Gene therapy is emerging as a potential strategy for the treatment of cardiovascular diseases such as peripheral arterial disease, myocardial infarction, restenosis after angioplasty, and vascular bypass graft occlusion, for which current therapy is often inadequate. The first federally approved human gene therapy protocol started on September 14, 1990, in patients with adenosine deaminase deficiency. Ten years after the commencement of the first trial, more than 30 clinical studies of gene therapy for cardiovascular disease are under investigation. First, Isner and colleagues demonstrated the potential utility of gene therapy using an angiogenic growth factor (vascular endothelial growth factor [VEGF]) for the treatment of critical limb ischemia in human patients. More recently, his group revealed the usefulness of gene therapy using VEGF to treat ischemic heart disease. Although there are still many unresolved issues, human gene therapy for cardiovascular disease is now becoming a reality.

Novel Therapeutic Strategy to Treat Vasospasm After Subarachnoid Hemorrhage (SAH)

In addition to the diseases cited above, the study of Toyoda et al in this issue of Circulation Research identifies vasospasm after SAH as another potential target for gene therapy. These investigators transfected the gene of calcitonin gene–related peptide (CGRP), a potent vasodilator, into the cisterna magna of rabbits using adenovirus. Interestingly, transfection of CGRP gene ameliorated cerebral vasospasm after experimental SAH. Although delayed, prolonged arterial constriction after SAH can lead to brain ischemia and infarction, there is no known effective pharmacotherapy. Vasospasm occurs in 30% to 40% of patients after SAH and is the leading cause of mortality and morbidity in SAH. Previous reports demonstrated arterial dilation after injection of recombinant CGRP in experimental SAH. The present studies emphasize the transfectability of cerebral vascular cells, by injection into the cerebrospinal fluid (CSF) of the cisterna magna.

Similarly, overexpression of endothelial nitric oxide synthase gene using an adenoviral vector also prevented angiopathy (vasospasm) after SAH. Alternatively, the introduction into target cells of synthetic double-stranded DNA with high affinity for a target transcription factor, as a decoy cis element, has been proposed. Using the decoy strategy, Ono et al reported that transfection of nuclear factor-κB decoy oligodeoxynucleotides into the subarachnoid space prevented angiopathy after SAH in a rabbit model using virus-liposome methods. These results clearly demonstrate the possibility of treating SAH using recombinant genes or oligodeoxynucleotides. Nevertheless, gene therapy still requires efficient in vivo gene transfer technology to achieve the final goal. During the past decade, many gene transfer methods have been developed, and some are being applied clinically in human gene therapy studies. In vivo gene transfer techniques for cardiovascular applications include (1) viral gene transfer with retrovirus, adenovirus, or HVJ (hemagglutinating virus of Japan, Sendai virus), (2) liposomal gene transfer with cationic liposomes, and (3) naked plasmid DNA transfer. These techniques have different advantages and disadvantages. Adenovirus-mediated transfer is a promising gene transfer method for the treatment of cardiovascular disease, as the adenoviral method is very effective for transfection into nonreplicating cells including vascular cells. The expression is temporary (weeks to months), suggesting that this transfer method may be particularly useful for treatment of self-limited diseases such as vasospasm after SAH, in which only temporary expression of the transgene is needed. However, for transfection into the central nervous system, adenovirus-mediated gene transfer is limited due to inflammatory changes. This undesirable adverse effect is particularly challenging for effective human gene therapy. Thus, further modification of vectors should be considered for human gene therapy in the central nervous system.

