brachial index) and TPI (toe pressure index).
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and improvement of intractable ulcers, indicating the effectiveness of the treatment for severe ischemic limbs, for which conventional treatments have been inadequate.

We demonstrated that an HGF (hepatocyte growth factor) discovered in Japan had a potent angiogenic action and began in June 2001 clinical studies (TREAT-HGF) using HGF genes to treat arteriosclerosis obliterans and Buerger disease. The subjects were patients with arteriosclerosis obliterans or Buerger disease who had pains at rest or ischemic ulcers and necrosis. We injected HGF gene plasmid into morbid limb muscle at four locations, preceded by administration of a preliminary dose and examination for side effects, whereupon we administered the therapeutic dose if no problems appeared. We have currently performed this therapy on six subjects, and observed increased ABI, improved pain, and improved ulcers. The gene therapy clinical studies review committee reported that there was no problem of safety in this therapy.

Since it remains difficult to perform viral gene transfer in clinical practice while the safety of virus vectors is yet to be established, at the present stage muscle tissue seems most appropriate to gene therapy in which genes are introduced in a plasmid form. Since VEGF and HGF are secretory proteins, the genes need not be introduced into all cells and it is possible to raise local concentrations with only partial introductions.

Isner et al. are also injecting VEGF genes directly into the myocardium of angina pectoris patients not adaptive to PTCA or CABG, and examining them for ischemic improvement from vascularization. They reported that the number of nitroglycerin dosages fell dramatically for each patient after gene therapy and that angiographic observations showed marked vascularization in each patient resulting from VEGF transgenesis. With SPECT they also observed an expansion of the normal blood-flow region and a decrease in the area of deficiency, indicating the utility of this treatment for ischemic heart disease.

Treatment for Post-Vasodilation Restenosis

A variety of drugs, primarily antiplatelets, have been tried to treat restenosis arising after transcutaneous vasodilation, but none has gained a consensus that it is effective. We have introduced a duplex nucleic acid compound (nucleic-acid-based therapeutic E2F decoy) that acts as a decoy on the transcription control...
element E2F binding arrays at promoter regions that are essential to the expression of regulator genes for the cell cycle of smooth muscle into smooth muscle cells in order to prevent the proliferation of smooth muscle cells, and have reported that it is possible to control neointimal growth in rat and pig models of restenosis.4)

In April 2000 we began clinical studies (J-PRAS) based on these results of treatments for post-vasodilation and post-stent restenosis with E2F decoy. The E2F decoy is applied to the hydrogel polymer coated on the surface of the vasodilatory catheter and administered after vasodilation. As of February 2002 we have performed five procedures and have observed no acute toxicity, nor any clear side effects in hematological or other examination. So far we have not observed any restenosis but in the future will add to the number of cases and examine the effects of the treatment.

On the other hand, Dzau et al. confirmed the effectiveness of the E2F decoy in graft models and in 1996 gained FDA approval for clinical studies of the E2F decoy. *Ex vivo* administration of the decoy to the vein grafts of patients improved the graft occlusion rate of 75% to 25%, confirming the utility of the E2F decoy. Phase III clinical trials are currently underway in the United States, and the decoy is scheduled to go on sale in 2003.

**Conclusion**

The clinical application of gene therapy for arteriosclerosis obliterans has only just begun in Japan on the basis of research conducted in the past few years and its effectiveness remains under investigation, but it is likely that genetic drugs will find a place in everyday medical examination and treatment in the future. We will be fortunate to be able, with new treatments, to enhance the quality of life of the patients with severe vascular diseases that had only palliative treatments up to now.

**REFERENCES**