Adventure of Gene Therapy Into the Brain

In addition to vasospasm after SAH, gene therapy may be used to treat other cerebrovascular diseases. Cerebral occlusive disease caused by atherosclerosis of the cerebral arteries or Moyamoya disease often causes chronic hypoperfusion of the brain. Such a condition leads to not only cerebral ischemic events but also neuropathological changes including dementia. An effective treatment to improve hypoperfusion has not...
yet been established. It is known that ischemic stroke induces active angiogenesis, particularly in the ischemic penumbra, which correlates with longer survival in humans. However, the natural course of angiogenesis is not sufficient to compensate for the hypoperfusion state. In the presence of obstruction of a major artery, blood flow to the ischemic tissue is often dependent on collateral vessels. When spontaneous development of collateral vessels is insufficient to allow normal perfusion of the tissue at risk, residual ischemia occurs. Recently, preclinical studies have demonstrated that angiogenic growth factors can stimulate the development of collateral arteries in peripheral and myocardial ischemia, a concept called therapeutic angiogenesis. Thus, therapeutic angiogenesis using angiogenic growth factors should be considered for the treatment of patients with cerebral ischemia.

Angiogenesis can be promoted in the rat brain using adenoviral vectors containing cDNA from basic fibroblast growth factor (bFGF), a well-known angiogenic factor. After intraventricular administration of the viral vector, bFGF gene transfer induced angiogenesis in normal rat brain accompanied by an extremely high concentration of bFGF in the CSF. In addition to bFGF and VEGF, hepatocyte growth factor, a potent angiogenic growth factor, might be useful to treat ischemic cerebrovascular disease. Stimulation of new vessel formation by angiogenic growth factors is likely to create new therapeutic options in angiogenesis-dependent conditions such as stroke, Moyamoya disease, and dementia, although a number of important issues, such as safety and side effects, have not yet been addressed.

Although it may be feasible to treat these diseases using recombinant proteins rather than nucleic acids, gene therapy has several potential advantages over protein therapy. (1) Gene therapy has the potential to maintain an optimally high and local concentration over time. This issue may be critical in the case of arterial gene therapy. In addition, in the case of therapeutic angiogenesis, it may be preferable to deliver a lower dose over a period of several days or more from an actively expressed transgene in the artery, rather than a single or multiple bolus doses of recombinant protein, to avoid side effects. (2) Regarding economics, which therapy would ultimately cost more to develop, implement, and reimburse, particularly for those indications requiring multiple or even protracted treatment, needs to be considered. (3) The feasibility of a clinical trial of recombinant protein is currently limited by the lack of approved or available quantities of clinical-grade recombinant protein, due in large part to the nearly prohibitive cost of scaling up from research-grade to human-quality recombinant protein. Moreover, the central nervous system is relatively inaccessible to circulating proteins and peptides, because an anatomical barrier (blood-brain barrier) exists to prevent the clinical utility of vasodilators such as CGRP or angiogenic growth factors such as bFGF. Given that the molecular size of numerous agents is too large to penetrate the blood-brain barrier, these agents seem to be ineffective without direct and continuous injection into the ventricle, striatum, or cerebral cortex by a surgical technique. From the standpoint of clinical use, it is clear that these methods are less useful compared with gene transfer into the cisterna magna, because they entail surgical insult and prolonged endurance for the patient. Indeed, previous studies used the infusion of recombinant protein continuously into the brain or the subarachnoid space, whose manipulation is risky in clinical situations. Such a procedure is necessary because of the rapid disappearance of recombinant factors into surrounding tissue.

**Perspectives in Gene Therapy for Treatment of Cerebrovascular Disease**

The report by Toyoda et al should stimulate additional investigations into gene therapy strategies including (1) how to overcome the presence of the blood-brain barrier that limits transgenes from reaching their cellular targets, (2) how to avoid adverse effects in the brain, and (3) how to maintain brain function. As the advent of gene therapy into the brain provides new information for the treatment of human cerebrovascular disease, further efforts to investigate the biology and pathophysiology of stroke, ischemic cerebrovascular disease, SAH, dementia, and atherosclerosis should be stimulated. In addition to these issues, it is time to take a hard look at practical issues that will determine the real clinical potential. These include (1) further innovations in gene transfer methods, (2) well-defined disease targets, (3) cell-specific targeting strategies, and (4) effective and safe delivery systems.

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